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Acute Toxicity of Synthetic Detergents to Snails: Effect of Sodium Lauryl Sulfate on *Limnaea peregra* Shells

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Detergents are required in a wide range of our daily life for diverse purposes. In many of these fields of application, the surfactants are used in interaction with water. In some Spanish rivers concentrations of anionic surfactants are higher than 5 mg/L (MOPU 1983; Tarazona et al. 1983). The aquatic toxicology of surfactants has been studied for many years. Most attention has been given to the survival, growth, and reproduction of fish in aqueous concentrations. In a previous work we found that Limnaea peregra is highly sensitive to the anionic surfactant sodium lauryl sulfate (SLS), with a 96 h LC_{50} of 0.54 mg/L (unpublished data).

Lethality is a very crude measure of toxic response. Therefore, there has been a search for sublethal effects that would occur at much lower concentrations of the toxic substance than those causing death so these concentrations might be monitored (Duffus 1980). One of the most characteristic and significant structure of the gastropods is the shell, which was neatly described as a conical tube spirally coiled for mechanical convenience (Ellis 1926) and which is also used for the taxonomic classification (Macan 1977). This paper shows the effects of the anionic surfactant SLS on the chemical composition and macroscopic aspect of shells of L. peregra exposed to different concentrations of the surfactant.

MATERIALS AND METHODS

One hundred L. peregra cultivated in our laboratory were acclimatized to water quality conditions for 72 h. They were 3 months old and weighed 0.1-0.2 g. During the acclimatization they were fed with an algal (i.e. Chlorella s.p.) diet.

Three-liter cylindrical, glass aquaria, each with a total volume of 2 L dechlorinated tap water, were used. A control and four SLS concentrations which ranged between 0.16 and 13.5 mg/L were used with ten animals per and control with water renewal every 24 h. Animals were not fed during the test.

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Water quality (hardness, alkalinity, calcium, magnesium, chloride, ammonia and nitrite) was analyzed before and after solution renewal using analytical methods described by Rodier (1981). SLS concentrations were measured by methylene blue method (APHA-AWWA-WPCF 1980) using a calibration curve constructed specifically for SLS in a Pye Unicam SP/1800 spectrophotometer at 650 nm. Detergent concentration were measured 10 min and 24 h after adding enough volume of 1 g/L Sodium Lauryl Sulfate (Serva research grade, purity 99%) water solution to obtain the desired concentration.

The animals were exposed to SLS during 6 days. Dead animals were collected and maintained in dechlorinated tap water without SLS for 18 days. The 18th day, all shells were collected and weighed. The shells were heated at 105°C for 2 h until water was evaporated. They were cooled in desiccator for 15 minutes and weighed. Dry shells were put in furnance at 550°C for 12 h, cooled and weighed. Then, wet and dry weight, organic and inorganic matter were determined as differences between weights.

The data were subjected to regression analysis. The correlation coefficients were evaluated statistically by the Student's t-test.

RESULTS AND DISCUSSION

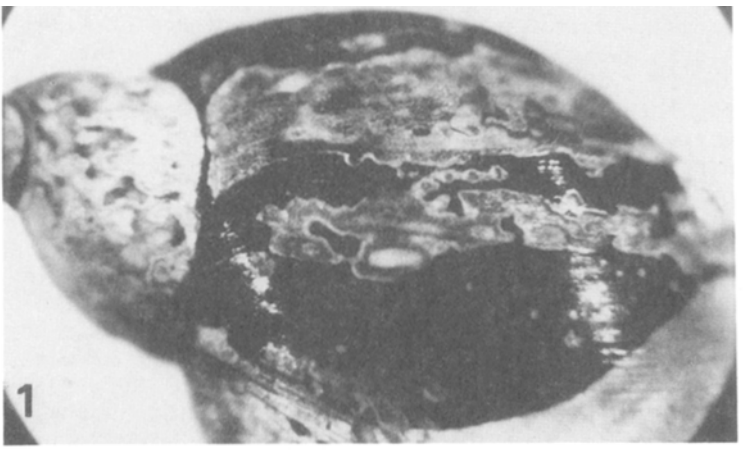
Water quality parameters analyzed during the test are shown in Table 1. Dissolved oxygen was not less than 80% saturation. There was no mortality in the control aquaria at the end of the exposure period. Mortality in SLS aquaria was higher than 50% .

Table 1. Water quality parameters.

Parameter	mean value[a]	stand.dev.[a]
Hardness (mg $CaCO_3$/L)	35.60	± 1.03
Calcium (mg CaO/L)	13.24	± 0.93
Magnesium (mg MgO/L)	4.94	± 0.65
Chloride (mg/L)	22.64	± 4.54
Alkalinity (mg $CaCO_3$/L)	2.41	± 0.12
Ammonia (mg/L)	N.D.[b]	
Nitrite (mg/L)	N.D.[b]	
Temperature (ºC)	22	± 1
pH	7.1	± 0.05

[a]n=12; [b]N.D.= not detected

The 20th day after beginning the bioassay there were three dead L. peregra in the control aquaria. Therefore, dead control animals were kept in dechlorinated tap water only for 4 days. During the bioassay shell discoloration and holes were observed on snails exposed to SLS (Figures 1 and 2). Discoloration was observed on all SLS treated animals but, holes were observed only in snails exposed to high concentrations.



