

## Histopathological changes of gill, liver and kidney in Caspian kutum exposed to Linear Alkylbenzene Sulfonate

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### Abstract

The histopathological effects of Linear Alkylbenzene Sulfonate on the gill, liver and kidney tissues of the Caspian kutum, *Rutilus frisii kutum* were studied. The fish were exposed to three sublethal concentrations of anionic detergent, Linear Alkylbenzene Sulfonate (LAS) for short term intervals (192 h). Gill, liver and kidney samples were collected after 192 h of exposure to LAS and lesions were analyzed by light microscopy. The histological changes to gills were edema, fusion of lamellae and lamellar aneurism. Some alterations like reduction of the interstitial haematopoietic tissue, tubular shrinkage, degeneration in the epithelial cells of renal tubule and necrosis were observed in the kidney. In the liver tissue, hepatocyte degeneration, congestion and dilation of sinusoid and vacuolar degeneration were seen. It seems that sublethal concentration of LAS may affect sever changes to gill, kidney and liver of *R. frisii kutum* specimens that leads to malfunction of these organs which cause damage to health of the fish.

**Keywords:** *Rutilus frisii kutum*, Linear Alkylbenzene Sulfonate (LAS), histopathology, gill, liver, kidney

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## Introduction

Linear Alkylbenzene Sulphonate (LAS) is the most widely utilized synthetic anionic surfactant for cleaning products (Hampel et al., 2004), with an annual global production rate of 4 mt (HERA-LAS, 2004). In waste water treatment plants, LAS is removed in up to 99%, but certain amounts of this compound may reach rivers and coastal waters, causing risk to aquatic life (Hampel et al., 2008). Most of domestic sewage flows into the Caspian Sea directly or through rivers without sewage treatment (UNIDO Caspian pollution report, 1998). In cases of untreated wastewater discharge, concentrations of LAS may reach higher concentrations at the impact zone (Hampel et al., 2004).

Water pollution produces pathological alterations in fish. These exposure concentrations may not be lethal for the affected species, but may influence internal functions and structures which under chronic exposure conditions could have effects on the effectiveness of vital functions and processes such as competitive stress and environmental resistance, reproduction, growth, etc (Duft et al., 2003; Hampel et al., 2008).

Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants (Thophon et al., 2003). Gill, kidney and liver are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining these specific target organs

(Gernhofer et al., 2001). Furthermore, the pathological changes discovered in these organs are normally easier to identify than functional ones (Fanta et al., 2003), and serve as warning signs of damage to animal health (Hinton and Laurén, 1990).

One of the most important and economic aquatics of Caspian Sea is Kutum (*R. frisii kutum*) (Babazadeh et al., 2008) which devoted approximately 78% of bony fish harvested (Afraei Bandpei et al., 2011). Research on the histopathological effects of LAS in the gill, kidney and liver of Caspian Sea kutum has not been done. So, this is the first report for assessment of histopathological changes in the gill, kidney and liver of Caspian kutum due to exposure to sublethal concentration of LAS.

## Materials and methods

The juvenile (0/5- 1 g) Caspian Kutum, *R. frisii kutum* were obtained from rearing unit of Shahid Ansari State, Rasht, Iran. Commercial LAS, a mixture of C10-C13 homologues with all positional isomers except 1-phenyl and an average molecular weight of 343 (sodium salt derivative), was supplied by Paxan Company (Tehran, Iran). Fishes were kept in a stock tank for one week for acclimation.

