

## Toxicity and Identification of LAS in Tissue of Rainbow Trout (*Oncorhynchus mykiss*)

### Alabalık Üzerine LAS' in Toksik Etkisi ve Dökuda Teşhisi

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#### Abstract

Toxicity and identification of LAS were investigated on rainbow trout (*Oncorhynchus mykiss*) exposed to LAS at various concentration levels in aquarium. The lethal level of LAS was 12.5 mg/L in single dose and 13.5 mg/L in progressive doses. The accumulation of LAS in the organs such as gill, heart, stomach and liver were determined by metachromatic method with azur A and toluidine blue. The LAS absorbed on tissue is colored red in examination on microscope. This is the first record in the literature using metachromasy for biological trace of LAS in tissue.

**Keywords:** Toxicity, LAS, metachromasy, rainbow trout

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#### Introduction

LAS, Linear alkylbenzene sulfonates is not a homogeneous compound. It is composed of homologues and isomers which occurred during its preparation from LAB. It contains alkyl groups of C<sub>8</sub> - C<sub>16</sub>.

Many papers have been published on the toxicity of LAS in marine organisms such as *Ceratopteris thalictroides* (Sing and Devi, 1989), *Gammarus olivii* (Topçuoğlu *et al.*, 1992), *Carcinus mediterraneus* (Venezia *et al.*, 1988); *Gobius*

*melanostomus* (Ünlü and Topçuoğlu, 1977), *Oryzian lapites* (Wakabayashi and Onizuka, 1986), goldfish (Tsai and McKee, 1978) and *Salmo gairdneri* (Spehar *et al.*, 1979). Labeled LAS (S<sup>35</sup>) was used to determine toxicity in carp (Kikuchi *et al.*, 1978 - 1980), in *Proterorhinus marmoratus* (Topçuoğlu and Birol, 1982). The chain lengths of LAS and hardness of water affected toxicity of LAS (Wakabayashi *et al.*, 1980).

Many papers were published on the effect of LAS on gill. The accumulation of LAS in fish organs was investigated at, as in gill (Abel, 1974). Gills were found to be covered with mucus in case of fish exposed to the detergent. (Misra *et al.*, 1985). The detergents thicken the gill epithelium that influences the rate of respiration, subsequently alter the respiratory function and the hydromineral balance (Rusas *et al.*, 1988). Sublethal concentration of surfactants is accumulated on the gill epithelium of fish and changed the lipid composition of the tissue and affected the production of mucus and also partially destroyed the epithelium chloride cells (Abel and Skidmore, 1974).

Higher concentration of surfactant was found in hepatopancreas and kidney than in gill. An additive toxicity was noticed in rainbow trout for mixture of anionic detergents ABS and LAS and copper and mercury (Calamari and Marchetti, 1973).

The effects of LAS on enzymatic systems were also investigated on liver and gill by Gupta *et al.*, (1988) and on plasma parameters of *O. mykiss* by Koc *et al.*, (2001).

The methods used for determination of LAS are MBAS (Standard Methods, 1995), HPLC (Marcomini and Giger, 1987; Terzic *et al.*, 1992; Terzic and Ahel, 1993; Koç *et al.*, 2001) and metachromatic (Güven *et al.*, 1994). Metachromasy phenomenon which was used in this work for identification of LAS in tissue was first indicated for histological staining of particular tissue elements in animal (Ehrlich, 1887). It was based on the change of the absorption band of dyes from long to shorter wavelengths (Lison, 1935). LAS was identified by thin-

layer chromatography (Akıncı and Güven, 1994) and also determined by the same methods using various metachromatic dyes (Güven *et al.*, 1994).

In this work lethal level and accumulation of LAS were investigated on the tissues of rainbow trout (*Onchorhynchus mykiss*).

### **Material and Method**

LAS (97%) was obtained from Lever, Gebze, Turkey.

The solvents used: Ethanol (Tekel, Turkey), the other solvents and chemicals were Merck products.

Dyes: Azur A (Gurr), toluidine blue (Fluka) of 0.1% solution in water.

Rainbow trout weighing 300-15g was obtained from a farm in Balıkesir, Turkey.

Aquarium in glass, containing 250 L tap water.

LAS was determined before and after its addition in aquarium by MBAS (Standard Methods, 1995). 800ml sample was taken from aquarium for each analysis. The standard curve of LAS was plotted by MBAS method in a concentration of 0.2-2 µg / ml. The absorbance was read at 652nm in a spectrophotometer (Shimadzu UV-Visible, 1601)

#### **Toxicity assay**

The test was made in aquarium containing 250 L tap water, which was passed through carbon filter for dechlorination, then aerated. The fish were inserted into the tank and aeration continued during the experiment.

