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Histopathological Changes Induced by Malathion in the Gills of Bluegill *Lepomis macrochirus*

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Malathion is a widely used broad spectrum organophosphorus insecticide. Its wide use provides many occasions for its entry into aquatic environments. Pesticides reach aquatic systems by direct application, spray drift, aerial spraying, washing from the atmosphere by precipitation, erosion and run-off from agricultural land, by discharge of effluents from factories, and in sewage (Edwards 1976). The presence of this chemical in the aquatic environment would adversely affect many non-target species like fish. As reported by Khan (1976), about 50 to 90% of the absorbed malathion can be eliminated in one to three days by the fish. According to Edwards (1976), about 25% of malathion remained in river water after 2 wk, and 10% remained after 4 wk from the time of its entry.

Respiratory distress is one of the early symptoms of pesticide poisoning (Murty 1986). According to Skidmore and Tovell (1972) these toxicants appear to cause a loss of adhesion between the epithelial cells and the underlying pillar cell system, accompanied by a collapse of the structural integrity of the secondary lamellae. Gills are important in respiration as well as osmoregulation of the fish. Therefore it was decided to study the effects of malathion on the gills of bluegill sunfish, Lepomis macrochirus.

Bluegills were selected for this study due to the following reasons: 1) Bluegills are more sensitive to malathion when compared to fathead minnows and goldfish (Pickering et al. 1962). 2) They are important both as edible and game fish. 3) They are easily available and easy to maintain in the laboratory.

MATERIALS AND METHODS

Bluegills, body length 18–20 cm, weight 32–36 g, were supplied by a local supplier. The fish were transferred in oxygenated containers to the laboratory. They were acclimated in glass aquaria (122cm x 52cm x 31cm) at a constant temperature (21 $\pm$ 1°C) for 30 days prior to the experiments. They were fed 3 times daily with live guppies and commercial fish food-sticks, Tetra Doro Min manufac-

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tured by Tetra Werke, West Germany. The 96-hr LC50 for malathion to bluegills was reported differently as 0.088 mg/L by Pickering et al. (1962), 0.013 mg/L by Macek and McAllister (1970), and 0.108 mg/L by Eaton (1970). Cook et al. (1976) reported concentrations ranging from 0.0008 to 5 mg of malathion in some surface waters. The concentration used in this study is within the range of reported concentrations. Replicates of 10 fish were exposed to a concentration of 0.05 mg/L malathion for 24, 48, 72, and 96 hr. Five fish were sampled at each testing period.

Test water was renewed after every 24 hr period. The fish were not fed during the experiments. The pH was monitored during the experiment and the mean was found to be 7.2. The average values for the water quality data were as follows: dissolved oxygen 7.55 mg/L, ammonia 0.3 mg/L, total hardness 105 mg/L as $CaCO_3$, alkalinity 38 mg/L as $CaCO_3$. The amount of nitrite was not detectable at a detection limit of 0.2 mg/L. There was about 2 L of water per gram weight of fish.

Fish were pithed and gills from the control and experimental animals were isolated. The gills were cleaned in physiological saline and fixed in Lillie's fluid for about 24 hr. The gills were washed overnight in running tap water, dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax. Sections (6-7u) were cut using a rotary microtome and stained with Harris hematoxylin and eosin.

RESULTS AND DISCUSSION

The gill is (Figure 1) made up of filaments or primary lamellae arranged in double rows. Secondary lamellae arise from these filaments. The secondary lamella is lined by a squamous epithelium. Inside this epithelium are lamellar blood sinuses separated by pillar cells. At the tip of the secondary lamella is a marginal blood sinus lined by an endothelium. In between the secondary lamellae, the primary filament is lined by a thick stratified epithelium. This region contains the mucous cells and chloride cells.

After 24 hr of exposure to 0.05 mg/L malathion some mild degenerative changes were seen in the interlamellar region and epithelial lining of the secondary lamellae. The lifting up of the epithelium, some necrosis, exudation (Figure 2) and bulging at the tip of the primary filament were noticed after 24 hr of exposure (Figures 3 and 4). Table 1 shows the histological changes noticed in the experimental and control animals.