**Exposure Conditions:** The fish were tested in a continuous flow-through system, comprising 3 treatments and one blank in 3 replications (totally 12 tanks). The exposure solution was renewed completely each day in order to ensure constant concentrations. The tests were done in 192 h. Physical and chemical factors of water, such as temperature, pH and dissolved oxygen were maintained at appropriate values

similar to standard conditions (temperature  $20 \pm 1^\circ\text{C}$ ; pH  $7.9 \pm 0.1$ ; %DO between 60-100%). Three sublethal concentrations (1:20, 1:10 and 1:5 of the 96 h LC50) were chosen, based on the 96 h LC50 value,  $11/6 \text{ mg L}^{-1}$  (Gholami et al., 2010). So, the fishes exposed to 0/58, 1/16 and 2/32  $\text{mg L}^{-1}$  concentrations of commercial LAS. The control tank was kept under the same conditions without adding of surfactant. Sampling was done after 192 h from onset 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:** Solid-phase Extraction technique with ODS SPE columns (SPE-C18 purchased from Applied Separations) was used for extraction of water samples (50 mL). LAS was eluted from the column with 5 mL of  $\text{CH}_3\text{OH}$  (Merck). After evaporation of the solvent, the sample was dissolved in 1 mL of  $\text{CH}_3\text{OH}$  (Merck) and transferred to HPLC vials. LAS concentration in water was determined by reversed-phase HPLC with fluorescence detection (Tolls et al., 1999).

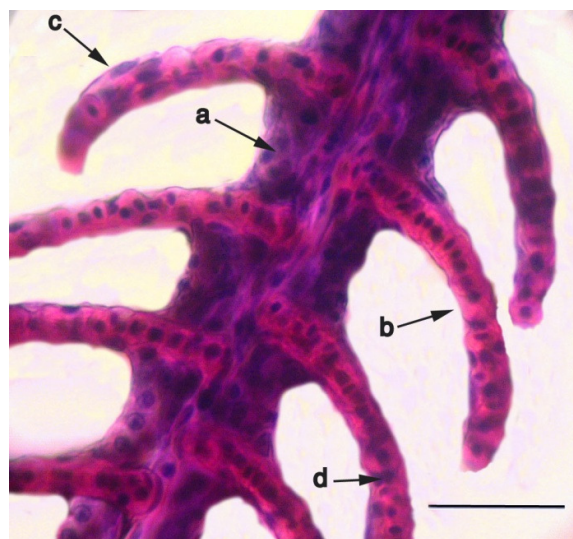
**Histological analysis:** Ten fish per treatment were anesthetized and the gills, livers and kidneys were removed. Tissues were placed in prelabeled, individual glass vials in which Bouin's fixative was added. After 24 h immersion fixation, tissues were transferred to coded glass vials containing 70% ethanol. Tissues dehydrated in concentrations of alcohol, and embedded in paraffin wax for microtome sectioning ( $5 \mu\text{m}$ ) (Poosti and Marvasti, 1999). Sections were mounted onto slides and after hydration in ethanol series of descending concentration, were stained with

standard haematoxylin and eosin (H&S) stain. 10 sections of each tissue from each fish were examined by light microscope (Kazemi and Bahmani, 1998).

## Results

**Water analysis:** Analysis of water samples showed that the concentration of LAS in water was maintained  $\pm 10\%$  of selected concentration.

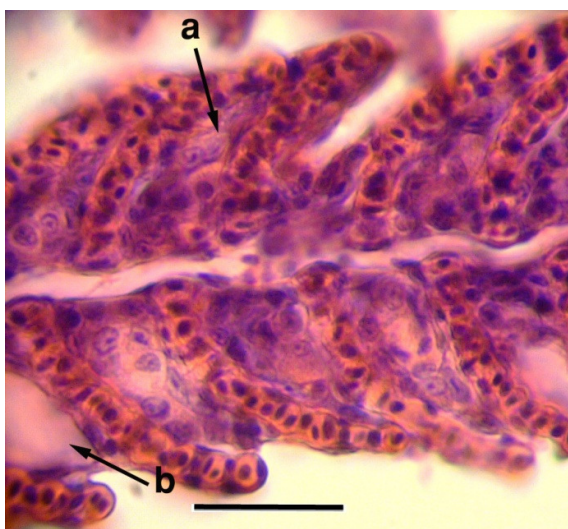
**Control gill tissues:** The gills from control specimens showed normal conditions. i.e. fresh red color and the gill lamella were arranged normally (Fig.1).