Each experiment was made on three samples of rainbow trout (*Onchorhynchus mykiss*) and totally 40 animals were used. The control was made on rainbow trout in another aquarium where LAS was omitted. Feeding stopped 2 days prior to and throughout the experiment.

Toxicity test was made by addition of LAS to tank as:

1. Cumulative addition 12.5 mg / L at single dose.
2. Progressive addition 4.5 mg / L of LAS per day.

The animals were frozen after death, the sections (3-10 $\mu$ m) of gills, heart, hepatopancreas and stomach were taken with Frigocut Reichert – Jung (Model 2800). They were frozen in a freezer Shandon Crxomatrix frozen specimen embedding medium cat. No. Far. 2800. One drop of azure A or toluidine blue was added to each section and examined under microscope, then photographed.

### Result and Discussion

The equations of the standard curves of LAS are:

$$\text{for MBAS method } y = 0.038 \times C + 0.0196 \quad r = 0.998$$

Lethal dose of LAS is found in cumulative assay as 12.5 mg / L in one day and in progressive assay totally 13.5 mg in 3 days.

According to these findings toxicity of LAS was similar when given in either cumulative or progressive dose.

LC<sub>50</sub> value of LAS was reported as 0.36 mg / L (untreated) and 29.5 mg / L treated water in activated sludge for 96 h in rainbow trout juvenile at 18°C (cited by Spehar *et al.*, 1979). Our results on adult rainbow trout are lower than this finding.

The accumulation of LAS in various organs was determined by autoradiography and liquid scintillation counting technique (Kikuchi *et al.*, 1978,1980; Topçuoğlu and Birol, 1982; Wakabayashi *et al.*, 1980; Wakabayashi and Onizuka, 1986. In this work a new technique was proposed based on metachromasy. The examination was made as on described above.

Bioaccumulation of LAS in tissues are shown in Fig. 1-6. The figures show the surface of these tissues in dark coloured pink, azur A.

In actual photographs the red tissues showed that LAS had been absorbed by the surface of gill, stomach and heart.

We found that the surfactants were readily absorbed and distributed throughout the body tissues of the fish.

The metachromatic dye, used in determination of LAS, appeared in the heart, thus indicating that LAS had been also absorbed by that organ. In our earlier paper, it was shown that LAS after absorption was transferred to blood and affected plasma parameters (Koç *et al.*, 2001).

On dyeing with metachromatic dyes the absorption ranked as:  
Gill > stomach > heart

The colour surface areas of gills, as dyed by azur A or Nile blue are larger than other tissues. This finding indicated greater absorption had occurred in gill, thus supporting the findings of Abel (1974).

No appreciable difference was noted between azur A and Nile blue or the other metachromatic dyes.

The proposed technique is quick and simple for identification of LAS accumulated in tissue. It is first to use azur A, a metachromatic dye, to trace the absorption of LAS in tissues for biological tracing.

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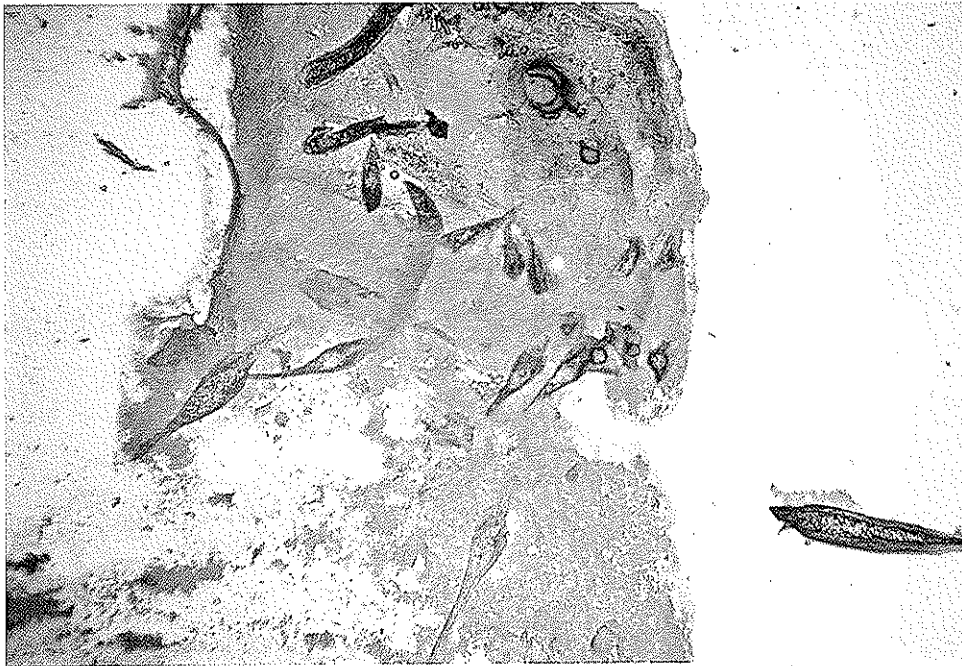
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a)



b)