The damage was more pronounced after 48 hr of exposure (Figure 5). Necrosis of the secondary lamella, further lifting up of the epithelium and intraepithelial "edema" were observed after 48 hr of exposure. The fusion of the secondary lamellae was seen after 72 hr of exposure (Fig. 6). Ninety-six hr of exposure resulted in a pathological condition called the bulbing of the secondary lamella or clavate lamella as seen in Figure 7.

Table 1. Summary of histological changes seen per group in the gills of Bluegills exposed to 0.05 mg/L malathion and control animals.

Time in hr	Epithelial Lifting & Necrosis	Edema	Fusion of Lamellae	Clavate Lamellae
24	++	+	None	None
48	+++	++++	None	None
72	++	None	++	None
96	++	None	None	+
Control	None	None	None	None

+ = mild ++ = moderate +++ = severe ++++ = very severe

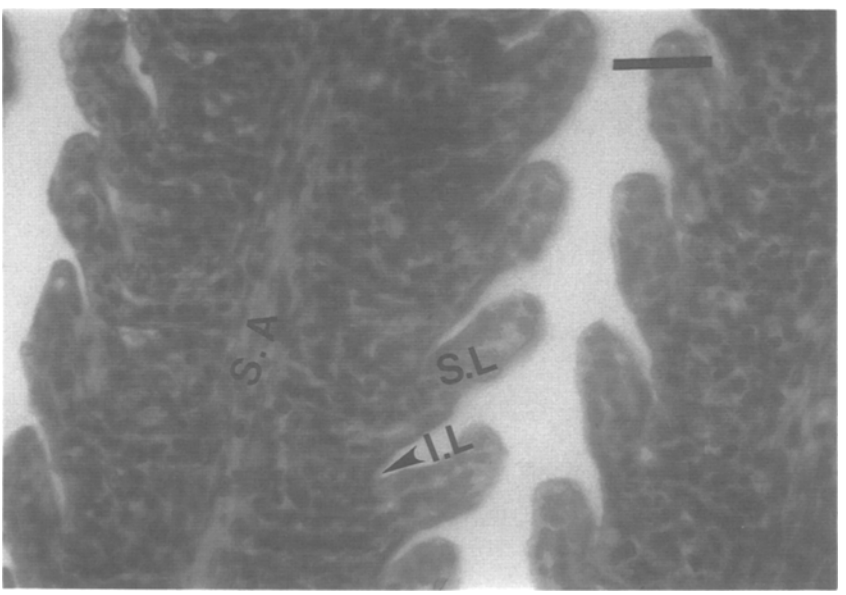

Figure 1. Section of primary lamella of gill from a control fish. S.A = Supporting axis, S.L = Secondary lamella, I.L = Interlamellar region, Scale bar = 0.025 mm, Hematoxylin & Eosin X 400.

