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Histopathological Changes in the gills of air breathing teleost *Clarias batrachus* Linn. Exposed to endosulfan

Somdutta Moitra, Ranjeeta Bhattacharjee and N.S.Sen*
Department of Zoology, Ranchi University, Ranchi-834008

Abstract: The impact of Endosulfan, an organochlorine pesticide on the gills of Indian freshwater catfish, *Clarias batrachus* was evaluated. The experimental group was exposed to 0.5µg/L of endosulfan for 30 days. Experimental and control fishes were sacrificed every 10 days for 30 days. The gills were fixed in Bouin's solution, dehydrated in graded ethanol, infiltrated in xylene, sectioned at 4-6µm and stained in hematoxylin and eosin. Epithelial necrosis, hypertrophy of epithelial cells, rupture of gill epithelium and hemorrhage at primary lamellae were observed in fishes after 10 days of exposure, while lifting of the epithelium, oedema and fusion of adjacent secondary lamellae were conspicuous in fishes of 20 days of exposure. No effects were however observed in control fishes. The authors believe that endosulfan is significantly toxic to the fish, so much so that it may lead to asphyxia and subsequent killing of the fish.

Keywords: Endosulfan, *Clarias batrachus,* Gill, Epithelial necrosis

Introduction

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6, 9a-hexahydro-6,9-methano-2,4,3-benzodioxyanthiepin -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 developed countries (Robinson and Mansingh, 1999; El-Kabbany *et al.,* 2000). Acute toxicity of endosulfan to fish was reported previously (Naqvi and Hawkins, 1988; Cengiz *et al*., 2001).

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 nitrogenous waste excretion (Health, 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 the 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 the fish gills (Singh and Sahai, 1990). Therefore, it was decided to determine the histopathological effects on the gills in the catfish *Clarias batrachus* Linn. exposed chronically to endosulfan.

A pesticide is any substance or mixture of substances intended for preventing or controlling any unwanted species of plants or animals. Endosulfan is an organochlorine pesticide used primarily on a variety of food crops like tea, coffee, fruits, vegetables as well as rice, cereals, maize, sorghum etc. Endosulfan is moderately persistent in the soil environment and *Clarias batrachus* being a bottom dweller increases its chance of being exposed and affected by endosulfan. Therefore, the authors presume that this is the ideal fish for selection.

Gills apart from being the primary respiratory organ in fishes, are also responsible for other vital physiological functions like excretion of nitrogenous wastes, acid base balance and ion regulation. So when fish are exposed to environmental pollutants, these vital functions are deleteriously affected and the functional impairment of gills can significantly damage the health of fish (Alazemi *et al*., 1996; Munshi, 1993; Kumar and Tembhre, 2010).

Materials and methods

Adult fish with an average body weight of 0.2±0.03 kg and total length of 20.8±0.4 cm were

***Corresponding author:** N S Sen, Department of Zoology, Ranchi University, Ranchi.
Email: *nisargasen2003@yahoo.co.in*

collected from the local market. The fish were transferred in oxygenated containers to the laboratory. They were acclimated in glass aquaria at a constant temperature (24± 1^0C) for 4 days prior to the experiment. Aged tap water was used for acclimation as well as preparing test solutions. Water was continuously aerated. Fish were fed two times daily with commercial food –sticks. Technical grade Endosulfan (Thiodan®, 33.70% endosulfan) was provided by Hoechst Co India.

The fish were divided into two groups in glass aquaria. Ten fish were used for each group. Group I was exposed to commercial formulations of the pesticide. The nominal concentrations tested were 0.5µg/l for endosulfan, being the $1/12^{th}$ fractions of the 96 h LC_{50} value. Group II was maintained in pesticide-free water to serve as control. Half the amount of test water was renewed every 24 hrs. The average values for the water quality data were as follows: temperature 24±1^0C, pH 7.9, dissolved oxygen 7.2 mg/l, and total hardness 168 mg/l as $CaCO_3$.

