

Estimation of zinc chloride contamination by histopathological analysis of the respiratory organs of the air breathing 'murrel' *Channa striata* (Bloch, 1797) (Channiformes, Pisces)

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ABSTRACT

Sub-lethal toxicity of zinc chloride (11.5 ppm) on the respiratory organs of the *Channa striata* has been analysed. The mucous cells show periodic fluctuations in their number, size and staining properties elaborating larger quantities of sulphated mucopolysaccharides. The respiratory epithelium (RE) of the respiratory (secondary) lamellae (SL) of the gills shows periodic lifting with deformity of the lamellar elements, haemorrhages due to necrosis and sloughing off of the RE, followed by hyperplasia and fusion of neighbouring SL. Other prominent alterations include increased thickness of the RE and subsequent dismantling of the vascular elements. Fusion of SL reduces the surface area for gaseous exchange, causing impaired branchial respiration. The alterations in the suprabranchial chamber include protrusion of RBC-engorged minute vascular papillae on the floor of its lumen. This brings the blood nearer to the air in the lumen. Congestion of the sub-epithelial blood vessels of the respiratory organs, especially the suprabranchial chamber, takes place following exposure.

Key words: air-breathing organs, *Channa striata*, gills, histopathology, respiration, zinc chloride toxicity

Introduction

The gills of the air-breathing teleost *Channa striata* that inhabits derelict water-bodies containing hypoxic waters fail to provide the basic O₂ requirements of the fish. To compensate for this the fish has acquired a bimodal respiratory mechanism for exploitation of the atmospheric O₂, also via its suprabranchial chambers (air-breathing organs, ABO). To test the quality of heavy metal contaminated waters, gills and air-breathing organs

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(ABO) of *Heteropneustes fossilis* have successfully been used as a potent bio-indicator (HEMALATHA and BANERJEE, 1997a; HEMALATHA and BANERJEE, 1997b).

In this paper the toxicity of water-borne zinc salt, $ZnCl_2$ on the gills and ABO of another important edible fish, *C. striata*, has been investigated because, unlike *H. fossilis*, the ABO in this fish is not modified gill structures. CHANDRA and BANERJEE (2003) have recently evaluated the toxicity of $ZnCl_2$ on the skin (a water-breathing organ) of the same fish, *C. striata*.

Materials and methods

Healthy specimens of *Channa striata* (18-20 cm length) acclimated for 35 days (d) in large plastic tubs containing plain tap water (having dissolved O_2 , 6.3 mg/l, pH 7.2 water hardness 23.0 mg/l and room temperature 30 ± 2 °C). They were regularly fed with minced goat liver on alternate days and the water was renewed after every feed.

Five groups of 5 fish each were exposed to $ZnCl_2$ (11.5 ppm, 10% of 96 h LC_{50} value detected by Spearman-Kärber method (HAMILTON et al., 1977) for a maximum period of 45 days. Each group was separately exposed to $ZnCl_2$ solution, prepared in tap water. Parallel control groups of 5 fish each were kept separately in plain tap water (without addition of $ZnCl_2$). Feeding was allowed during exposure. After 0 h, 7 d, 15 d, 30 d and 45 d of exposure, 3 fish each from the respective experimental groups, as well as control group, were sacrificed. The second gills and the entire ABO (supra-branchial chamber) from left side of the fish were fixed in 10% neutral formalin, aqueous Bouin's fluid and absolute ethanol. Paraffin sections of 6 mm were stained with Ehrlich's haematoxylin and alcoholic eosin (H/E) for routine histopathological study. Certain carbohydrate moieties were located by periodic acid-Schiff (PAS), alcian blue pH 2.5 (AB 2.5), AB 2.5/PAS, alcian blue pH 1.0 (AB 1.0) (PEARSE, 1985) techniques (Tables 1, 2). The entire experiment was repeated twice.

Results

Air-breathing organs (ABO) (supra-brachial chamber). Control ABO. The supra-brachial chambers are developed dorsal to the gill arches and are placed above the pharynx (MUNSHI, 1962). A respiratory membrane containing islets of vascularized tissue lined the supra-brachial chamber (Fig. 1). The sub-epithelial area is richly vascularized with large and small blood vessels. Fine blood capillaries from the sub-epithelial connective tissues entered deep into the epithelial lining where they anastomosed extensively and reach to the surface of the supra-brachial cavity (Fig. 1). These minute blood channels/capillaries (BLCs) terminated in the form of vascular papillae (VP) where gaseous exchange take place (MUNSHI, 1962). Supporting epithelial cells (ECs) separated the neighbouring VP.