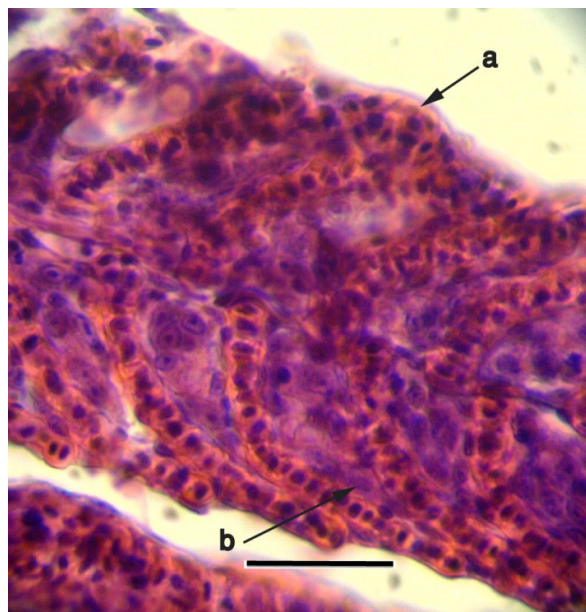
**Figure 1:** normal Gill tissue of *Rutilus frisii kutum* exposed to 0.00 ppm LAS. H&E. Bar:  $25 \mu\text{m}$ ; (a) primary lamellae (b) secondary lamellae (c) lamellar epithelial cells (d) pillar cell

**Treated gill tissues:** In the fish exposed to  $0.58 \text{ mg L}^{-1}$  of LAS for 192 h, hyperplasia of the lamellar epithelial cells, lamellar fusion and edema were observed (Fig. 2). Lamellar aneurysm, increasing the number of epithelial

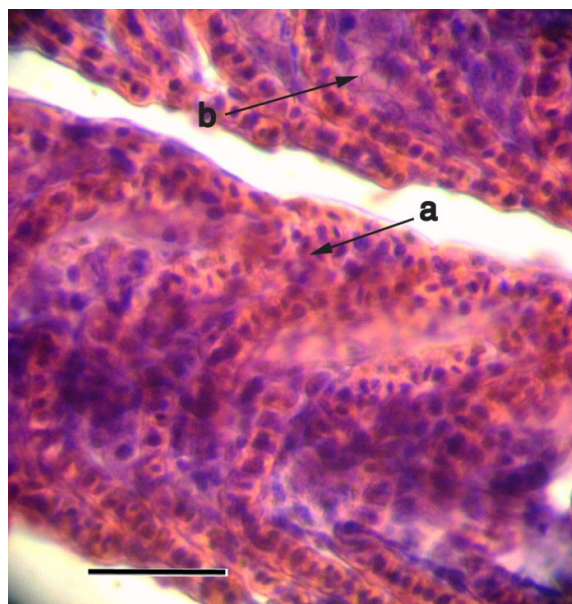
cells and fusion of adjacent lamellae were seen in the gills of fish examined after 192 hours of exposure to  $1.16 \text{ mg L}^{-1}$  of LAS (Fig.3). In the case of gills that exposed to  $2.32 \text{ mg L}^{-1}$  LAS, the gills were more damaged that individual lamellae were indistinguishable (Fig. 4).