Fig 1. The section of rainbow trout gill a) in aquarium LAS added b) control: LAS omitted

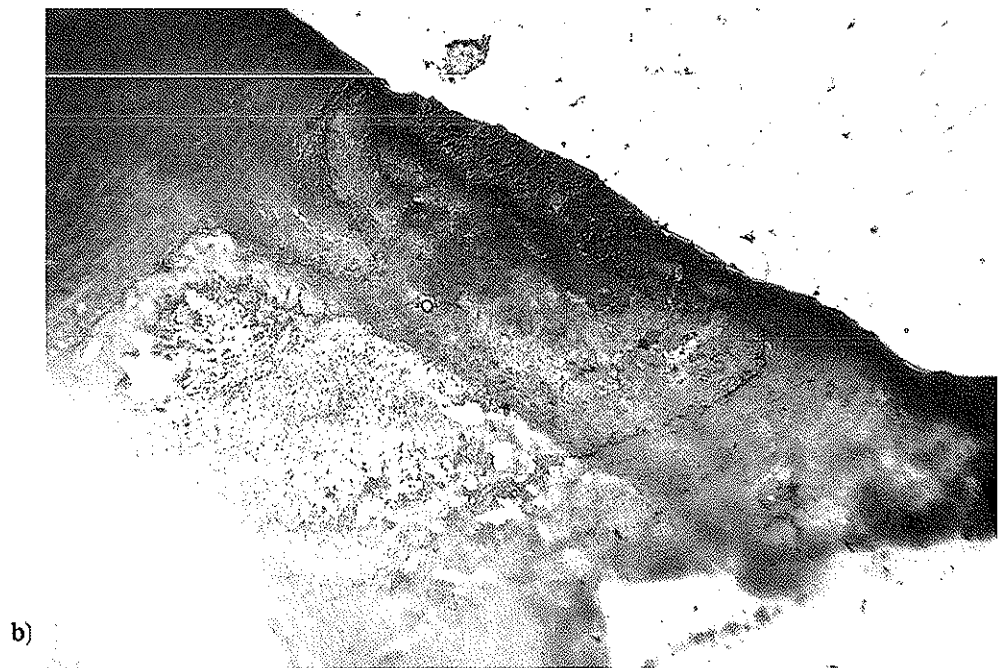
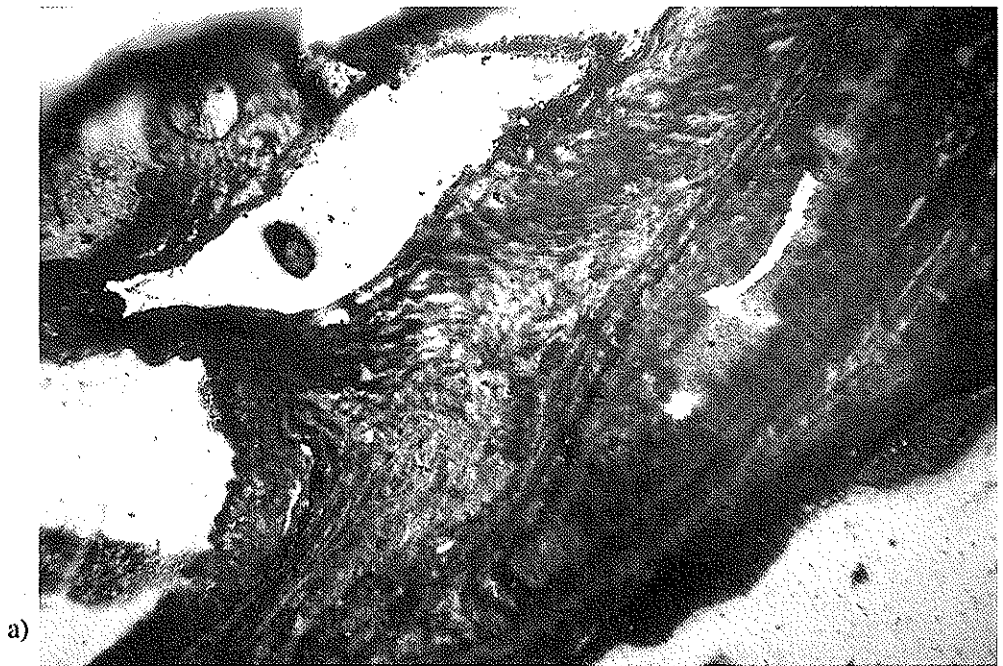
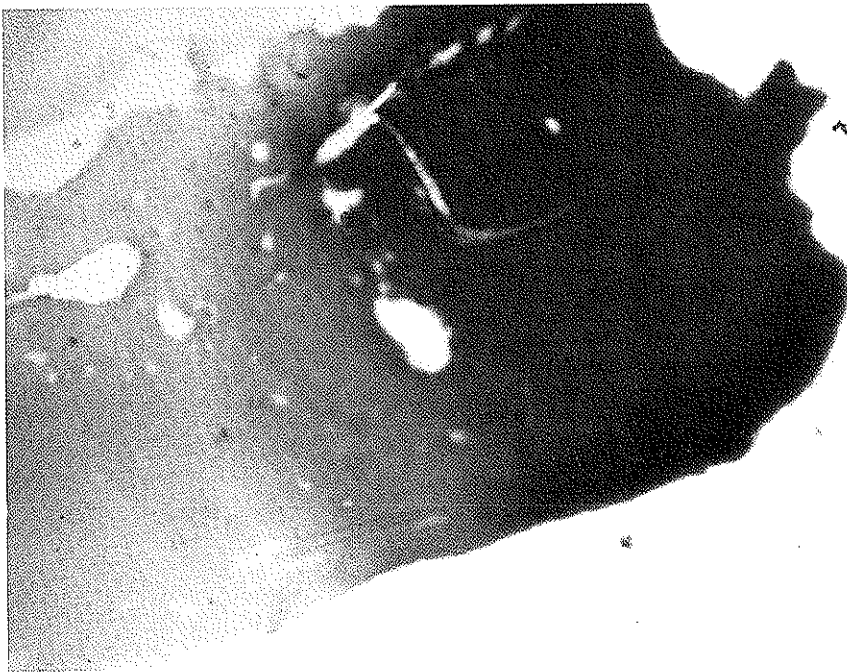


