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Branchial and Renal Pathology in the Fish Exposed Chronically to Methoxy Ethyl Mercuric Chloride

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Pathological manifestations causally related to pesticide poisoning have been described in both surficial and internal tissues of the fishes (Eller 1971; Couch 1975; Anees 1978). Konar (1970) observed extensive nuclear degeneration in liver, intestinal tract, and kidney of the rohu, Labeo rohita exposed to sublethal levels of heptachlor. Agallol and thiodan evoked histopathological disorders in the widowtetra, Gymnocorymbus ternetzi (Amminikutty & Rege 1978). Hepatic lesions were noticed after exposure to diazinon, methyl parathion, and dimethoate in Channa punctatus (Anees 1978).

Among the various organomercurials are phenyl mercuric acetate, methyl mercuric dicyanidiamide, methoxy ethyl mercuric chloride, methoxy ethyl mercuric silicate etc. Of these, the methoxy ethyl mercuric chloride (MEMC) is used in agriculture as an antifungal seed dressing, and its toxicity is primarily manifest in the Hg^{2+} ion. This report describes pathogenesis of branchial and renal lesions in the common freshwater fish, Puntius conchonius exposed chronically to sublethal levels of MEMC. Prior to this, alterations in the peripheral blood and metabolite levels in response to experimental MEMC poisoning have been demonstrated in this species (Gill & Pant 1985).

MATERIALS AND METHODS

Acquisition and maintenance of experimental fish, Puntius conchonius, have been described earlier (Gill & Pant 1985). The laboratory acclimatized fish were exposed to commercial formulation of MEMC (Agallol 30% W.P., Bayer's India Ltd., Bombay) at 3.6 and 6.0 ppm, representing 20 and 33% of the 96-h LC50. A parallel control group of fish was maintained in the toxicant-free tapwater (pH 7.4; hardness 370 mg/L as $CaCO_3$; dissolved oxygen 7.7 mg/L). Both the control and MEMC exposed fish were fed ad libitum, and small batches (n=8–10) sacrificed by decapitation, without using anaesthesia, at the end of 7, 21, 35, and 56 days.

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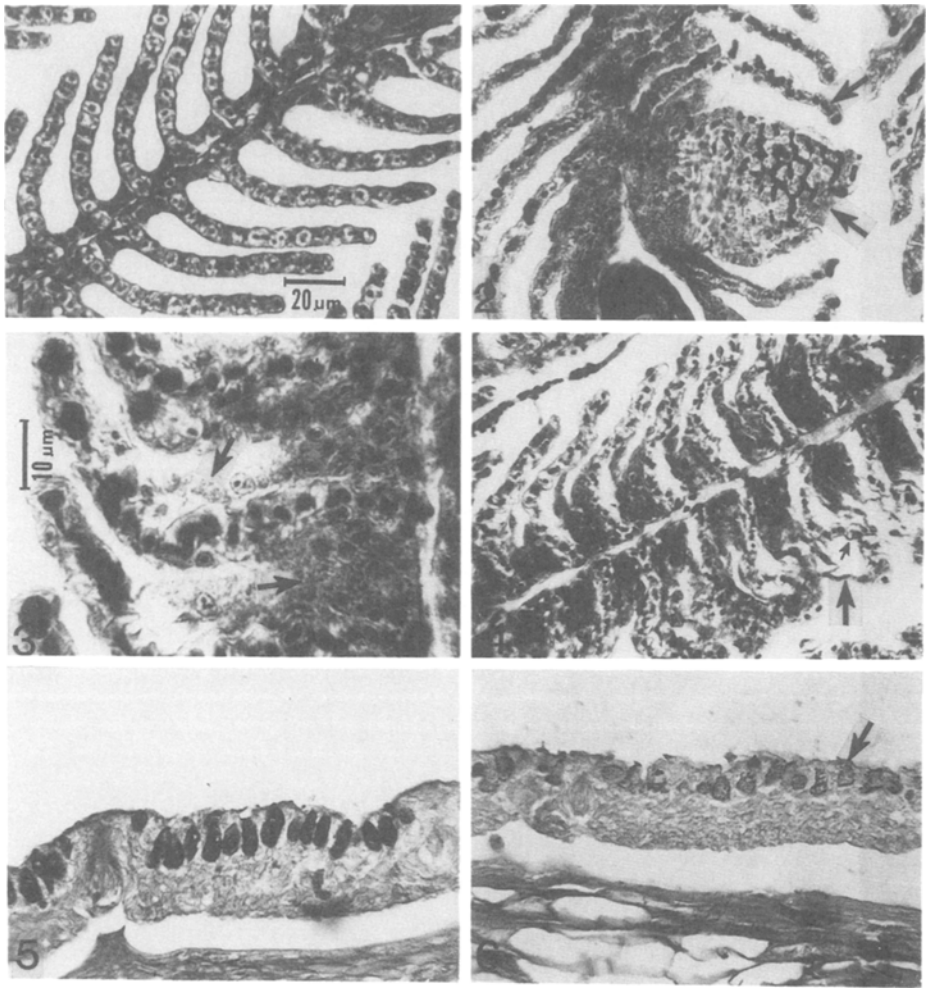
Tissue samples were collected immediately after sacrifice. The gills were carefully removed after lifting the opercula and placed in freshly prepared aq. Bouin's fluid for at least 24 h. Thereafter, the gill arches were separated and treated with a decalcifier for about 4 h followed by thorough washing in running tapwater (12-24 h) and stored in 70% alcohol. The trunk kidneys being fragile, were removed only after the whole trunk region had remained immersed in aq. Bouin's fluid for 24-30 h. Both, the gill arches and kidneys, were dehydrated through an ascending ethanol series (Humason 1971), cleared in xylene (15-20 min) and infiltrated with paraffin. Sectioning of paraffin blocks was done on an American Optical microtome at 4-6 µm, and the sections stained with Delafield's hematoxylin or Heidenhain's iron hematoxylin (Humason 1971) with eosin as an optional cytoplasmic stain. Histological lesions were located light microscopically and photographed with an Olympus PM6 camera.