**Figure 2:** Gill tissue of *Rutilus frisii kutum* exposed to 0.58 ppm LAS after 192h. H&E. Bar: 25 $\mu\text{m}$ . (a) fusion of adjacent lamellae (b) edema



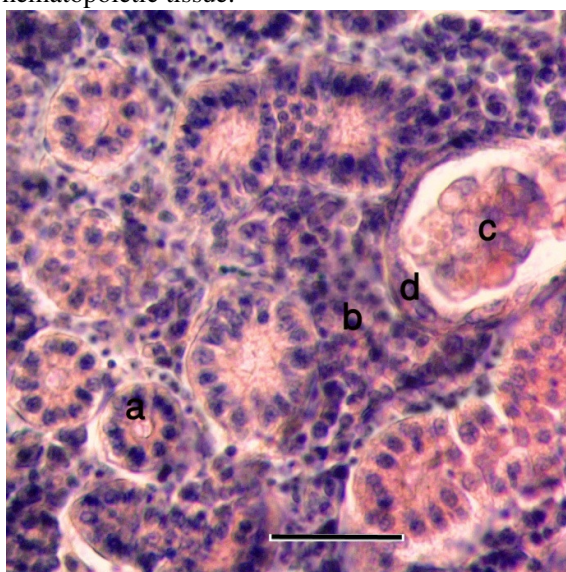
**Figure 3:** Gill tissue of *Rutilus frisii kutum* exposed to 1.16 ppm LAS after 192h. H&E. Bar: 25 $\mu\text{m}$  (a) Lamellar aneurysm (b) fusion of lamellae



**Figure 4:** Gill tissue of *Rutilus frisii kutum* exposed to 2.32 ppm LAS after 192h. H&E. Bar: 25 $\mu\text{m}$ . (a) lamellar aneurysm (b) full fusion of lamellae



**Control of kidney tissues:** Histopathological changes were not observed in the kidney of the control fishes. The structural details of the kidney of control *R. frisii kutum* are shown in Figure 5. Like other teleosts, the basic unit of kidney is composed of a renal corpuscle, Bowman's capsule and glomerulus and various segment of the renal tubules. These tubules are surrounded by an interstitial lymphoid – hematopoietic tissue.

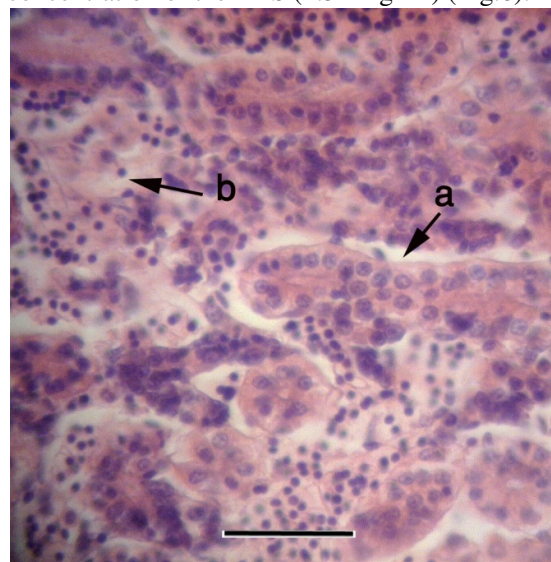


**Figure 5:** Normal kidney tissue of *R. frisii kutum* exposed to 0.00 mg L<sup>-1</sup> LAS after 192h. H&E.Bar:25µm; (a) renal tubules (b) hematopoietic tissue (c) glomerulus (d) Bowman's capsule

