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# Histopathological change induced in the liver of the Caspian kutum fry after acute exposure to the anionic surfactant

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## ABSTRACT

Surfactants such as linear alkylbenzene sulfonates (LAS) are widely used in the formulation of detergents in commercial products. After utilization, they are discharged to aquatic ecosystems, causing risk to aquatic life. In the present study, the liver histological damage of the Caspian Kutum, Rutilus frisii kutum, exposed to three sublethal concentrations of Surfactant detergent, Linear Alkylbenzene Sulfonate (LAS) for short terms intervals (24,48,72 and 96 hours) is assessed. Histopathological changes observed in liver structure include irregular-shaped nuclei, congestion and dilation of sinusoid. Results demonstrated that exposure duration affected the liver tissues of Caspian kutum more than the concentration of LAS did.

Key Words: Rutilus frisii kutum; Linear Alkylbenzene Sulfonate (LAS); liver; histophatology

# INTRODUCTION

Linear alkylbenzene sulphonates (LASs) are highly water soluble surface active agents widely used in synthetic laundry detergent formulation and household cleaning products [1]. Most of the detergents consumed are discharged via urban sewer systems into the marine medium, to a large extent without prior treatment of the sewage [14]. Most of domestic sewage flows into the Caspian Sea directly or through rivers without sewage treatment [16]. In the cases of untreated wastewater discharge, concentrations of LAS may reach higher concentrations at the impact zone [11]. *Rutilus frisii kutum* is important and economical species of Caspian Sea [2]. Nowadays, migration of Caspian Kutum has been reduced because of excessive pollution of rivers [16]. The environmental risk assessment and ecotoxicological involve the use of biomarkers designed to highlight an early stage of pollution [8]. Histopathological studies have been conducted to help establish causal relationships between contaminant exposure and various biological responses in the laboratory studies. These histopathological investigations have been proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments [15]. The liver plays a primary role in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions [12]. Limited papers were published on the histopathological effect of LAS on fish liver [7, 11]. This is the first assessment of the effects of sublethal

concentration of LAS in the liver of the Caspian kutum. In addition, the effects of duration with concentration of exposure of LAS on fish liver in a flow-through system have compared.

## MATERIALS AND METHODS

Paxan company (Tehran, Iran) supplied commercial LAS, a mixture of  $C_{10}$ - $C_{13}$  homologues with all positional isomers except 1-phenyl and an average molecular weight of 343 (sodium salt derivative). Caspian Kutum fry, *Rutilus frisii kutum* (0/5-1 g) were obtained from rearing unit of Shahid Ansari State, Rasht, Iran. Acclimatization of fishes was done for one week in a stock tank.

## **Exposure** Conditions

A continuous flow-through system, using city dechlorated water spiked with commercial LAS was prepared according to the OECD (1992) [9]. The exposure solution was renewed completely each day in order to ensure constant concentrations. Physical and chemical factors of water, such as temperature, pH and dissolved oxygen were maintained at appropriate values similar to standard conditions (temperature  $20\pm 1$ ; pH 7.9  $\pm 0.1$ ; %DO between 60-100%). Based on the 96 h LC<sub>50</sub> value of LAS for Caspian Kutum fry, 11/6 mg L<sup>-1</sup> [6], three sublethal concentrations (1:20, 1:10 and 1:5 of the 96 h LC50) were chosen. So, the fishes exposed to 0/58, 1/16 and 2/32 mg L<sup>-1</sup> concentrations of commercial LAS. Exposure to the different concentrations was conducted in triplicates and three control assays were run simultaneously. The control tanks were kept under the same conditions without addition of surfactant. Sampling of fishes was done after 24, 48, 72 and 96 hours from the beginning of experiment. Daily Samples of water were taken and measured for ensuring that the concentration of toxicant maintained as near as possible to the nominal value.

## Water analysis

Water samples (50 mL) were extracted by solid-phase Extraction with ODS SPE columns (SPE-C18 purchased from Applied Separations) as described by Tolls *et al.* [17]. LAS was eluted from the column with 5 mL of CH3OH (Merck). The samples were dissolved in 1 ml of CH3OH and transferred to HPLC vials, after evaporation of the solvent. LAS concentration in water was determined by reversed-phase HPLC with fluorescence detection.

## Histological analysis

After anesthetizing of fishes, the livers were removed and fixed by Bouin's solution. Then tissues were processed in a routine paraffin embedding procedure. Sections of 5  $\mu$ m thick were taken which were later stained by haematoxylin and eosin method. Stained sections of were examined by light microscope.