Fig 2. The section of rainbow trout stomach a) in aquarium LAS added b) control: LAS omitted



a)



b)

Fig 3. The section of rainbow trout liver a) in aquarium LAS added b) control: LAS omitted

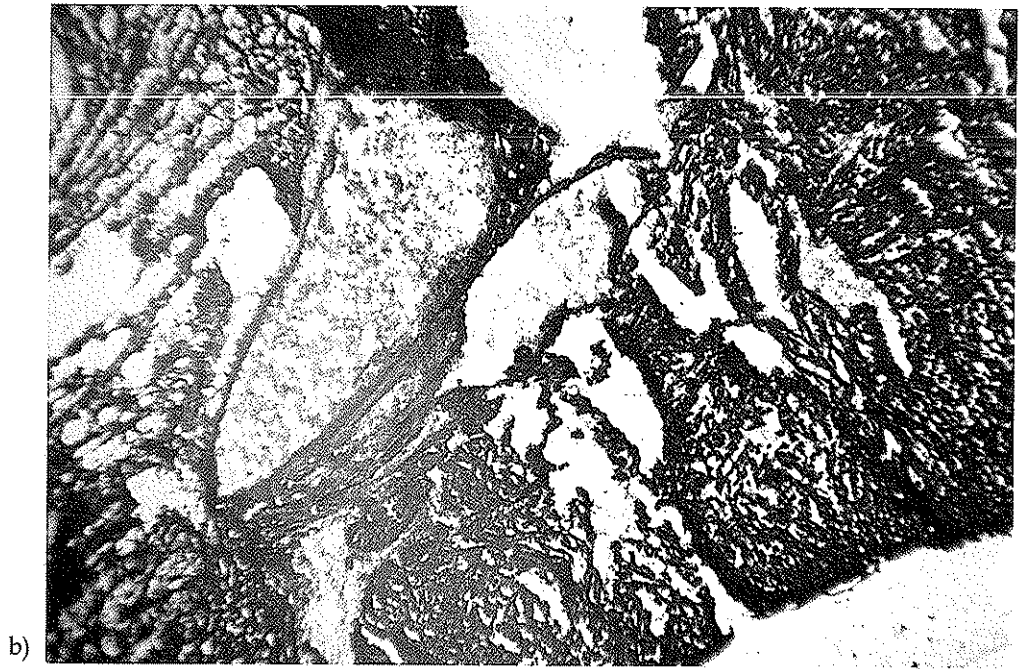


Fig 4. The section of rainbow trout heart a) in aquarium LAS added b) control: LAS omitted