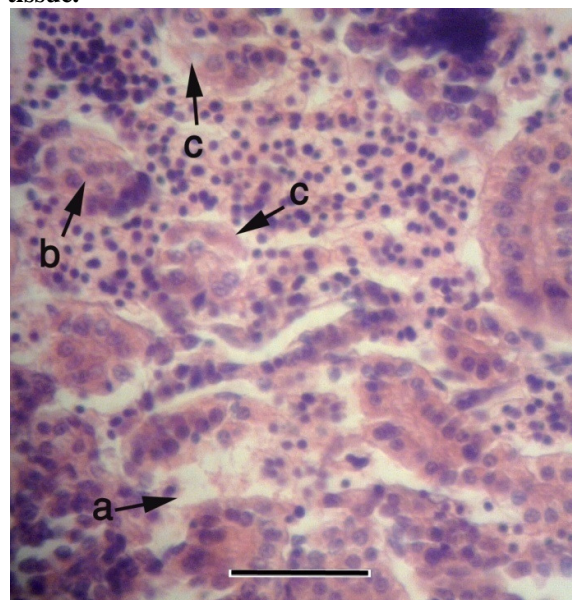
**Treated kidney tissues:**

The fish exposed to 0.58 mg L<sup>-1</sup> LAS for 192 h showed a moderate reduction of the interstitial haematopoietic tissue and tubular shrinkage (Fig. 6). These alterations as well as degeneration in the epithelial cells of renal tubule were observed in the fish exposed to 1.16 mg L<sup>-1</sup> of LAS (Fig.7). More reduction of the interstitial haemocytopoietic tissue, necrosis, tubular degeneration and tubular

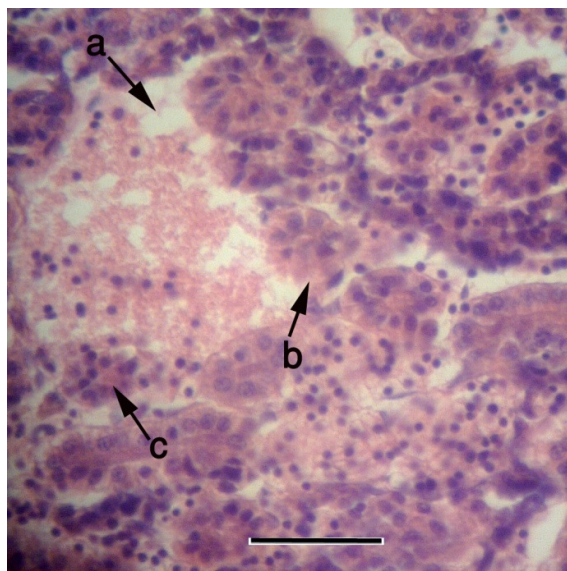
shrinkage were seen at the highest exposure concentration of the LAS (2.32 mg L<sup>-1</sup>) (Fig.8).



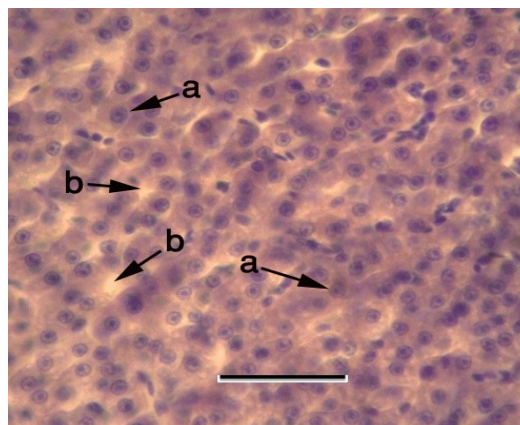
**Figure 6:** Kidney tissue of *R. frisii kutum* exposed to 0.58 mg L<sup>-1</sup> LAS after 192h. H&E.Bar:25µm. (a) tubular shrinkage (b) reduction of the interstitial haematopoietic tissue.



**Figure 7:** Kidney tissue of *R. frisii kutum* exposed to 1.16 mg L<sup>-1</sup> LAS after 192h. H&E. Bar:25µm. (a) reduction of the interstitial haematopoietic tissue (b) tubular shrinkage (c) degeneration in the epithelial cells of renal tubule



