

# Effect of Linear Alkylbenzene Sulfonate on Some Biochemical Parameters in Rainbow Trouts (*Oncorhynchus mykiss*)

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### Summary

Changes of some biochemical parameters after exposing rainbow trout to linear alkylbenzene sulfonate (LAS) are reported in this study. Alterations in the activities of lactate dehydrogenase (LDH), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (AP), total protein, albumin, cholesterol, and glucose have been examined after exposure to sublethal concentrations (0.2 mg/L and 0.4 mg/L) of linear alkyl benzene sulfonate for 54 days.

The results from fish exposure to two different sublethal doses of LAS were compared with those from untreated control fish. Statistically significant increases in experimental plasma LDH and AP activities were found, whereas AST and ALT activities in treated groups tended to be higher without significant differences. Plasma cholesterol levels in exposed fish were significantly lowered, but alterations in total protein, albumin, and glucose levels were insignificant compared to controls. The observed increases in the plasma activities of LDH, AST, ALT and AP suggested that low LAS concentrations might cause cellular damage.

**Keywords:** rainbow trout, *Oncorhynchus mykiss*, linear alkylbenzene sulfonate, biochemical parameters

Synthetic detergents cause toxicity in aquatic environment by adversely affecting the fauna and flora particularly for fish (11, 20, 21). Nevertheless, in recent years there has been a rapid increase in the production of synthetic detergents used in industries and for household purposes. Linear alkyl benzene sulfonate (LAS) is the most widely used anionic surfactant in modern world. LAS is particularly harmful due to its effects on microalgae, invertebrates, and fish, all of which are living in aquatic environment (15). These detergents may also damage metabolic processes in the fingerling carp (11).

Lower concentrations of LAS play a role as stress factor and cause decrease in weight and behavioural abnormalities characterized by different swimming moves (10). Enzyme activities in the serum of fish have been used to indicate water pollution (1, 13, 23). Serum enzyme activities reflect very sensitively the health condition of fish, so their use in diagnosis has been recommended (9, 23). Enzymes such as alanine amino transferase (ALT) and aspartate amino transferase (AST) occur fundamentally in liver cells, so an increase of their serum levels in is clearly indicating a liver cell damage (7, 17).

Gluth and Hanke (6) have reported that levels of serum cortisol and glucose increased, the amount of

liver and muscle glycogen decreased and protein and cholesterol concentrations were reduced after exposure of carps to the pollutants. Parameters of high and low stress response (e.g. cortisol and glucose levels in blood) were also found to be associated with disease resistance in fish (4, 5). The aquaculture environment exposes the fish to a regimen of repeated acute stress, which has detrimental effects on growth, reproduction and the immune system (16). Levels of blood plasma ions and enzymes with important metabolic functions can give an indication to the general health of the fish (1, 3, 8, 22, 24).

The purpose of this work was to study the effect of sublethal concentrations (0.2 mg/L LAS and 0.4 mg/L LAS) of LAS on some selected plasma enzymes and some biochemical parameters of rainbow trout under *in vivo* conditions.

### Material and methods

In this study 120 rainbow trout (*Oncorhynchus mykiss*) with a mean weight of 40-60 g were used. Fish were obtained from Sapanca Fisheries Station of Istanbul University. Fish were weighed before the study and separated into groups of 40 fish each. During the course of the study fish were held in 2000 L Fibreglas tank. The photoperiod was 12 h light/12 h dark. The water in the tank was saturated

with oxygen ( $11 \pm 2^\circ\text{C}$ ). While no chemical compound was applied to the water of the first group, 0.2 and 0.4 mg LAS/L water were added for 54 days to the water of the second and the third groups, respectively. LAS levels applied to the experimental groups were measured with the Anilin Blue method (2) and were kept constant throughout the study. Linear alkylbenzene sulphonates (LAS C<sub>11-13</sub>) was donated by Henkel Company (Henkel Co. Ltd., Istanbul, Turkey).

At the end of experimental period, blood samples of randomly chosen animals from each group were obtained by caudal puncture after being anaesthetized. Collected blood samples were centrifuged at 4,000 rpm for 10 min. Plasma glucose, total protein, albumin and cholesterol levels and the enzymes aspartate amino transferase (AST), alanine amino transferase (ALT) alkaline phosphatase (AP) and lactate dehydrogenase (LDH) were spectrophotometrically determined by commercial reagent kits (Bio-Clinica).

The differences among the groups were statistically tested with analysis of variance (ANOVA) and the Duncan's test.

## Results and discussion

The results of the experiment are given in Table 1. Sublethal concentrations of LAS (0.2 mg/L and 0.4 mg/L) altered some of the enzyme activities. LDH and AP levels were significantly higher vs. controls. AST and ALT showed a tendency towards increase without being statistically different from untreated fish. Similar changes were observed for plasma total protein and glucose levels in the exposed groups (0.2 and 0.4 mg/L). Total plasma cholesterol levels were significantly lower in experimental groups compared to controls. The level of plasma albumin decreased insignificantly in experimental groups.

Many works have aimed to use fish as indicator organisms for the measurement of the effects of environmental pollution. A primary problem in these works was the establishment of reliable indicators of sublethal intoxication in fish. In many studies, blood enzyme activities in fish and some changes resulting from such abnormal states as stress the blood of fish and, intoxication, disease, changed water quality, etc. have been examined (1, 9, 17, 23, 25). Blood serum contains a number of enzymes whose concentrations may vary as a result of normal metabolic processes. The increased enzyme activities in the blood may indicate a specific pathological process (17).