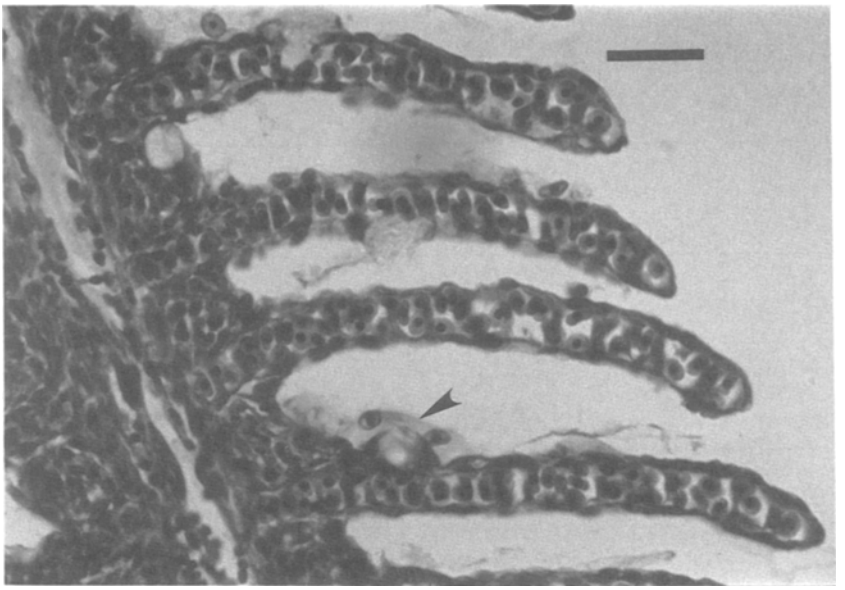

Figure 2. Section through secondary lamellae after 24 hr of exposure to 0.05 mg/L malathion showing necrosis and exudation. Scale bar = 0.025 mm, H & E X 400.

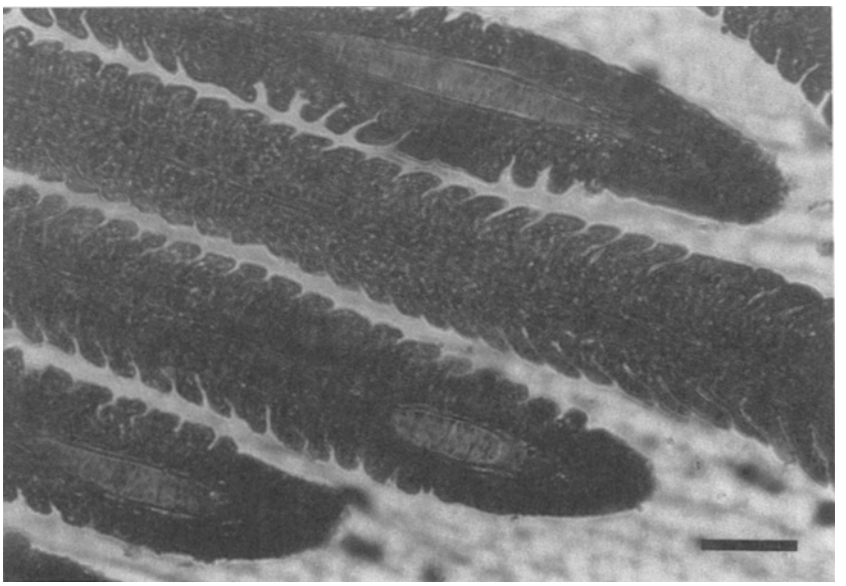

Figure 3. Section of a gill showing the tips of primary filaments from a control fish. Scale bar = 0.025 mm, H & E x 400.

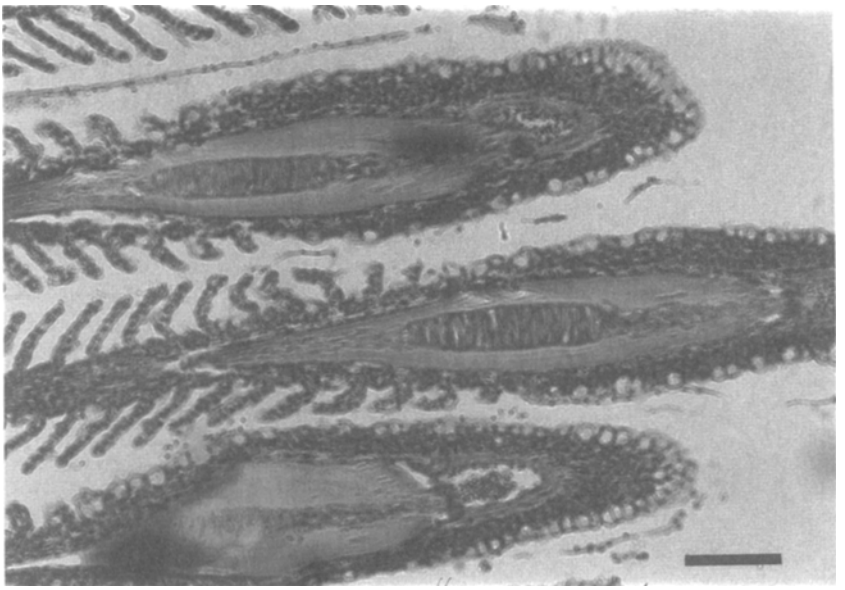

Figure 4. Section of a gill showing the tips of primary filaments from a fish after 24 hr exposure to 0.05 mg/L malathion. Scale bar = 0.025 mm, H & E X 400.