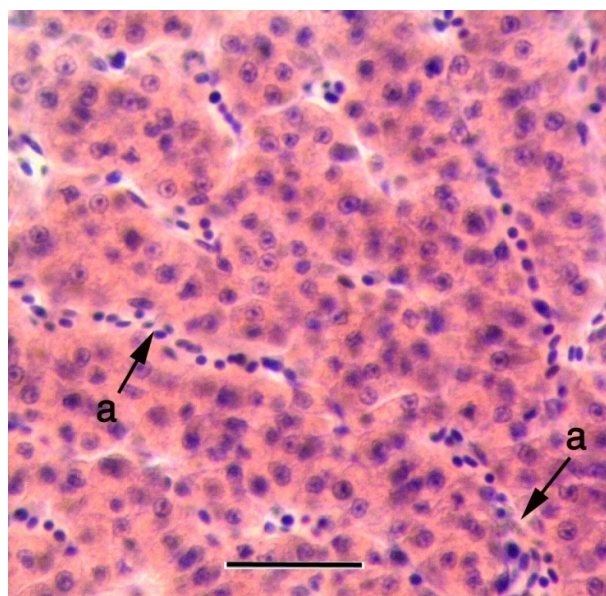
**Figure 8:** Kidney tissue of *R. frisii kutum* exposed to 2.32 mg L<sup>-1</sup> LAS after 192h. H&E. Bar: 25µm. (a) necrosis and reduction of the interstitial haematopoietic tissue (b) tubular degeneration (c) luminal occlusion and tubular shrinkage.



**Figure 9:** Normal liver tissue of *R. frisii kutum* exposed to 0.00 mg L<sup>-1</sup> LAS after 192h. H&E. Bar: 25µm; (a) hepatocytes with a nucleus (b) sinusoid.

**Control of liver tissues:** Liver histology from control fish is illustrated in Figure 9. No histopathological changes were observed in the liver of the control fish and the hepatic parenchyma shows regular distribution of hepatocytes arranged around the vascular system (sinusoids) (Fig.9). *Treated liver tissues*

In the liver tissues of fish exposed to 0.58 mg L<sup>-1</sup> LAS, congestion of sinusoid was observed (Fig.10). At exposure concentrations of 1.16 mg L<sup>-1</sup>, more congestion and dilation of sinusoid were seen (Fig.11). In fish exposed to 2.32 mg L<sup>-1</sup> of LAS, the histological alterations of liver were more evident and some other changes such as hepatocyte degeneration and vacuolar degeneration were detected (Figs.12 and 13).



**Figure 10:** Liver tissue of *R. frisii kutum* exposed to 0.58 mg L<sup>-1</sup> LAS after 192h. H&E. Bar: 25µm. (a) congestion of sinusoid.



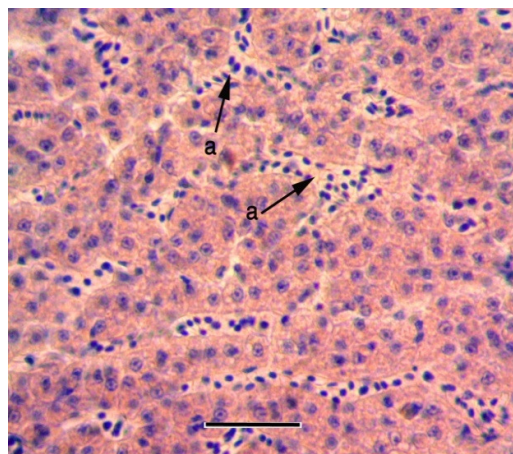


Figure 11: Liver tissue of *R. frisii kutum* exposed to 1.16 mg L<sup>-1</sup> LAS after 192h. H&E. Bar: 25μm. (a) congestion and dilation of sinusoid.

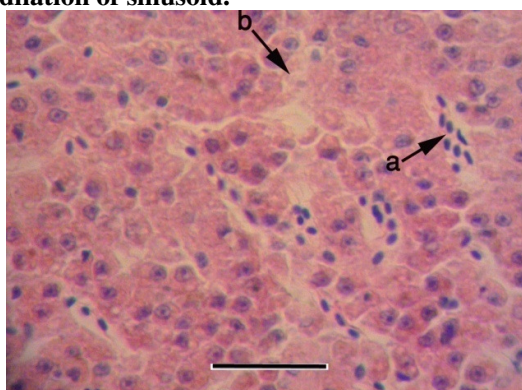


Figure 12: Liver tissue of *R. frisii kutum* exposed to 2.32 mg L<sup>-1</sup> LAS after 192h. H&E. Bar: 25μm. (a) congestion and dilation of sinusoid (b) hepatocyte degeneration.

