*Zoological Studies* **34**(1): 41-46 (1995)

Zoological Studies

# Corneal Damage in Young Tigerperch (*Terapon jarbua*) Exposed to the Surfactant Linear Alkylbenzene Sulfonate (LAS)

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*(Accepted November 7, 1994)*

**Bao-Quey Huang and Dar-Yi Wang (1995)** Corneal damage in young tigerperch (*Terapon jarbua*) exposed to the surfactant linear alkylbenzene sulfonate (LAS). *Zoological Studies* **34**(1): 41-46. Investigations were conducted, using three different approaches, to learn how the detergent LAS (linear alkylbenzene sulfonate) influences the structure and function of the corneal epithelium in the estuarine-dwelling tigerperch (*Terapon jarbua*). The membrane potentials of corneal epithelium exposed to concentrations of LAS of 0, 5, and 10 ppm changed from $-7.4 \pm 1.1$ mV ($n = 24$) to $-4.5 \pm 0.8$ mV, ($n = 24$) and $-3.1 \pm 1.0$ mV ($n = 24$), respectively. Exposure to LAS (5 or 10 ppm) caused dramatic thinning of the corneal epithelium tissue. The retinomotor responses of the pigmented epithelium also shifted from $81.53 \pm 1.72\%$ to $68.03 \pm 6.21\%$, and $67.77 \pm 3.42\%$ ($n = 3$), respectively. LAS likely caused the morpological and electrophysiological damage to the corneal epithelium and the resulting abrasion or partial opacity of corneas, thus reduced retinomotor responses.

**Key words:** Detergent effects, Depolarization, Corneal abrasion, Retinomotor response.

The cornea of all vertebrates consists of three major structures: the epithelium (outermost), stroma, and endothelium (innermost). The corneal epithelium of fishes is directly exposed to their aquatic environment and is impermeable to both water and ions (Edelhauser et al. 1968, Ubels and Edelhauser 1987). The stroma consists of an orderly arrangement of collagen fibrils (Ferguson 1989). Removal of and damage to the corneal epithelium may cause stromal swelling and opacity (Edelhauser et al. 1981). Edema in the stroma disrupts the orderly fibrillar array causing the cornea to lose its transparency. Therefore, the structural integrity of the corneal epithelium is crucial to maintaining its transparency.

In response to changes in ambient light, the retinal pigmented epithelium and retinal photoreceptors (cones and rods) can vary their relative location. The pigment migration response is synchronized with the intensity of light incident on the retina and is generally known as the retinomotor response, or photomechanical movement. It is an adaptive function found in most teleosts (Fernald 1988, Huang and Hu 1991). The mechanism and regulation of pigment migration may be mediated exogenously and endogenously (Guma 1982, Meer and Anker 1986).

Linear alkylbenzene sulfonate (LAS) is an anionic surfactant that is widely used in domestic detergents and for industrial purposes. It commonly finds if way through rivers to estuarine and coastal sea waters. This pollutant is known to exhibit acute toxicity in the range 0.4 – 40 ppm (Abel 1974, Lewis and Suprenant 1983, Conti 1987, Chawla et al. 1988, Kobuke 1988). Most research has focused on the effects of LAS on gills directly exposed to the pollutant (Zaccone et al. 1985, Bielinska 1987, Byrne et al. 1989, Roy 1989a, b, Huang and Wang 1994), rather than on corneas that are also directly exposed to the aquatic medium.

Corneal damage is believed to impair vision, possibly affecting the ability of fish to perceive and to escape from predators (Brandt et al. 1986, Ubels and Edelhauser 1987, Browman et al. 1990).

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Tigerperch (*Terapon jarbua*) inhabits the estuarine waters around Taiwan except along the east coast where land drops precipitously into the ocean (Miu et al. 1990) and therefore it can be a significant indicator of the effects of LAS. Little is known about the influence of the surfactant LAS on morphological structures and physiological functions of visual organs directly exposed to aquatic pollutants. Three approaches were used to understand the integrated effects of LAS on visual functions. The morphological and electrophysiological alterations of the corneal epithelium were the subjects of two examinations, and the retinomotor response was diagnosed by an histological morphometric method, with statistical analysis.

