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Histopathological Changes in the Gills of Mosquitofish, *Gambusia affinis* Exposed to Endosulfan

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Endosulfan (6,7,8,9,10,10- hexachloro -1,5,5a,6,9a- hexahydro-6,9 -methano-2,4,3-benzo-dioxyanthiepin-3-oxide) is among the most toxic pesticides for aquatic life, especially fish, and therefore has been registered as a 'priority pollutant' by the US Environmental Protection Agency. Particularly in developing countries, endosulfan is in general use for pest control in jute, cotton, sugar cane and vegetables. Due to agricultural activity, endosulfan has repeatedly been reported in surface waters and soil of developing and established countries (Robinson and Mansingh 1999; El-Kabbany et al. 2000). Acute toxicity of endosulfan to fish was reported previously (Naqvi and Hawkins 1988; Cengiz and Ünlü 1999).

Previous histopathological studies of fish exposed to pollutants revealed that fish organs are efficient indicators of water quality (Cardoso et al. 1996; Barlas 1999; Cengiz et al. 2001). The gills are important organs in fish to perform respiration, osmoregulation, acid base balance and nitro-genous waste excretion (Heath 1987). Fish gills are also vulnerable to pollutants in water because of their large surface area and external location. For this reason, fish gills are considered to be most appropriate indicators of water pollution levels (Alazemi et al. 1996). Many investigators have reported the histopathological changes in the gills of different fish species exposed to pesticides (Sinhaseni and Tesprateep 1987; Gill et al 1988; Richmonds and Dutta 1989; Alazemi et al. 1996; Erkmen et al. 2000). However, there has been little information on the histopathological impact of endosulfan on fish gills (Singh and Sahai 1990). Therefore, it was decided to determine the histopathlogcal effects of gills in a mosquitofish, *Gambusia affinis* exposed chronically to endosulfan. This species of fish was studied because they are commonly present in the ponds and streams near agricultural areas.

MATERIALS AND METHODS

One years old adult mosquitofish with an average body weight of 0.2±0.03 gr and total length of 2.8±0.4 cm were collected from the local river. The fish were transferred in oxygenated containers to the laboratory. They were acclimated in glass aquaria at a constant temperature (20±1°C) for 4 days prior to the experiment. Aged top water was used for acclimation as well as preparing test solutions. Water was continuously aerated. Fish were fed 2 times daily with commercial fish food-

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sticks. Technical grade endosulfan (Thiodan®, 33.70% endosulfan) was provided by Hoechst Co-Turkey.

The fish were divided in to three groups and placed in separate glass aquaria. At least ten fish were used for each group. Groups I, II were exposed to commercial formulations of pesticide. The nominal concentrations tested were 0.5 µg/L and 0.75 µg/L for endosulfan, being the 1/12th and 1/8th fractions of the 96 h LC_{50} value respectively. Group III was maintained in pesticide-free water to serve as control. Half the amount of test water was renewed every 24 hr. The average values for the water quality data were as follows: temperature 20±1°C, pH 7.9, dissolved oxygen 7.2 mg/L, total hardness 168 mg/L as $CaCO_3$.

Gambusia affinis exposed to endosulfan did not show any alteration in behavioural patterns and feeding activity. Likewise, growth was not retarded following exposure to endosulfan, and a part from mucus secretion no macroscopically overt signs of pathology could be discerned during dissection.

Both the experimental and control fish were sacrificed every 10 days for 30 days. Immediately after decapitation the gills were removed and dropped into aqueous Bouin's fluid. After fixation for 24-30 hr, tissues were dehydrated through a graded series of ethanol, cleared in xylene and infiltrated in the paraffin. Four-6 µm thick sections were cut on microtome and stained Hematoxylin-Eosin. Pathological lesions were examined under optical microscope.

RESULTS AND DISCUSSION

The histopathological changes were more evident in specimens exposed to endosulfan and were not observed in the control fish. After exposure, an excessive amount of mucus observed over the gills of live specimens. It has been reported that the stress caused by variations in the environment and pathologic agents induce the proliferation of mucous cells and increase secretion (Richmonds and Dutta 1989; Cardoso et al. 1996).