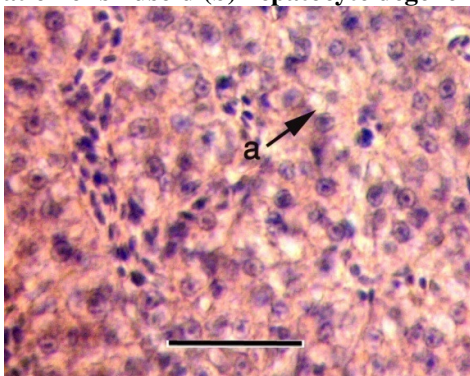


Figure 13: Liver tissue of *R. frisii kutum* exposed to 2.32 mg L<sup>-1</sup> LAS after 192h. H&E. Bar: 25μm. (a) vacuolar degeneration.

## Discussion

According to Roberts and Rodger (2001), massive lamellar hyperplasia may lead to secondary lamellar fusion. Thickening of the primary filament epithelial padding due to edema, cellular hyperplasia and fusion of the secondary lamellae are defense mechanisms that could impair blood-water exchange by reducing the surface area of the secondary lamellae that is in contact with the water (Kelly, 2007). Such alterations are non-specific and may be induced by different types of contaminant (Mallatt, 1985). The formation of an aneurysm is related to the rupture of the pillar cells (Martinez et al., 2004) due to a bigger flow of blood or even because of the direct effects of contaminants on these cells. This is a severe type of lesion, recovery from which is more difficult than the epithelial changes (Poleksic and Mitrovic-Tutundzic, 1994).

In the present study several changes of gill tissue like hyperplasia, edema, fusion of lamella and lamellar aneurysm were detected in the gills of fish after 192 hours of exposure to three sublethal concentrations of LAS. Some changes in the gill architecture of fish have been observed after acute exposure to a sublethal concentration of LAS, such as epithelial lifting and fusion of gill lamellae and stagnation of gill vessels (Alvarez-Munoz et al., 2009). However hypertrophy, hyperplasia, fusion of adjacent lamella and telangeasies of the gill were noticed when fish exposed to LAS for chronic test (Hampel et al., 2008; Rejeki et al., 2008).

The teleostean kidney is one of the first organs to be affected by contaminants in the

water (Thophon et al., 2003) and receives the largest proportion of postbranchial blood. Therefore renal lesions might be expected to be good indicators of environmental pollution (Cengiz, 2006; Hampel et al., 2008). In the kidney of *R. frisii kutum* after acute exposure to LAS, histological changes like reduction of the interstitial haematopoietic tissue, tubular shrinkage, degeneration in the epithelial cells of renal tubule and necrosis were noticed. Reduction of the interstitial haematopoietic tissue and epithelial hyperplasia with luminal occlusion of renal tubules have been reported in kidney of *Solea senegalensis*, when exposed to LAS in a chronic test (Hampel et al., 2008). Degeneration in the epithelial cells of renal tubule and narrowing of the tubular lumen has been shown in kidney of fishes exposed to deltamethrin (Cengiz, 2006). Rosety et al. (2002) reported tubular retraction in the kidney of *Scophthalmus maximus* L. exposed to the sodium dodecyl sulfate. The shrunk lumen of tubules are suggestive of hindered tubular reabsorption (Bhatnagar et al., 2007). Camargo and Martinez (2007) reported some alteration like tubular degeneration and luminal occlusion in the Neotropical fish species *Prochilodus lineatus*, caged in an urban stream. In severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995). In the present study tissue necrosis in *R. frisii kutum* specimens was seen when exposed to the highest concentrations of LAS.