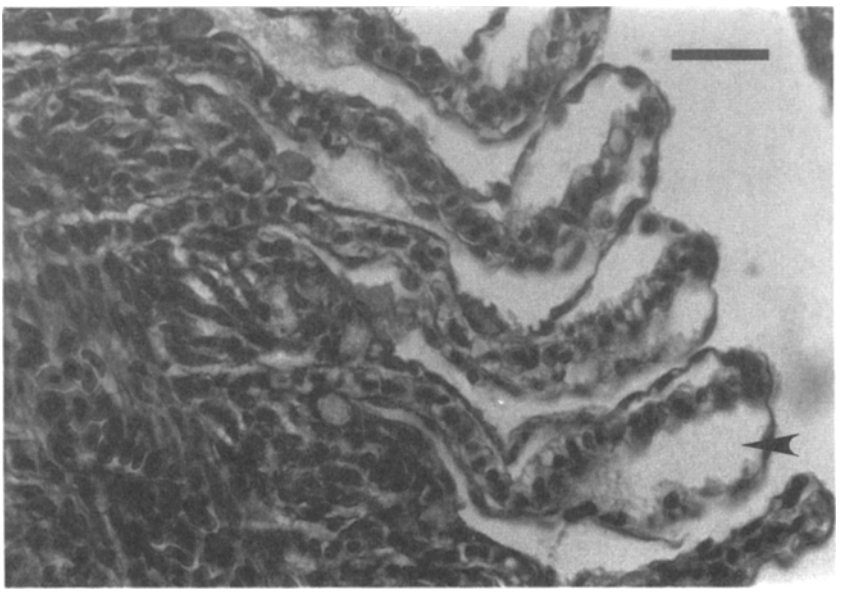

Figure 5. Section through the secondary lamellae after 48 hr of exposure to 0.05 mg/L malathion showing "edema". Scale bar = 0.025 mm, H & E x 400.

One fish died around 78 hr and two died around 84 hr of exposure. None of the control fish died during this period. After exposure, an excessive amount of mucus was found over the gills of live specimens. Khangarot (1982) reported that the gills of live animals treated with zinc were covered by a film of coagulated mucus.

In this study, under the same exposure conditions different gills and lamellae showed different degrees of degenerative changes. We have reported the predominant and constantly observed changes. Many authors have noted that, under any given set of conditions, each kind of gill lesion tends to vary widely in intensity (Mallatt 1985). Different fish also tend to be affected in varying degrees (Van Valin et al. 1968).

Past authors have divided the commonly reported gill lesions into two groups: 1) "the direct deleterious effects of the irritants" (Temmink et al. 1983) and 2) the defense responses of the fish (Morgan and Tovell 1973). The observed epithelial necrosis and rupture of the gill epithelium are direct responses induced by the action of malathion. The defense responses noticed are excessive mucus secretion, lifting up of the epithelium, lamellar fusion and clavate lamella. The lifting of the epithelium increases the distance through which the toxicant has to travel to reach the blood stream. Lamellar fusion could be protective in that it diminishes the amount of vulnerable gill surface area (Mallatt 1985). Branchial responses