RESULTS AND DISCUSSION

The secondary gill lamellae in control fish were found to be slender, erect with an uninterrupted epithelium covering the pillar cells, and contain interspersed sinuses (Fig. 1). Chronic MEMC intoxication caused drooping of secondary lamellae as well as capillary congestion. A 7 day exposure to 3.6 ppm MEMC lead to formation of aneurysms containing intact or lysed RBCs (Fig. 2). With higher MEMC concentration, 6.0 ppm, majority of the secondary lamellae were grossly deranged and showed wilting of pillar cell system which resulted in severe capillary congestion. Such lamellae revealed lysed RBCs stagnating in the lamellar blood sinuses. The epithelium was hardly discernible and the lamellar sinuses were engorged with degenerated RBCs. Besides, hypertrophied chloride cells in the interlamellar crypts and debris from cellular sloughing was observed in the affected gills (Fig. 3). After 56 days exposure to 6.0 ppm MEMC, an extensive curling and fusion of adjacent lamellae occurred due to separation of lamellar epithelium, and hypertrophy and hyperplasia of epithelial and chloride cells (Fig. 4). By comparison to those of the control fish (Fig. 5), the mucocytes in MEMC-exposed fish were found to be smaller and depleted (Fig. 6).

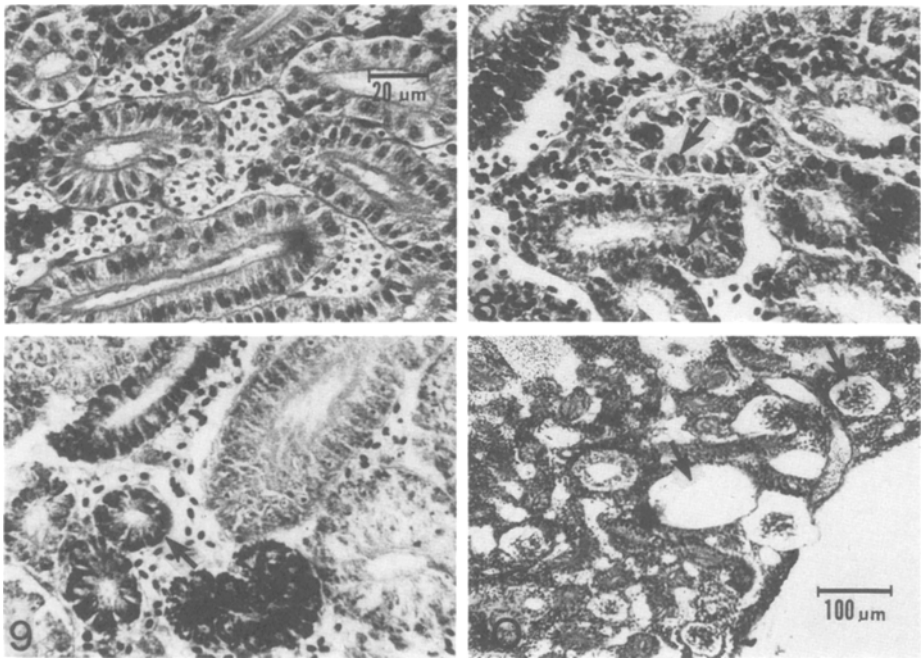
Descriptions of pesticide-induced gill lesions are available in the literature. Exposure of 6-month old carp, Cyprinus carpio to 0.025 mg/L methoxychlor and 1 mg/L propoxur for 30 days lead to swelling of gill epithelium and congestion in secondary lamellae (Lakota et al. 1978). Treatment of Herotilapia multispinosa with fenthion, at concentration 1.1 and 7.2 mg/L for 96 h, resulted in gill lesions including hyperplasia and desquamation of the epithelium and thrombosis in the secondary gill lamellae (Jauch 1979).