The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply (Van der Oost et al., 2003), it is



also one of the organs most affected by contaminants in the water (Rodrigues and Fanta, 1998). Alterations in the liver may be useful as markers that indicate prior exposure to environmental stressors (Velmurugan et al., 2009). Congestion and dilation of sinusoid, hepatocyte and vacuolar degeneration were detected in *R. frisii kutum* exposed to sublethal concentrations of LAS. Several studies demonstrated that alterations in number, size and shape of the hepatocyte nucleus can be due to contaminants (Paris-Palacios et al., 2000; Camargo and Martinez, 2007; Figueiredo-Fernandes et al., 2007; Hampel et al., 2008). Congestion of sinusoid, shrinkage of hepatocytes, slight atrophy and vacuolar degeneration have been reported in liver of fishes after chronic exposure to LAS (Hampel et al., 2008; Rejeki et al., 2008). Congestion is a blood circulation disturbance due to the increase volume of the blood in the blood capillary. Vacuolar degeneration is known as an acute swelling of the organ (Rejeki et al., 2008).

In this research, the gill, liver and kidney damages were dependent to the concentrations of the LAS. When comparing histopathological changes in the three concentrations, 2/32 mg L<sup>-1</sup> shows more damages than 1/16 mg L<sup>-1</sup>, and 0/58 mg L<sup>-1</sup> had the lowest irritation. So, the higher concentration of LAS shows more damage than the lower ones. Some other studies have shown this result for LAS (Hampel et al., 2008; Rejeki et al., 2008) and some other pollutants (Cengiz and Nlu, 2002; Regar and Bhatnagar, 2006). The tissue damages of the fish exposed to LAS detergent

may be due to the accumulation of the detergent in them (Rejeki et al., 2008).

All the histopathological observation indicated that exposure to sublethal concentrations of LAS caused destructive effect in the gill, kidney and liver tissues of *R. frisii kutum*. However, complementary studies are necessary for a better understanding of its deleterious effects.

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## تغییرات بافت شناسی آبشش، کبد و کلیه ماهی سفید دریای خزر در معرض آلاینده آلکیل بنزن سولفونات خطی

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### چکیده:

در تحقیق حاضر اثرات بافت شناسی آلاینده آلکیل بنزن سولفونات خطی (Linear Alkylbenzene Sulfonate) بر بافتهای آبشش، کبد و کلیه ماهی سفید دریای خزر (*Rutilus frisii kutum*) مطالعه گردید. ماهیان در معرض سه غلظت تحت کشنده شوینده آنیونی، آلکیل بنزن سولفونات خطی، به مدت ۱۹۲ ساعت قرار گرفتند. نمونه گیری از بافت های آبشش، کبد و کلیه پس از قرارگیری در معرض آلاینده LAS انجام شد و آسیب ها توسط میکروسکوپ نوری بررسی گردیدند. تغییرات بافت شناسی آبشش شامل ادم، اتصال لاملاها و آنورسم بودند. در بافت کلیه کاهش بافت بینابینی، چروکیدگی توبولها، دژنراسیون سلولهای اپی تلیالی توبولها و نکروز مشاهده گردید. بافت کبد دژنراسیون هیپاتوسیتها، پرخونی و تورم در سینوزوئیدها و دژنراسیون واکوئولی را نشان داد. طبق نتایج به نظر می رسد که غلظت تحت کشنده آلکیل بنزن سولفونات خطی (LAS) تغییرات شدیدی را در بافتهای آبشش، کبد و کلیه گونه ماهی سفید دریای خزر ایجاد میکند که می تواند منجر به اختلالات عملکردی این اندامها و در نهایت باعث آسیب به سلامت ماهی گردد.

**کلمات کلیدی:** ماهی سفید دریای خزر (*Rutilus frisii kutum*)، آلکیل بنزن سولفونات خطی، آسیب بافتی، آبشش، کبد و کلیه

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