## METHODS AND MATERIALS

Young tigerperches (*Terapon jarbua*), weighing 0.67 – 2.12 g, were obtained from the northeast coast of Taiwan and maintained under natural photoperiod. They were fed commercial feed once per day but all feeding stopped 24 hr before the experiment. The fish were kept in sea water at pH 7.91 – 7.99, 4.1 – 4.4 ppm DO, 35‰ salinity, and 27 – 28 °C.

Ten fish were kept in each experimental tank (10L) that contained LAS (linear alkylbenzene sulfonate, Sigma) in concentrations of 5 and 10 ppm, with 0 ppm serving as a control group. Dead fish of the experimental groups (15 min and 30 min of exposure in 10 and 5 ppm, respectively) were removed from the tank. The control group was sampled immediately following the withdrawal of dead fish in the 5 ppm group. Corneas and retinae were dissected from the excised eyes, fixed in Bouin's solution and embedded in paraffin, following routine procedures. Transverse sections (7 $\mu$m) were cut and stained with haematoxylin and eosin.

Retinomotor responses were measured (mean thickness of pigment epithelium/mean thickness of photoreceptor layer, Fig. 1) from light-adapted retinae by the use of a microimage processing system (Hamamatsu Photonics C2400, Japan and JAVA soft, U.S.A.). Experiments were carried out in triplicate and one-way ANOVA (analysis of variance) was applied to examine the effects of LAS concentration.

For electrophysiological recording, the isolated corneas were placed in a recording chamber. Glass microelectrodes (outer diameter, o.d. 1 mm, fibre-filled, borosilicate, AM System Co. U.S.A.) with tip resistance 40 – 60 M Ω were used for

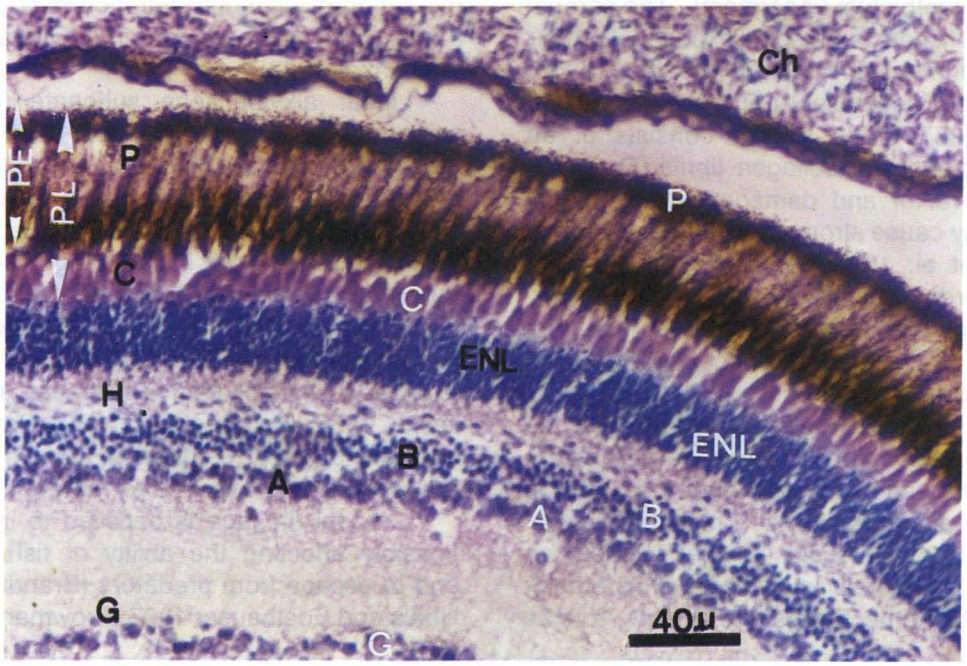


**Fig. 1.** The retina of a juvenile tigerperch. The retinomotor response of pigment epithelium was shown by PE/PL (see mark at the top left corner). Ch: Choroid; PE: Pigment epithelium; P: Epithelial pigment; PL: Photoreceptor layer; C: Cone; ENL: External nuclear layer; H: Horizontal cell; B: Bipolar cell; A: Amacrine cell; G: Ganglion cell.

intracellular recording of membrane potentials from the corneal epithelium (Wang and Huang 1994). Voltage responses were fed to a preamplifier (MEZ 8201, Nihon-Kohden, Japan) which was connected to a chart recorder (BD Kipp & Zonen, the Netherlands). A micromanipulator (Campden Instrument LTD, London) was used to manipulate the microelectrode to approach the corneal epithelium.