The epithelial lining of the ABO also became studded with a large number of strongly PAS, moderately to strongly AB 2.5 positive mucous cells (MCs) (Fig. 2). These MCs stained faintly with AB 1.0 and assumed a dark greenish-violet colour with AB 2.5/PAS techniques (Table 1). The secretion of these MCs often formed patches of slimy layer over the epithelial surface. The suprabranchial chamber of this fish communicated freely with the pharynx (MUNSHI, 1962) and came into direct contact with the aquatic environment.

Table 1. Periodic alterations in the staining properties of the mucous cells of the suprabranchial chamber of *Channa striata* subjected to zinc chloride exposure

Histochemical techniques applied	Intensity of reaction (after various intervals of exposure)				
	Control	Experimental			
	0 day	7 days	15 days	30 days	45 days
PAS	3	0	0	3	3
AB 2.5	2~3	3	3	3	3
AB2.5/PAS; **	3	3	3	3	3
AB 1.0	3	2~3	2~3	2~3	3

Symbols and abbreviations: ** = variously stained reactions ranging from various shades of magenta, violet to greenish blue, in the same or different MCs; ~ = to; AB 2.5 = alcian blue at pH 2.5 for acidic mucopolysaccharides; h = hour; PAS= periodic acid/Schiff reaction for neutral glycoproteins; AB 1.0 = alcian blue pH 1.0; AB 2.5/PAS = alcian pH 2.5/periodic acid Schiff for acidic and neutral glycoproteins; 0 = negative reaction; 1 = weak reaction; 2 = moderate reaction; 3 = strong reaction.

Exposed ABO. After 7d of exposure, the inner epithelial lining got thickened by hyperplasia (Fig. 3).

The fine BLCs formed an intensive network, especially in the middle and outermost layers, from where they showed congestion and coursed upwards into the epithelium to terminate in the VP. At the floor of the lumen the distal projecting parts of the VP protruded further on the surface of the epithelium as numerous fine globular bulging, engorged with RBCs (Fig. 3). The sub-epithelial blood vessels also showed congestion. The MCs also developed in the middle and lower layers and showed great hyperplasia (Fig.4), giving negative PAS and strong AB 2.5 reactions. With the AB 2.5/PAS technique all the MCs assumed a dark greenish-blue colour (Table 1) (Fig. 4). Most of the MCs appeared fully loaded with their secretory material. No slimy secretion was found to lubricate the floor of the lumen.

After 15 d the network of the BLCs via the epithelial lining became less extensive. Simultaneously, the density of the VP bulged at the floor of the lumen also decreased with a decreased number of RBCs within the VP (becoming 1 to 2) (Fig. 5) between 15 to 30 d.

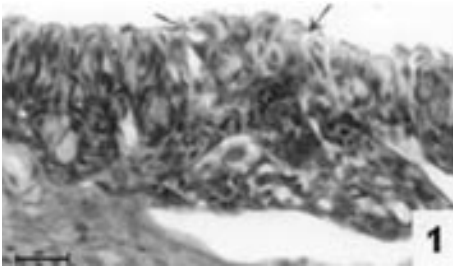


Fig. 1. Part of epithelial-lining of the suprabranchial chamber of control fish, showing the distribution of minute blood channels (BLCs) (arrows) at its surface (H&E; $\times 240$; scale bar = 42 μm).

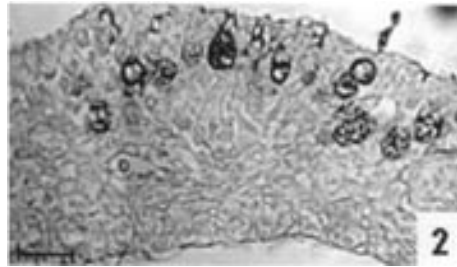


Fig. 2. Distribution of mucous cells (MCs) in the outer stratum of epithelial lining of the suprabranchial chamber of the control fish (AB 2.5/PAS; $\times 240$; scale bar = 42 μm).