Clarias batrachus exposed to endosulfan, they did not show any alteration in behavioral patterns and feeding activity. Likewise, growth was not retarded following exposure to endosulfan, and apart 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 paraffin. 4-6µm thick sections were cut on microtome and stained in 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 was observed over the gills of live specimens. It has been reported that the stress caused by the variations in the environment and pathologic agents induced the proliferation of mucus cells and increased secretion (Richmonds and Dutta, 1989; Cardoso *et al*., 1996).

In this study, after 10 days of exposure to 0.5µg/l endosulfan, epithelial necrosis, hypertrophy of the epithelial cells, rupture of gill epithelium, hemorrhage at primary lamellae and sloughing of respiratory epithelium were noted (Fig.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 the fish examined after 30 days of exposure to 0.5µg/l (Figs. 2, 3).

Epithelial necrosis and rupture of gill epithelium are direct deleterious effect of the irritants. The fish's defence responses are excessive mucus secretion. Lifting of the epithelium, lamellar fusion and club-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 can result in hypoxia, respiratory failure problems with ionic and acid-base balance (Alazemi *et al.,* 1996).

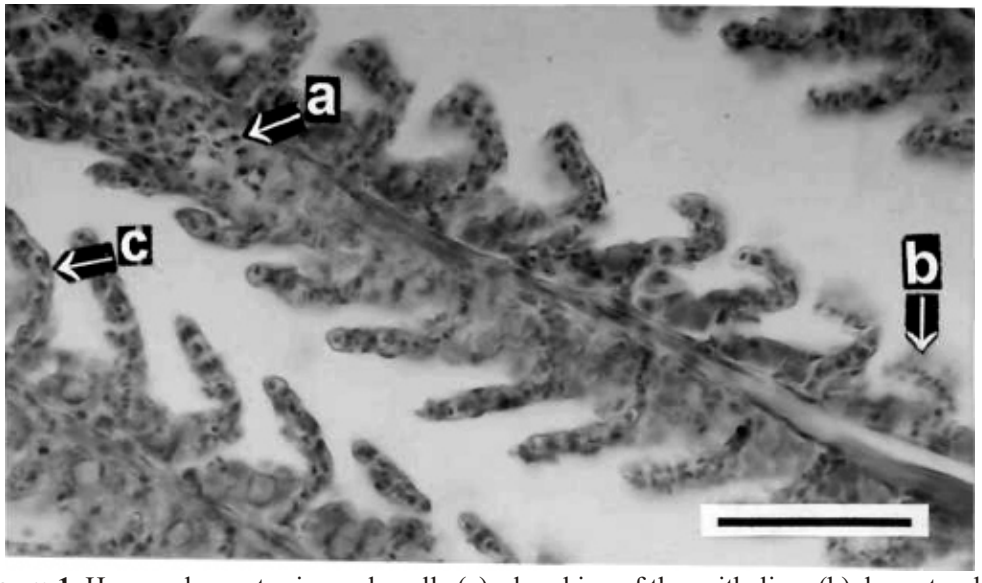

Figure 1. Haemorrhage at primary lamella (a), sloughing of the epithelium (b), hypertrophy of epithelial cells (c) (10 days; 0.5 ug/L endosulfan). Bar: 50um, Hematoxylin & Eosin.