In this study, after 10 days of exposure to 0.5 µg/L endosulfan, epithelial necrosis, rupture of gill epithelium, haemorrhage at primary lamellae, sloughing of the respiratory epithelium and hypertrophy of epithelial cells were noted (Figure 1). The lifting of the epithelium, oedema, epithelial necrosis, fusion of adjacent secondary lamellae, and haemorrhage at primary lamellae were observed in the gills of fish examined after 20 days of exposure to 0.5 µg/L (Figure 2). Haemorrhage at primary lamellae and fusion of secondary lamellae, club-shaped lamellae, sloughing of the respiratory epithelium were noticed after 30 days of exposure to same concentration (Figure 3). In the fish exposed to endosulfan concentration of 0.75 µg/L for 10 days, hypertrophy of epithelial cells, aneurysms in secondary lamellae were seen (Figure 4). Epithelial necrosis, fusion of secondary lamellae, haemorrhage at primary lamellae, sloughing of the respiratory epithelium, aneurysms in secondary lamellae, hypertrophy of epithelial cells were observed in the gills examined after 20 days of exposure to 0.75 µg/L endosulfan (Figure 5). At

same concentration, gills were so severely damaged that fusion of secondary lamellae and aneurysms were observed after 30 days of exposure (Figure 6). In addition, individual secondary lamellae were indistinguishable due to extensive necrosis of epithelium.

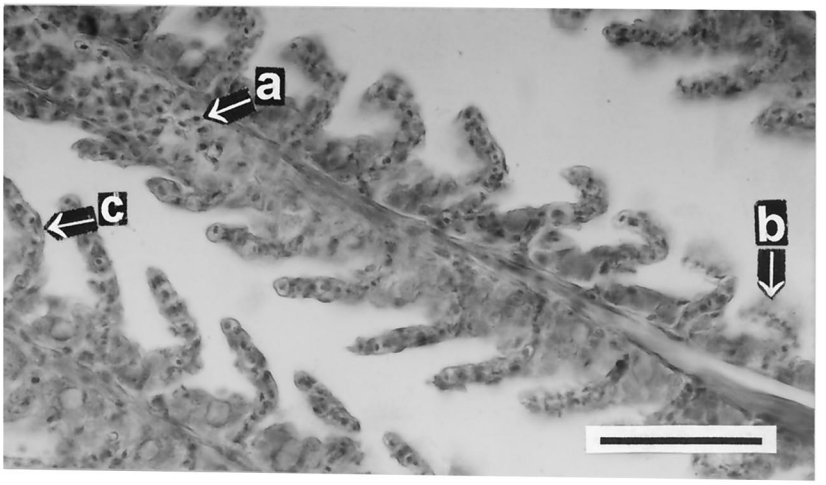

Figure 1. Haemorrhage at primary lamella (a), sloughing of the epithelium (b), hypertrophy of epithelial cells (c) (10 days; 0.5 µg/L endosulfan). Bar: 50µm, Hematoxylin & Eosin.

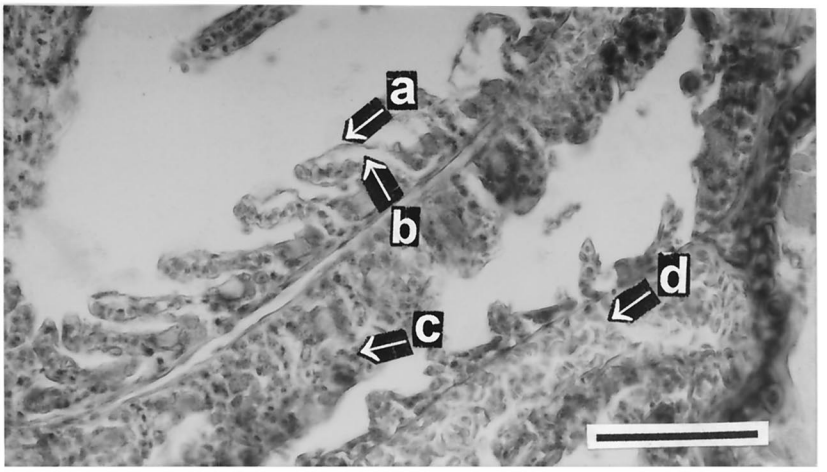

Figure 2. Lifting up of the epithelium (a), oedema (b), epithelial necrosis and fusion of adjacent lamellae (c), haemorrhage at primary lamella (d) (20 days; 0.5 µg/L endosulfan). Bar:50µm, Hematoxylin & Eosin.