## RESULTS

Representative recording data of membrane potentials of the corneal epithelia appear in Table 1. These intracellular recordings were obtained from the corneas of fish exposed to LAS at various concentrations. All recordings were made from only the most superficial cellular layer, i.e. the epithelium. The membrane potentials of the corneal epithelium from the control group ranged from $-6$ to $-10$ mV ($-7.4 \pm 1.1$ mV, $n = 24$). After exposure to LAS (5 and 10 ppm), the potentials changed to $-4.5 \pm 0.8$ mV ($n = 24$) and $-3.1 \pm 1.0$ mV ($n = 24$), respectively. Table 1 illustrates the decline of membrane potentials, or depolarization, (from $-7.4$ mV to $-3.1$ mV). The depolarizing effects of LAS on the membrane potentials of the corneal epithelium were significant ($p < 0.05$, Table 1).

The retinomotor responses of pigmented epithelium varied among the concentration groups. Table 2 shows that the variation was dose-related. In the control group (LAS, 0 ppm), responses reached $81.53 \pm 1.72\%$. Abrupt changes occurred when concentrations of LAS was increased to 5 and 10 ppm. Retinomotor responses became $68.03 \pm 6.21\%$ and $67.77 \pm 3.42\%$, respectively. Differences were significant ($p < 0.05$, Table 2).

Histological examination of the corneas indicated significantly different thicknesses of epithelial layers between the control and experimental groups (Fig. 2). Histopathological corneal examinations of young tigerperch exposed to concentrations of LAS of 5 and 10 ppm revealed significant abnormalities. At 0 ppm LAS, the corneal epithelium consisted of multiple layers of squamous epithelial cells overlaying the upper (outer) limit of the corneal stroma (Figs. 2A, B). After exposure to LAS (5 or 10 ppm), the corneal epithelium exhibited a significant thinning of the tissue and loss of epithelial cells, so that it appeared to be composed of fewer layers (Figs. 2C, D). The loss of epithelial cells was not severe enough to the uncover or expose the underlying corneal stroma.

**Table 1.** Means and standard deviations of membrane potential of cornea epithelium after treatment with LAS. The notes a, b and c mark the significant differences as determined by *t*-test ($p < 0.05$)

| LAS Conc. | Membrane potential (mV) | *t*-test |
|---|---|---|
| 0 ppm | $-7.4 \pm 1.1$ ($n = 24$) | a |
| 5 ppm | $-4.5 \pm 0.8$ ($n = 24$) | b |
| 10 ppm | $-3.1 \pm 1.0$ ($n = 24$) | c |

**Table 2.** Means and standard deviations of retinomotor responses (%) at various concentrations of LAS. Stars mark the values with significant difference from the control group

| LAS Conc. | Retinomotor response (%) | Significance |
|---|---|---|
| control (0 ppm) | $81.53 \pm 1.72$ ($n = 3$) | |
| 2.5 ppm | $72.43 \pm 5.78$ ($n = 3$) | * |
| 5.0 ppm | $68.03 \pm 6.21$ ($n = 3$) | * |
| 10.0 ppm | $67.77 \pm 3.42$ ($n = 3$) | * |

## DISCUSSION

This study indicates that LAS causes pathological shifts in the eyes of young estaurine tigerperch, which may affect the survival of the young. Loss of corneal epithelium in a variety of marine and freshwater species results in transient corneal edema (Ferguson 1989) and the loss of corneal transparency (Edelhauser et al. 1981). It is uncertain whether the decline of the retinomotor response is due to the loss of corneal transparency because of direct effects of the surfactant LAS on the corneal epithelium, or whether it is due to other indirect effects (Roy 1988a, b).

Brandt et al. (1986) found that corneal cloudiness and perforations could be repeatedly observed in largemouth bass (*Micropterus salmoides*) subjected to handling stress. They also found this cloudiness to be reversible. Ubels and Edelhauser (1987) suggested that repeated mechanical contact of the eye may cause corneal epithelial abrasion, resulting in corneal cloudiness. Hence, stress may evoke acute corneal damage but the damage may not be permanent. In the present study, LAS acted as a possible agent of general