Fig. 3. Extensive hyperplasia of the epithelial lining showing projection of numerous BLCs on the floor of the lumen after 7 days of exposure (H&E; $\times 240$; scale bar = 42 μm).

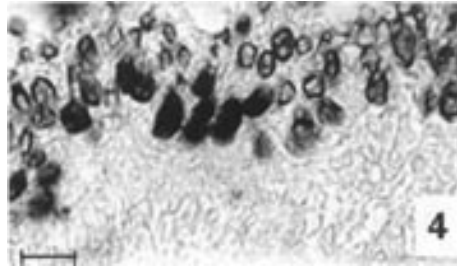


Fig. 4. Extensive hyperplasia of MCs after 7 days of exposure (alcian blue 2.5/periodic acid-Schiff (AB 2.5 / PAS; $\times 240$; scale bar = 42 μm).

Figs. 3.-4. Parts of ABO showing toxic impact of sublethal concentration of zinc chloride solution at different stages of exposure.



Fig. 5. Decrease in the number of bulgings of the BLCs at the surface of the epithelial lining after 30 days. Note the darkly stained nucleic of the RBCs present within the blood vessels passing through the epithelium (H&E, $\times 240$; scale bar = 42 μm).

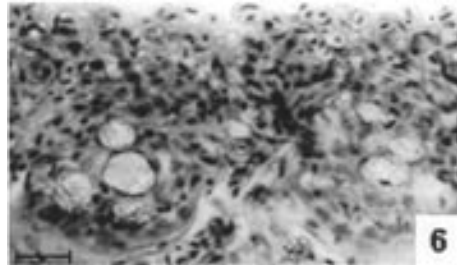


Fig. 6. Re-appearance of large number of BLCs on the surface of the epithelium after 45 days. Note prominent blood vessels connecting the BLCs at the surface, passing through the entire thickness of epithelium (H&E; $\times 240$; scale bar = 42 μm).

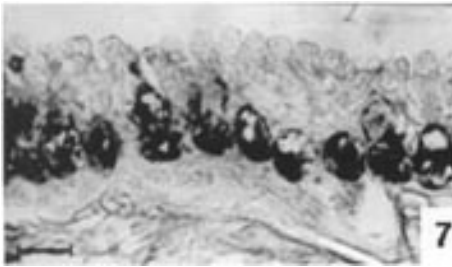


Fig. 7. A large number of MCs appeared in the epithelium, whose bulky bodies lodged in the lower middle layer (AB2.5/PAS; ×240; scale bar = 42 μm).

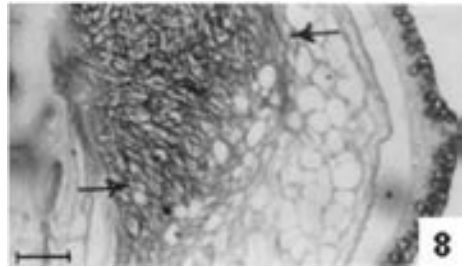


Fig. 8. Hyperplasia of MCs in epithelial lining after 45 days. Note the strong (greenish-blue reaction) shown by the sub-epithelial connective tissues (arrow) (AB2.5/PAS; ×60; scale bar = 167 μm).

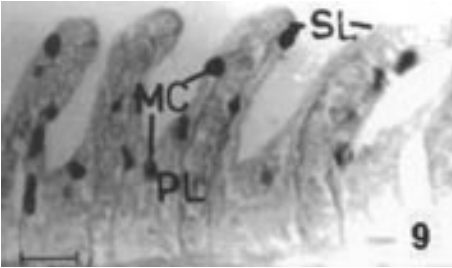


Fig. 9. T. S. of 3rd gill of control *C. striata* showing its structural organisation. (H&E; ×240; scale bar = 42 μm).

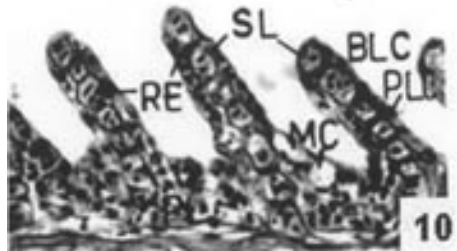


Fig. 10. T. S. of 3rd gill of control *C. striata* showing distribution of MCs both in gill filament (PL) & SL (Alcian blue 2.5 / Periodic Acid Schiff; ×240; scale bar = 42 μm).