In this study rainbow trouts were exposed to two different sublethal doses (0.2 and 0.4 mg/L) of LAS in order to determine its effect of LAS on plasma enzyme activity and other parameters. The activities of AP and LDH were increased statistically ( $p < 0.05$ ) whereas AST and ALT increased insignificantly. The above enzymes play an important role in the metabolic functions. The toxicity of surfactants arises from its

**Tab. 1. Plasma biochemical parameters of rainbow trouts following exposure to sublethal concentrations (0.2 mg/L and 0.4 mg/L) of linear alkylbenzene sulfonate. Data are presented as mean  $\pm$  SEM, n = 15**

Parameters	Control group (Unexposed)	Experimental Group I (0.2 mg/L LAS)	Experimental Group II (0.4 mg/L LAS)
AST (U/L)	22.66 $\pm$ 5.04 <sup>a</sup>	31.75 $\pm$ 4.75 <sup>a</sup>	36.5 $\pm$ 7.20 <sup>a</sup>
ALT (U/L)	3.33 $\pm$ 0.79 <sup>a</sup>	4.38 $\pm$ 1.00 <sup>a</sup>	4.60 $\pm$ 0.42 <sup>a</sup>
AP (U/P)	144.41 $\pm$ 14.03 <sup>a</sup>	197.00 $\pm$ 15.64 <sup>b</sup>	215.41 $\pm$ 15.33 <sup>b</sup>
LDH (U/L)	582.25 $\pm$ 39.50 <sup>a</sup>	641.18 $\pm$ 41.12 <sup>ac</sup>	716.00 $\pm$ 31.57 <sup>bc</sup>
Total protein (g/dl)	2.51 $\pm$ 0.30 <sup>a</sup>	2.59 $\pm$ 0.10 <sup>a</sup>	2.66 $\pm$ 0.30 <sup>a</sup>
Albumin (g/dl)	1.12 $\pm$ 7.10 <sup>a</sup>	1.10 $\pm$ 3.65 <sup>a</sup>	1.07 $\pm$ 2.50 <sup>a</sup>
Cholesterol (mg/dl)	168.40 $\pm$ 9.87 <sup>a</sup>	142.60 $\pm$ 8.029 <sup>b</sup>	136.00 $\pm$ 7.27 <sup>c</sup>
Glucose (mg/dl)	73.16 $\pm$ 3.75 <sup>a</sup>	76.75 $\pm$ 3.42 <sup>a</sup>	76.90 $\pm$ 2.91 <sup>a</sup>

Explanation: a, b, c – means with different superscripts in the same row are significantly different ( $p < 0.005$ )

action on biological system. An effect of surfactants is generally attributed to their ability to react directly with proteins leading to enzyme inhibition. The inhibition of membrane bound enzymes following treatment with LAS indicated that even the lower concentration of surfactants probably caused a cellular damage and inhibited the activities. The detergents interact with proteins and possibly with the phospholipid component and alter membrane permeability (7). It appears that they act as general cell toxic agents. The increased plasma LDH levels however, showed only the degree of tissue damage without indicating whose tissue or organ necrosis was responsible for the increased activities in the blood. The increased LDH activity showed that LAS had a high potential to interfere with aerobic mechanisms; however, the mode of its action has yet to be clearly defined (25).

Silbergeld (19) claims that blood glucose measurements are a sensitive, reliable indicator of environmental stress in fish. Blood glucose was the only parameter in which significant differences in stress response were detected (4, 5). Increase in glucose concentration is a secondary response to stress, so the increase in glucose level is an indicator for stress response (16). In this study, plasma glucose levels were insignificantly increased in both trial groups compared to the control group. This could be attributed to a stress response because fish may be more sensitive for stress (16). The elevations in serum glucose and cortisol were the most frequent changes occurring after exposure to the pollutants (12).

A decline in plasma protein and cholesterol content is also frequently observed after exposure to the several pollutants at sublethal concentrations (6). In this

study, plasma protein levels increased insignificantly and total cholesterol levels decreased significantly in treated groups. The insignificant increase in the level of total protein may result from the stress caused by toxic effect of LAS. Rehulka (18) suggested that clinical chemistry analyses in the diseased fish showed decline in the levels of total protein, cholesterol, triacylglycerol and total calcium and an increase in the urea level.

In conclusion, sublethal concentrations of LAS may cause some changes in blood biochemical parameters. Increases in enzyme activities and the decreases in the level of cholesterol, observed in the present investigation, may be an indicator of metabolic changes, which suggests that different biochemical parameters could serve as diagnostic instruments to reveal sublethal (different doses LAS) toxic responses in natural populations of fish.

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## STAN ZAKAŻNYCH CHOROBY ZWIERZĄT W POLSCE

według danych Głównego Inspektoratu Weterynarii w lutym 2004 r.\*

1. **Wścieklizna zwierząt domowych** – wystąpiła w 2 województwach: podkarpackim (1-1) i warmińsko-mazurskim (1-1). Zanotowano ją u 1 kota i 1 psa.
2. **Wścieklizna zwierząt dzikich** – wystąpiła w 5 województwach: kujawsko-pomorskim (1-1), lubelskim (3-3), świętokrzyskim (1-1), warmińsko-mazurskim (2-2) i wielkopolskim (1-2). Zanotowano ją u 8 lisów i 1 jenota.
3. **Zgnilec amerykański pszczoł** – wystąpił w województwie łódzkim (1-1).

\* W nawiasach podano liczbę powiatów i miejscowości, w których choroba została stwierdzona w okresie sprawozdawczym.