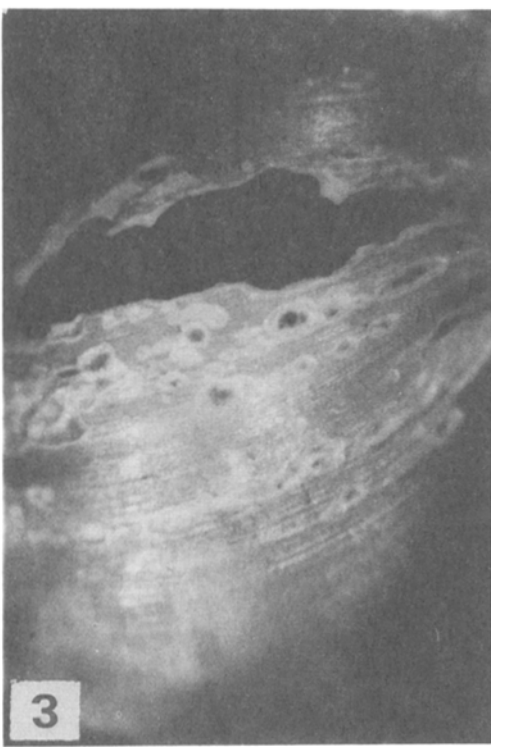


Figure 1. A normal shell of Limnaea peregra x 20.

Figure 2. Discoloration, as evident by changes in shade and pattern, and holes in the shells of sodium lauryl sulfate treated L. peregra x 5.

Figure 3. A detail of a hole in the shell of a L. peregra exposed to 12 ppm of sodium lauryl sulfate x 30.

Table 2. Dry weight (DW), organic matter (OM) and inorganic matter (IM) of shells of L.peregra exposed to the surfactant sodium lauryl sulfate and of snails of the culture aquarium.

SLS measured concen. ppm	DW mg/shell	OM mg/shell	IM mg/shell	OM/IM
Control	1.66	0.50	1.16	0.42
0.606	1.00	0.21	0.78	0.28
0.838	0.67	0.16	0.50	0.33
4.111	0.58	0.16	0.41	0.40
11.985	0.50	0.20	0.30	0.66
Culture Aquar.	1.25	0.17	1.08	0.15

Dry weights, results of organic and inorganic matter determinations and their ratios are shown in Table 2.

Shell's dry weights and organic matter were higher in control snails than in culture aquaria snails. Inorganic matter was similar in both groups. Organic matter was similar for all shells that were kept in water for 20-25 days (SLS-treated and culture aquaria shells), but higher in the control shells. After dying, control snails were kept in water only for 4 days, this event would likely explain the differences. Because of these differences, all calculations were made using culture aquarium snails as SLS-free group.

Dry weight (DW) was correlated with SLS concentration according to:

$$DW=0.770-0.252\log[SLS]$$

The correlation coefficient was $r=0.94$ with a statistical significance of $p<0.01$. A relation between organic matter and SLS concentration was not observed; similar weights for all groups were found. As for the dry weights, a log relation between organic matter (IM) against SLS-concentration was found. The relation was:

$$IM=0.583-0.258\log[SLS]$$

with a correlation coefficient of $r=0.96$ and a statistical significance of $p<0.005$. These results are very close to that found using shells of control groups as SLS-free group:

$$IM=0.596-0.285\log[SLS]$$

$$r=-0.97 \quad ; \quad p<0.005$$

It is clear that exposure to SLS causes a decrease of shell's dry weights due to a decrease in the inorganic components of the shells. This decrease depends on the logarithm of SLS concentration. Because discoloration and holes were observed in live animals, the shell's inorganic matter loss had to be measured for live animals.

The decrease of shell's inorganic matter due to SLS can be explained in two different ways. The first way is a direct effect on formation or loss of shell's inorganic matter. The primary inorganic component in molusca shells is $CaCO_3$. It has been proposed that SLS (Tovell et al. 1974) and other anionic surfactants (Bolle et al. 1963; Marchetti 1965) have a chelating

effect on calcium; so it is posible that SLS should be able to rob shells of calcium. The second way is an effect on epithelium and cells that play an important role in shell's formation and maintenance. The effect of SLS on epitheliums of aquatic animals has been extensively studied mainly in gills (Abel and Skidmore 1975; Abel 1976) and several papers about the effect of SLS on permeability have been recently published (Siegel and Gordon 1985a; 1985b ; 1986) this kind of physiological alterations should lead to a dose-dependent diminution of the capability of L.peregra to maintain shell's calcium.

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