Even though the density of MCs decreased substantially after 15 d, the quantity of slimy secretion in most of them increased further. These MCs lodged in the middle layer (ML) opened on the surface via the narrow neck and continued to show negative PAS, strong AB 2.5 and moderate to strongly AB 1.0 reactions. With AB 2.5/PAS, they took on a dark greenish blue staining (Table 1). However, patches of slimy secretion were noticed around the orifices of the MCs.

Even though the thickness of the epithelium varied greatly at different places after 30 d, the size of the ECs also decreased. While no apparent rupture of the VP or BLCs at the surface of the epithelium was noticed, a few RBCs on the surface of the epithelium were observed at this stage. The number of the RBCs in the sub-epithelial blood vessels also decreased. A few eosinophilic granular cells (EGCs) (ionocytes/chloride cells) also appeared in the epithelial lining. The basal cells in the lower layer of the epithelium became tall and columnar. The AB 1.0 staining intensity, density and dimension of the

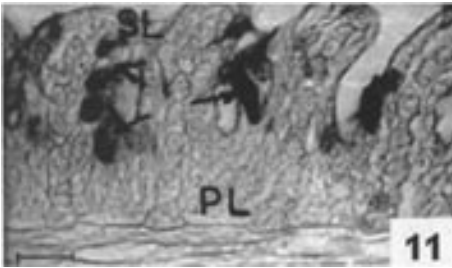


Fig. 11. Increased density of MCs (arrows) in the SL & PL after 7 days of exposure (AB 2.5 / PAS; $\times 240$; scale bar = 42 μm).



Fig. 12. Negative reaction shown by the MCs and the other components of the gill after 7 days of exposure (PAS; $\times 60$; scale bar = 167 μm).



Fig. 13. Lifting of the RE at the surface of the SL after 7 days of exposure H&E; $\times 240$; scale bar = 42 μm).

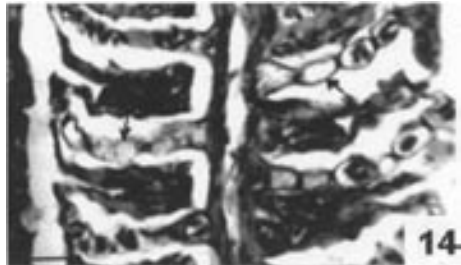


Fig. 14. Extensive thickening and lifting of the epithelial lining of the PL and SL after 30 days of exposure. Note the marked depletion of blood cells in the BLCs (arrows) (H&E; $\times 240$; scale bar = 42 μm).

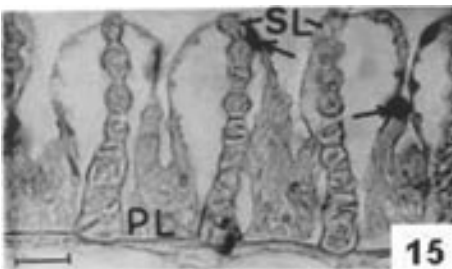


Fig. 15. Marked decrease in the density of MCs (arrows) in PL & SL after 45 days of exposure. Note the lifted RE from the surface of SL (AB 2.5 / PAS; $\times 240$; scale bar = 42 μm).



Fig. 16. Adhesion and fusion of neighbouring SL after 45 days of exposure (H&E; $\times 240$; scale bar = 42 μm).

Figs. 11.-16. Parts of the 3rd gill of *C. striata* showing toxic impact of sublethal concentration of zinc chloride solution at different stages of exposure.

MCs again increased substantially after 45 d, when these cells also acquired a flask shape with narrow necks. The voluminous ML gave strong PAS and AB 2.5 reactions (Table 1) with AB 2.5/PAS. While most of these cells assumed a dark bluish-violet colour, a few also became stained greenish-blue. However, a few small-sized MCs were also present in the superficial layer of the epithelial lining.

After 45 d of exposure the fine blood capillaries again formed an intensive network, especially in the middle and outermost layers (Fig. 6). Numerous, minute globular bulgings of the VP engorged with large numbers of RBCs protruded further in to the lumen (Fig. 6). A great degree of similarity in the structure of the ABO of the fish exposed to 7d and 45 d of exposure was noticed. The wall of the blood capillaries in the lower layer of the epithelium became thickened. Sub-epithelial blood vessels also showed extensive congestion.