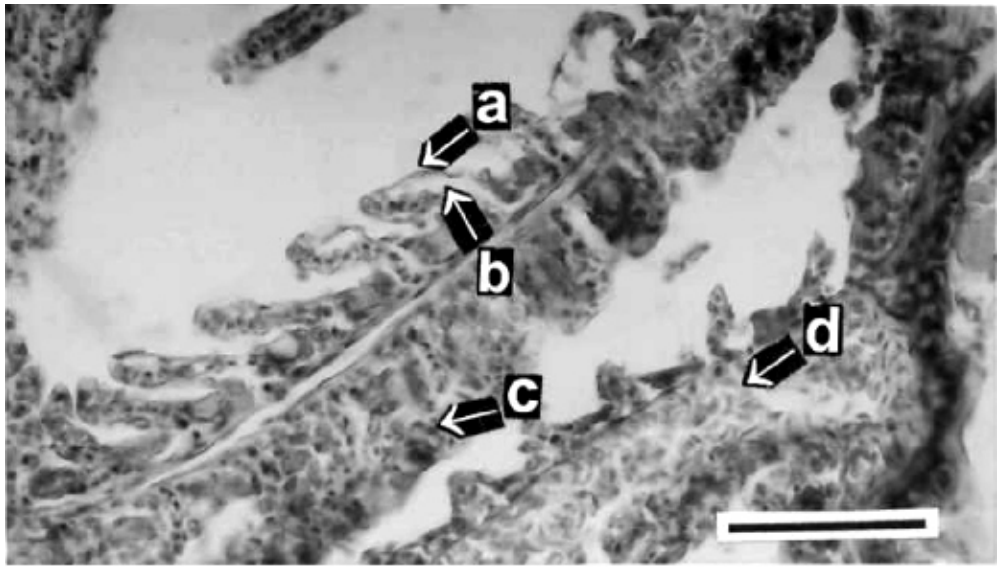

Figure 2. Lifting up of the epithelium (a), oedema (b), epithelial necrosis and fusion of adjacent lamellae (c), haemorrhage at primary lamella (d) (20 days; 0.5 ug/L endosulfan). Bar:50um, Hematoxylin & Eosin.

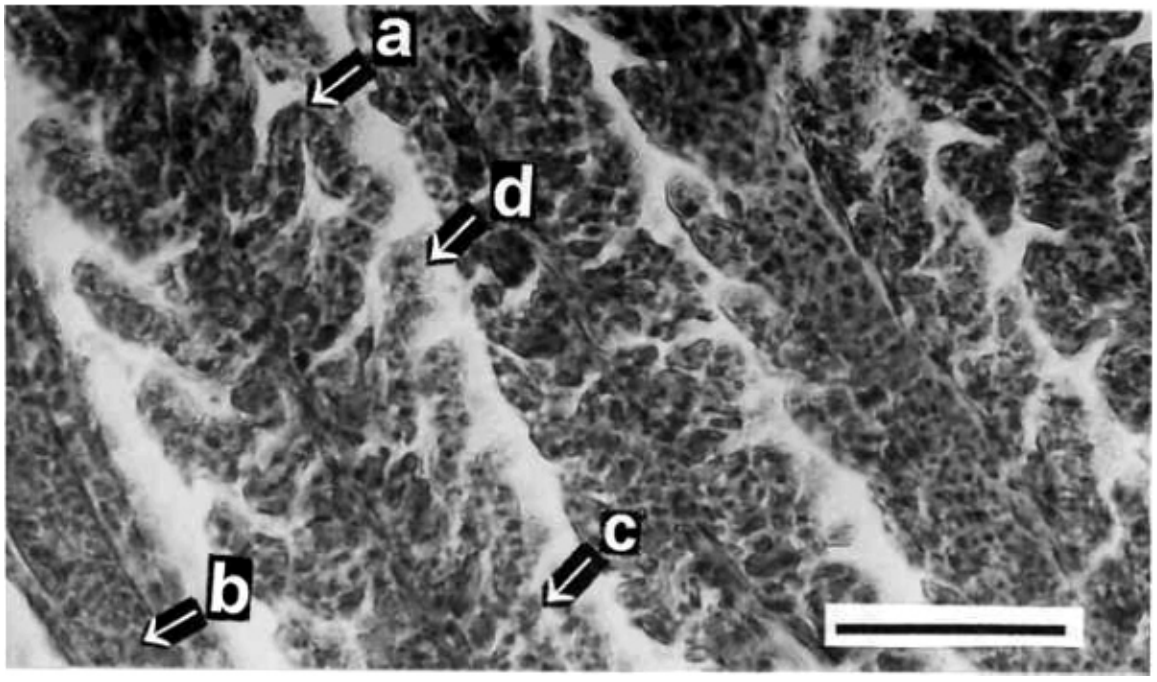

Figure 3. Club-shaped lamella (a), haemorrhage at primary lamella (b), fusion of secondary lamellae (c), sloughing of epithelium (d) (30 days; 0.5 ug/L endosulfan). Bar: 50um, Hematoxylin & Eosin.

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