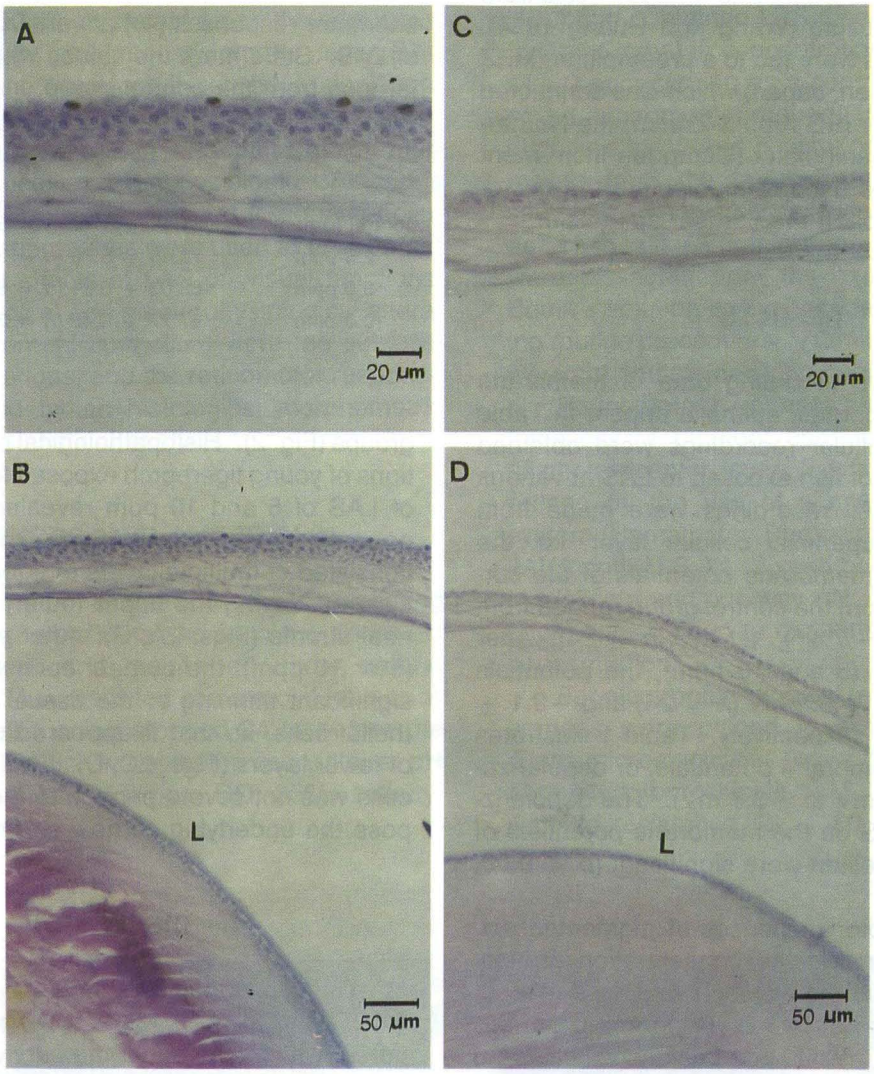


**Fig. 2.** Morphopathological effects of LAS on the cornea showing the severity of damage. Lenses (L) are at the bottoms of photographs (B) and (D). (A) and (C) are magnified from (B) and (D), respectively, to show the corneal epithelium.
(A), (B) Control group showing the multiple-layered epithelium. (C), (D) Effects of 10 ppm LAS on corneal showing damage.

stress, resulting in thinner corneal epithelium and lower retinomotor responses. These modifications increased qualitatively with higher concentrations of LAS. Histological examinations to quantify corneal damage were not performed in this study.

Bodammer (1985) found that corneal epithelial cells of larval striped bass (*Morone saxatilis*) appeared to be in the process of sloughing and the tissue thinning after exposure to 80 or 110 $\mu$g $Cu^{++}$/L. Daye and Garside (1976) studied the effects of low pH on corneal epithelial cells of brook trout (*Salvelinus fontinalis*) and reported a similar reaction. Therefore, Bodammer (1985) proposed that the cytopathological responses and intense sloughing of cells found in these studies demonstrated a generalized response of the corneal epithelial cells exposed to a toxic or hostile medium. Similar observations of thinning of the corneal epithelium have been obtained from the present work, thus supporting Bodammer's interpretation concerning LAS as a toxic or hostile factor.