Severe hyperplasia/hypertrophy MCs was noticed between 30 to 45 d of exposure (Figs. 7, 8). These sac-like goblet cells extended deep into the lower layers, giving strong PAS and AB 2.5 reactions. With AB 2.5/PAS, while most of these cells assumed a dark bluish-violet colour, a few of them also stained greenish-blue (Table 1). Most of the MCs appeared loaded with their secretory material. A thin layer of AB 1.0 positive (and PAS negative) slime almost invariably covered the bulged VP at the surface of the epithelium. Ionocytes continued to be present. The sub-epithelial connective tissues, which stained strongly with AB 1.0, also appeared spongy due to the presence of a significant number of fat cells. During experimentation only two fish died.

Gills. Control/after 0 h exposure. The vascular components of the SL were made up of alternately arranged pillar cells (PLCs) - blood channels (BLCs), which remained covered by a thin layer of respiratory epithelia (RE) (Fig. 9). The MCs were mostly located in epithelium lining the PL between two SL (Figs 9, 10). A few MCs were also present in the SL (Fig. 10) that assumed a dark greenish-black colour with AB 2.5/PAS (Table 2). They stained moderately to strongly with AB 2.5, and weakly to moderately with AB 1.0 techniques. No PAS positive MC was noticed either in the PL or in the SL (Table 2). A large number of EGCs (ionocytes) were also present in the PL as well as SL.

Exposed gill. An overall increase in the density of the MCs in the PL as well as in the SL was noticed after 7 d of exposure (Fig. 11). These fully loaded MCs stained negatively with PAS (Fig. 12), weakly to moderately with AB 1.0, moderately to strongly with AB 2.5 and greenish-blue with AB 2.5/PAS techniques (Fig. 11) (Table 2). However, the RE lining the SL showed hyperplasia and sometimes became detached and lifted from the underlying vascular components. The BLCs showed great congestion (with 5 or more numbers of RBCs). Further, neighbouring SL became completely fused, causing a substantial decrease in their height and free surface areas on them. Even though there was a slight decrease in the density of the AB 2.5 positive MCs after 15 d, the number of AB 1.0 positive MCs increased enormously. The RBC-engorged BLCs of the SL acquired a

Table 2. Summary of the histochemical alterations in the staining properties of the mucous cells of the gills of *Channa striata* subjected to zinc chloride exposure

Histochemical techniques applied	Intensity of reaction (after various intervals of exposure)				
	Control	Experimental			
	0 day	7 days	15 days	30 days	45 days
PAS	0	0	0	0	0
AB 2.5	2~3	2~3	1~2	2~3	2
AB2.5/PAS; **	1~3	1~3	1~2	2~3	1~2
AB 1.0	1~2	1~2	3	3	1~2

Symbols and abbreviations: ** = variously stained reactions ranging from various shades of magenta, violet to greenish-blue, in the same or different MCs; ~ = to; AB 2.5 = alcian blue at pH 2.5 for acidic mucopolysaccharides; h = hour; PAS = per iodine acid/Schiff reaction for neutral glycoproteins; AB 1.0 = alcian blue pH 1.0; AB 2.5/PAS = alcian pH 2.5/periodic acid Schiff for acidic and neutral glycoproteins; 0 = negative reaction; 1 = weak reaction; 2 = moderate reaction;

round, bead-like appearance. Due to lateral pressure the PLCs became very thin without exhibiting many cytoplasmic details. Hyperplasia of the epithelial linings of the PL and SL became more pronounced, causing extensive alteration in their cellular arrangement and morphology. Lifting of the RE from the surface of the SL, as well as PL, became more prominent (Figs 12-15). After 30 d the density of the AB 1.0 and 2.5 positive MCs decreased. While the staining intensity of the MCs in the PL with AB 2.5 increased, their intensity in the SL decreased and became moderately stained. The congestion in the BLCs diminished. The ladder-like vascular system of the SL became greatly disturbed (Fig. 16). Very often, the haphazardly distributed BLCs appeared empty, dilated and filled with an amorphous substance. Often, fusion of neighbouring BLCs was also noticed. A large number of chloride cells were seen in the PL at this stage. Once again, the density of the MCs decreased, with a decline in their staining intensity with AB 2.5, AB 2.5/PAS and AB 1.0 techniques after 45d. For the first time, MCs staining weakly PAS were noticed in the SL at this stage (Table 2). The congestion in the BLCs intensified once again. Very often, neighbouring SL coalesced throughout their entire height, leaving little free surface for gaseous exchange.