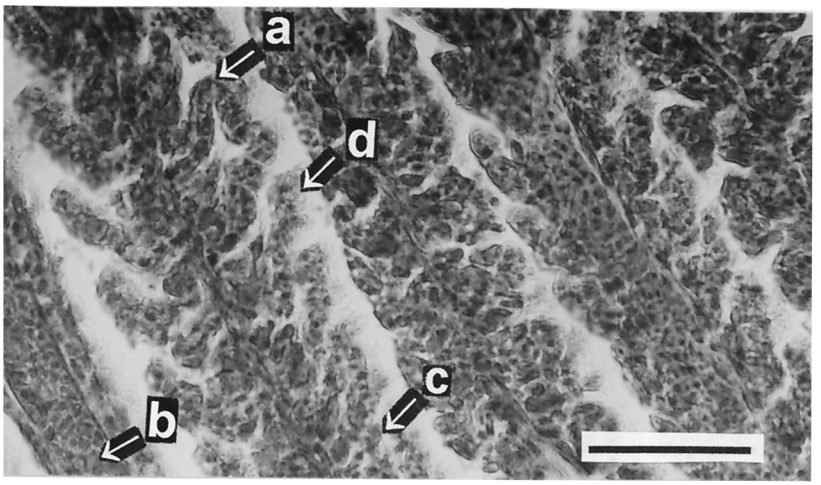

Figure 3. Club-shaped lamella (a), haemorrhage at primary lamella (b), fusion of secondary lamellae (c), sloughing of epithelium (d) (30 days; 0.5 µg/L endosulfan). Bar: 50µm, Hematoxylin & Eosin.

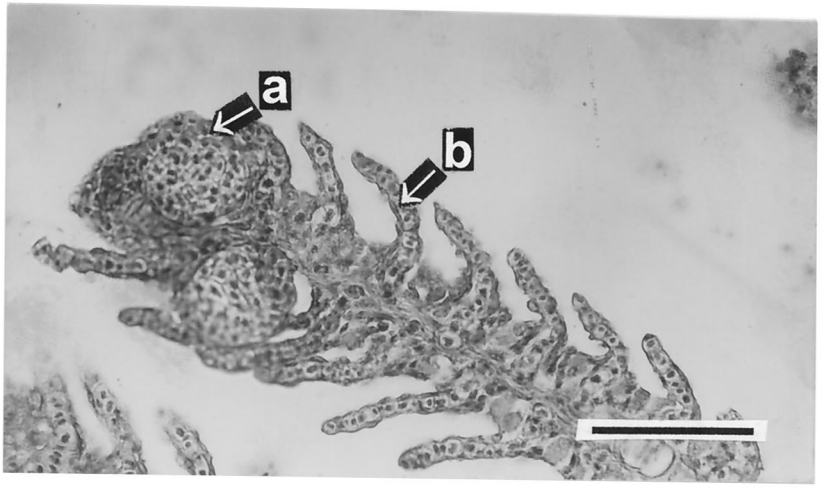

Figure 4. Aneurysm in secondary lamella (a), hypertrophy of epithelial cells (b) (10 days; 0.75 µg/L endosulfan). Bar: 50µm, Hematoxylin & Eosin.

There is increasing evidence that toxic compounds have a potential to cause the most harm to tissues and organs that contacted first (Timbrell 1991). The reason is that gills are very important absorption place for the toxic compounds. The histological examination revealed several structural and functional changes in the

gills. Similar findings were also observed by Singh and Sahai (1990) in *Puntius ticto*, which was exposed to endosulfan (0.20 mg/L).