The membrane potentials of the corneal epithelium appeared to experience greater depolarization as the concentration of LAS increased; i.e. the membrane potentials of corneal epithelium appeared dependent on dose. The altered membrane potentials in the corneal epithelium presented in this study are similar to those of the gill epithelium in tigerperch treated with LAS (Wang

and Huang 1994). The membrane potentials were close to the values from the gill epithelium of the same species. Similar blockage of function and breakdown of structure in both gills and corneas are consistent since both tissue were directly exposed to LAS (Huang and Wang 1994). Thus, we propose that the pathological modification of these epithelial tissues reflects a generalized response to a toxic medium. The visual ability of treated fish is assumed to be influenced by this epithelial damage, because persistent absence of the epithelial cell layer disrupts the normalcy of the underlying stromal layer and deeper tissues (Bodammer 1985).

By using a series of histochemical techniques, the gradual loss of lipid components and alteration of the physiological properties of lipid bilayers in various cell types of the gill epithelium were discovered to possibly be related to toxicity and death when fish were exposed to an anionactive detergent (Roy 1989a). LAS, an anionactive detergent, caused changes of membrane potential in gill epithelium (Wang and Huang 1994), necrosis of gill epithelium, and death of tigerperch (Huang and Wang 1994). At different levels of exposure, corneal damage may consist of tissue necrosis, cell rupture, and thinning of epithelial layers (McDonald and Wood 1993, Huang and Wang, 1994). However, it is difficult to trace the corneal epithelial cells undergoing the process of sloughing by light microscopic preparation and observation used in the present study. It has been proposed that sloughing and thinning of corneal epithelium are generalized responses to exposure to toxins (Bodammer 1985). Therefore, by providing evidence of thinned tissues, it is possible to link the sloughing of superficial tissue with roughness of the corneal surface. Necrosis and cell rupture may also induce abrasion of the corneal surface and lead to an increase in roughness of the cornea. Total integrated scattering (TIS) can be used to measure roughness ($\delta$) (Bennett and Mattson 1989), $\delta = \lambda\sqrt{TIS}/4\pi$ ($\lambda$: wavelength of light). The emerging beam is increasingly diffused as the light is increasingly scattered. Therefore, light reaching the retina may have decreased after exposure to LAS due to increased surface abrasion (roughness) of the cornea. However, the deterioration of retinomotor responses of treated fish is possibly affected by the elimination of exogenous light. Thus, there is compelling logic to link superficial corneal damage, and surface roughness, to the retinomotor response. The tracing of sloughing cells and the measurement of corneal surface roughness would be crucial parameters to establish such a linkage in future work.

Thus, the acute toxicity of the surfactant LAS destroys structure and blocks functions of the corneal epithelium and leads to functional alteration of retinomotor responses. The toxic mechanisms of LAS on retinomotor responses cannot be completely understood from the present experiment. Further investigation is needed to explain these retinal defects.

**Acknowledgements:** This work was partially supported by the National Science Council, R.O.C. (NSC 83-0409-B-019-028). We are grateful to two anonymous reviewers for critically reading the manuscript and offering valuable suggestions. Thanks are also due to Dr. D.F. Huang of the Department of Food Science and Mr. M.K. Fu of the Institute of Marine Biology and Mr. J.Y. Huang, National Taiwan Ocean University for their help in this manuscript.

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# 清潔劑LAS對花身雞魚幼魚角膜之破壞作用

黃寶貴[1] 王達益[1]

爲瞭解清潔劑LAS (linear alkylbenzene sulfinate)對河口生活之花身雞魚(*Terapon jarbua*)的角膜上皮構造與功能產生的影響。本實驗利用組織切片與電生理技術分別探討其作用。當LAS濃度由0 ppm增加至5 ppm與10 ppm時，角膜上皮的膜電位則從 -7.4±1.1 mV ($n$=24)分別改變爲 -4.5±0.8 mV ($n$=24)與 -3.1±1.0 mV ($n$=24)。5 ppm 與 10 ppm 濃度的LAS亦使得角膜上皮組織顯著變薄；網膜黑色素移動反應(retinomotor responses)亦由81.53±1.72%分別降低爲68.03±6.21%與67.77±3.42% ($n$=3)。LAS極可能引起花身雞魚角膜上皮組織在形態與電生理方面受到傷害，並造成角膜摩損或使得部份角膜變得不透明，因而降低網膜黑色素之移動反應。

**關鍵詞**：清潔劑作用，去極化，角膜摩損，網膜黑色素移動反應。

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