## RESULTS

Fig.1 normal liver tissue of *R. firrisi kutum* exposed to 0.00 mg L<sup>-1</sup> LAS after 96h. H&E.Bar:25µm; (a) hepatocytes with a nucleus (b) sinusoid.

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## Result of water analysis

The concentration of LAS in water was maintained  $\pm$  10% of selected concentration.

## Result of histological analysis

Histopathological changes were not observed in the liver of the control fish. The hepatic parenchyma shows regular distribution of hepatocytes disposed around the vascular system (sinusoids) (Fig.1).

In the case of 24 hours of exposure to 0.58 mg  $L^{-1}$  of LAS, the liver didn't show any histopathological changes (Fig. 2-1). For 48 hours congestion, i.e. the increase of the blood volume in the blood capillaries was observed (Fig. 2-2). After 72 and 96 hours of this concentration of LAS, congestion and dilation of sinusoids were seen (Fig. 2-3 and 2-4).



Fig.2 Liver tissue of *Rutilus firrisi kutum* exposed to 0.58 mg  $L^{-1}$  of LAS.

H&E. each Bar:25µm. (2-1) After 24h; (2-2) after 48h- (a) congestion; (2-3) after 72h- (a) congestion and dilation of sinusoids; (2-4) after 96h-(a) congestion and dilation of sinusoids.

In the liver of fish examined after 24 hours of exposure to 1.16 mg  $L^{-1}$  of LAS congestion was observed (Fig. 3-1) After 48 and 72 hours of exposure to the same concentration of LAS, congestion and dilation of sinusoids were noticed (Fig.3-2 and 3-3). In the cases of exposure to 96 hours, there was more congestion and dilation of sinusoids (Fig. 3-4).



**Fig.3 Liver tissue of** *Rutilus firrisi kutum* **exposed to 1.16 mg L<sup>-1</sup> of LAS.** *H&E. each Bar:25μm.* (3-1) *After 24h- (a) congestion; (3-2) after 48h- (a) congestion and dilation of sinusoids; (3-3) after 72h- (a) congestion and dilation of sinusoids; (3-4) after 96h- (a) congestion and dilation of sinusoids.* 

In the fish exposed to 2.32 mg  $L^{-1}$  LAS for 24 h, irregular-shaped nuclei was seen (Fig. 4-1). After 48 hours, congestion and dilation of sinusoids were noticed (Fig. 4-2). In the case of 72 and 96 h congestion and dilation of sinusoids and irregular-shaped nuclei of hepatocytes have taken place (Fig. 4-3 and 4-4).





# Fig.4 Liver tissue of *Rutilus firrisi kutum* exposed to 2.32 mg L<sup>-1</sup> of LAS.

H&E. each Bar:25µm. (4-1) After 24h - (a) irregular-shaped nuclei; (4-2) after 48h (a) congestion of sinusoids; (4-3) after 72h- (a) congestion and dilation of sinusoids (b) irregular-shaped nuclei; (4-4) after 96h- (a) congestion (b) congestion and dilation of sinusoids (c) irregular-shaped nuclei.

## DISCUSSION

The liver is the main organ for detoxification [4], and due to its function, blood supply and position [18], it is one of the organs most affected by contaminants in the water [13]. In the present study dilation of sinusoids, congestion and irregular-shaped nuclei were detected in *R. frisii kutum* exposed to sublethal concentrations of LAS. Congestion and blood stagnation have been observed in liver of fishes exposed to LAS in the previous studies [7, 11]. Congestion is a blood circulation disturbance due to the increase volume of the blood in the blood capillary [11]. Some studies demonstrated that alterations in shape, number, size and of the hepatocyte nucleus can be caused by contaminants [3, 5, 7, 10]. Alterations in size and shape of nucleus have often been regarding as signs of increased metabolic activity but may be of pathological origin [5]. In this study, when comparing histopathlogical changes of livers in the three concentrations, the higher concentration of LAS shows more damage than the lower ones. Some other studies have shown this result for LAS [7, 11]. The liver damages of the fish exposed to LAS surfactant may be due to the accumulation of the surfactant in this tissue [11]. According to results the longest time interval of lowest concentration was more damaged compared to the shortest time intervals of highest concentrations. So, it seems that the duration of exposure affects more than the concentration of LAS on the liver tissues of Caspian kutum.

## CONCLUSION

In the liver tissues of *R. frisii kutum* exposed to sublethal concentrations of LAS, congestion and dilation of sinusoids and irregular-shaped nuclei were detected. Also, the findings of the present histological investigations demonstrated a direct correlation between detergent exposure and histopathological disorders observed in liver tissue. So, histopathological alterations in the liver may be useful biomarkers for the toxicity of sublethal concentrations of LAS.

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