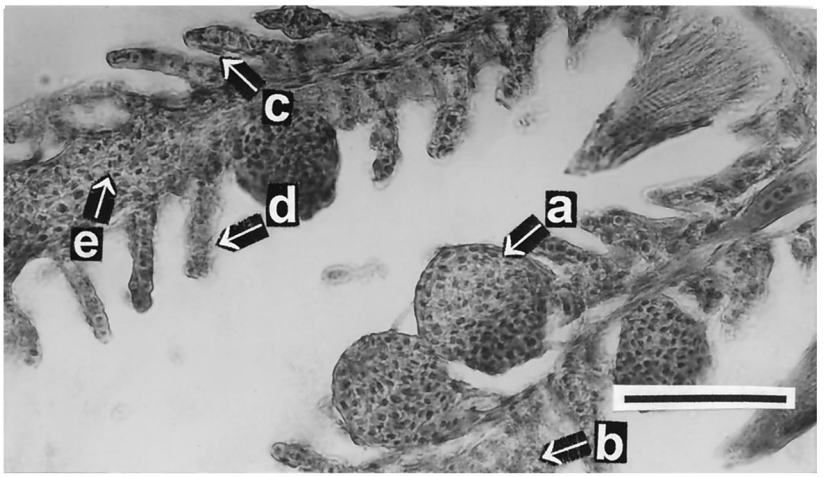

Figure 5. Aneurysm in secondary lamella (a), fusion of secondary lamellae (b), hypertrophy of epithelial cells (c), sloughing of epithelium (d), haemorrhage at primary lamella (e) (20 days; 0.75 µg/L endosulfan). Bar: 50µm, Hematoxylin & Eosin.

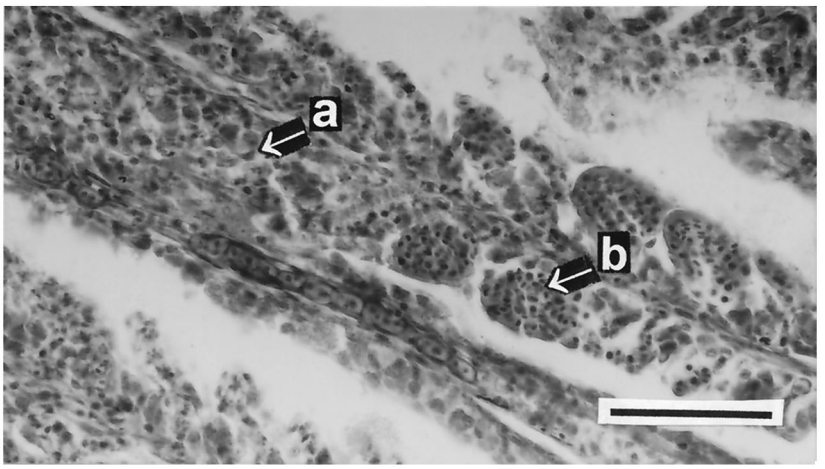

Figure 6. Epithelial necrosis and fusion of secondary lamellae (a), aneurysm (b) (30 days; 0.75 µg/L endosulfan). Bar: 50µm, Hematoxylin & Eosin.

The main histological changes observed haemorrhage at primary lamellae, fusion of secondary lamellae, club-shaped lamellae, hypertrophy of epithelial cells and aneurysms has been also reported in fish gills exposed to various kind of pesticides

(Gill et al 1988; Richmonds and Dutta 1989; Erkmen et al. 2000). Sinhaseni and Tesprateep (1987) described only marked swelling of secondary lamellae and hydropic vacuolation of epithelial cells in *Puntius gonionotus* following exposure to paraquat. Furthermore, Gill et al. (1988) revealed lysed blood cells in the sinuses and degenerated chloride cells in the inter lamellar crypts in the gills of *Puntius conchonius*, exposure to carbaryl.

Epithelial necrosis and rupture of gill epithelium are direct deleterious effects of the irritants. The fish's defence responses are excessive mucus secretion. Lifting the epithelium, lamellar fusion, and clup-shaped lamellae could be protective in that it diminishes the amount of vulnerable gill surface area (Richmonds and Dutta 1989). The histopathological changes of gill result in hypoxia, respiratory failure problems with ionic and acid-base balance (Alazemi et al. 1996).

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