Discussion

Both the respiratory organs of exposed *C. striata* showed extensive MCs hypertrophy and hyperplasia (Figs 4, 7, 8, 11). HEMALATHA and BANERJEE (1997a, b) mentioned that binding of zinc with the various S-containing proteinaceous moiety of the mucus assist its

subsequent elimination due to sloughing of the mucus into the medium, as evidenced by fluctuation in the secretory activity of the epithelial lining. BRADLEY and SPRAGUE (1985) found that acute toxicity of dissolved zinc is reduced at low pH. The shift in the nature of the mucous towards acidity and/or weak sulphation as revealed by increased AB 2.5/AB 1.0 reactions in the MCs is thus significant, as the mucous films with acid moieties over the respiratory surfaces perhaps reduce the acute toxicity of the zinc by providing a layer of acidic slimy coating.

Due to prolonged exposure the protective device provided by slimy coating collapses, and the zinc salt penetrates into the cellular constituents of the gills and other respiratory organs causing various degrees of wear and tear, such as detachment and lifting of the RE from the underlying vascular elements (PLC-BLC) of the SL, thereby causing formation of non-tissue spaces and hyperplasia of the ECs of the epithelial linings of PL as well as SL (Figs 12-15). All these factors temporarily widen the diffusion distance between the blood in the BLCs and dissolved O₂.

Increases in the density of ionocytes in the epithelial linings of the ABO (apart from the gills) of *C. striata* which also come in direct contact with the ambient zinc salt have been regularly observed. The ionocytes of the gills showed periodic fluctuation in their density in exposed fish. Similar hypertrophy and hyperplasia of the chloride cells in the gills of fishes following exposure to copper have been observed by RAJBANSHI and GUPTA (1988), PELGROM et al. (1994) and MAZON et al. (1999).

Another compensatory manifestation is noticed in the gills of *C. striata* following ZnCl₂ exposure when the BLCs exhibit extensive enlargement. The enlarged BLCs of the gills of zinc chloride exposed *C. striata* showed great congestion and engorgement with a large number of RBCs. This brings a larger quantity of blood material in the BLCs nearer to the aquatic environment, perhaps to compensate for the increased thickness of the SL caused by active regeneration of the damaged sites.

Numerous fine blood capillaries engorged with RBCs form an extensive network in the hyperplastic inner epithelial lining of the ABO of *C. striata* following ZnCl₂ exposure. Simultaneously, the minute blood capillaries at the inner layers of the epithelium lining the ABO also become extensively engorged with RBCs. These RBC-loaded BLCs acquire a globular shape and bulge out to protrude into the lumen, bringing the RBCs nearer to the air in the lumen (Figs 3-5) for more efficient extraction of O₂. This increases the efficacy of the supra-branchial chamber and compensates for the impeded branchial respiration caused by the direct toxic impact of the zinc salt, and perhaps prevented mortality due to toxicity of the ambient zinc salt.

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SAŽETAK

Analizirana je subletalna toksičnost cinkova klorida (11,5 ppm) na dišne organe riba *Channa striata*. Sluznične stanice pokazivale su povremene promjene u broju, veličini i osobitostima bojanja s izraženom većom

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količinom sulfatnih mukopolisaharida. Epitel dišnih (sekundarnih) nabora škrge povremeno se otkidao što je dovelo do njihova oštećenja te krvarenja zbog nekroze i ljuštenja dišnog epitela s posljedičnom hiperplazijom i spajanjem susjednih dišnih listića. Druge promjene očitovale su se u vidu zadebljanja dišnog epitela i oštećenja krvožilja. Spajanje dišnih listića smanjilo je površinu za izmjenu plinova dovodeći do oslabljenog bronhijalnog disanja. Promjene na suprabronhijalnoj komori dovele su do zadebljanja sićušnih papila na bazi lumena žila. Time se krv našla bliže zraku u lumenu. Nakon izloženosti došlo je do zadebljanja subepitelne krvnih žila dišnih organa posebice suprabronhijalne komore.

Ključne riječi: organi zračnog disanja, *Channa striata*, škrge, patohistologija, trovanje cinkovim kloridom