In P. conchonius, gross hypertrophy and hyperplasia of the chloride cells partially occluded the interlamellar space through which the respiratory current normally passes. This must have seriously impaired the oxygen uptake by the gills resulting in hypoxia at the tissue level. Further, the gills of all the MEMC-



Branchial pathology of P. conchonius chronically exposed to MEMC. Hematoxylin and eosin stain.

Figure 1. Control. Scale of magnification is common to all figures except where otherwise stated.
Figure 2. Showing aneurysm and lysed RBCs in lamellar sinuses. (7 days; 3.6 ppm MEMC).
Figure 3. Showing epithelial necrosis, hypertrophied chloride cells, and cellular debris. (7 days; 6.0 ppm MEMC).
Figure 4. Secondary lamellae showing fusion, lifting of epithelium, and wilting of pillar cell system. (56 days; 6.0 ppm MEMC).
Figure 5. Mucocytes on the branchial epithelium of control fish.
Figure 6. Depleted and atrophied mucocytes. (56 days; 6.0 ppm MEMC).



Renal pathology of P. conchonius chronically exposed to MEMC. Hematoxylin and eosin stain.

Figure 7. Control. Scale of magnification is common to all figures except where otherwise stated.
Figure 8. Showing necrosis of tubular epithelium and pycnotic and karyorhectic nuclei. (7 days; 3.6 ppm MEMC).
Figure 9. Renal tubules showing hyperchromatic deposits in the epithelium. (7 days; 6.0 ppm MEMC).
Figure 10. Part of the kidney showing edematous spaces and several shrunken glomeruli. (56 days; 3.6 ppm MEMC).

treated fish were found to be veiled by coagulated mucus causing coagulation-film anoxia which too might have aggravated the respiratory distress. Since there was no mortality during the stipulated 56-day experimental period, it is likely that compensatory respiratory, ionoregulatory, and cardiovascular changes manifest themselves in the MEMC-exposed fish although overall activity of the animal and its capacity to resist infections may be considerably curtailed.

The present findings are also indicative of the nephrotoxic effect of MEMC in P. conchonius. In the control fish the kidneys revealed intact renal tubules surrounded by extensive hematopoietic tissue and sinusoids (Fig. 7). Following MEMC exposure for 7 days at 3.6 ppm, the tubular epithelial cells revealed pycnotic and karyorhectic nuclei and a disrupted luminal surface (Fig. 8). In some of the tubules, deposition of unidentified hyperchromatic substance

as well as vacuolation was observed in the tubular epithelium (Fig. 9). The kidneys in fish, which evidently were morbid after 56 days exposure to 3.6 ppm MEMC, appeared spongy and friable. The glomeruli were conspicuously shrunken and collapsed thus leaving large Bowman's spaces (Fig. 10). Contrary to the findings of Amminikutty & Rege (1978), MEMC did not cause thickening of the wall of Bowman's capsule in P. conchonius.

The nephrotoxic effects of MEMC are primarily due to the presence of Hg which has been shown to induce renal lesions in fishes (Trump et al. 1975). Possibly, the collapse of epithelial cell infrastructure supporting the glomerulus occurs due to the known affinity of Hg^{2+} ions for binding to the -SH groups on the cell membranes and the subsequent changes in the cellular permeability leading to swelling and autolysis. Besides, it has been suggested that the negative charge on the carbohydrate moiety of the glycoproteins, which lie on the outer side of the plasma membranes, is altered in the presence of heavy metal ions resulting in an attraction between the adjacent cells. In the rat kidney, marked alterations in the glomerular epithelium including distortion of epithelial cell configuration and close apposition of adjacent foot processes occurred following perfusion with polycations (Seiler et al. 1977). A reversal of the polycation-produced epithelial changes occurred in the presence of heparin (polyanion). However, in the P. conchonius, further investigations may indicate the pathognomonic significance of the MEMC-induced renal lesions.

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