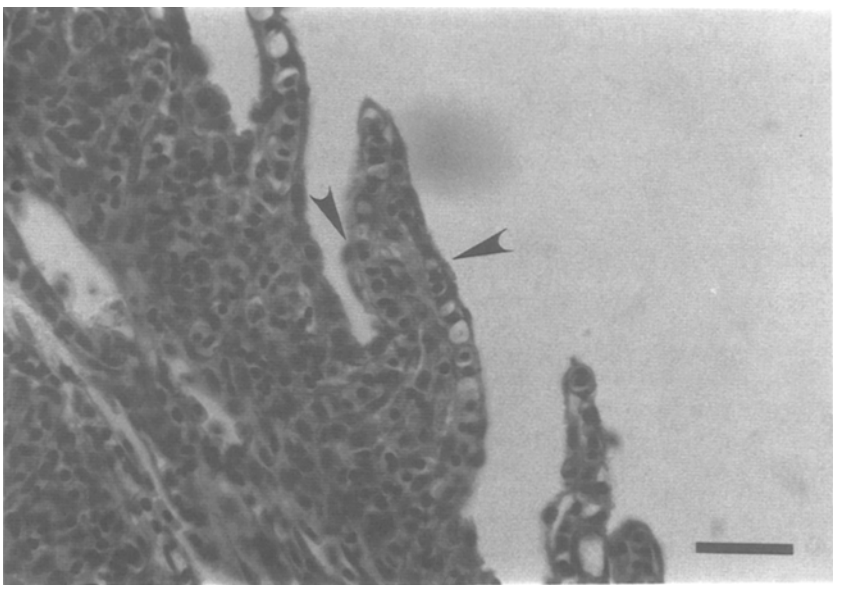

Figure 6. After 72 hr of exposure to 0.05 mg/L malathion. Two lamellae (arrows) are seen fused together. Scale bar = 0.025 mm, H & E X 400.

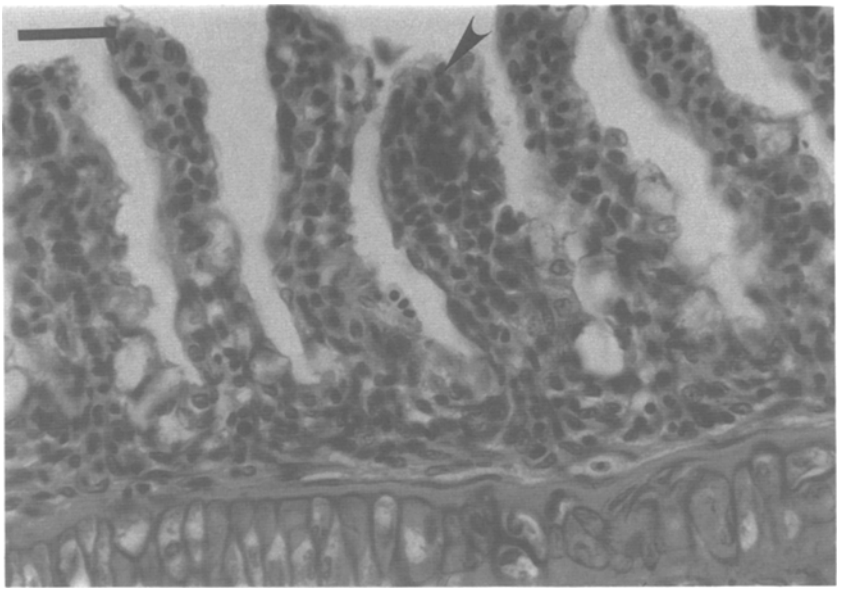

Fig. 7. Clavate lamella or bulbing of the lamella after 96 hr of exposure to 0.05 mg/L malathion. Scale bar = 0.025 mm, H & E x 400.

that serve to slow the entry of toxicant have the undesirable effect of threatening to suffocate the fish (Skidmore 1964). According to Leino et al. (1987) gills of pearl dace and fathead minnows from experimentally acidified Canadian lakes exhibited various cellular, histological, and histopathological changes, which may signify or contribute to problems with respiration and ionic and acid-base balances. The severe damage in terms of necrosis and rupture of the gill epithelium results in hypoxia and respiratory failure. In addition to this the fish encounters problems with ionic and acid-base balance.

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