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Gill, Liver, and Kidney Lesions Associated with Experimental Exposures to Carbaryl and Dimethoate in the Fish (*Puntius conchonius* Ham.)

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The environmental pollution due to extensive usage of the pesticides without proper management has far reaching effects on the survival potential of aquatic animals for some of these toxic chemicals may persist in the environment for long periods, often unchanged. Fishes have been monitored for pesticide contamination (Johnson 1968). Certain pesticides, e.g., organochlorines and their metabolites, accumulate in the wild fish, particularly in liver and fatty tissues (Duke & Wilson 1971). Results of controlled laboratory exposures of fishes to pesticides and related chemicals have revealed that liver is often the organ with highest pesticide concentration (Duke & Wilson 1971). Although more than 900 commercial pesticide formulations are in general use, fewer than 30 have been examined for their adverse effects on fish liver (Pimentel 1971; Gupta 1986).

Carbaryl is a broad spectrum insecticide used extensively in agriculture for foliar pests and for control of ectoparasites on livestock and pets. Dimethoate has been recognized as an effective systemic and/or contact insecticide against a wide range of insects and mites. In contrast to the high toxicity of most other organophosphorus systemics, dimethoate toxicity is rated as moderate. A perusal of literature reveals very few published reports on the biological effects of these two pesticides on the non-target species such as fishes (Carlson 1971; Kaur & Toor 1977; Pant & Gill 1983). The present report describes cytopathology of branchial, hepatic and renal lesions in a common freshwater fish, Puntius conchonius exposed chronically to carbaryl and dimethoate in water.

MATERIALS AND METHODS

Specimens of Puntius conchonius (Order Cypriniformes), 5.66±0.15 cm mean total length, were collected from the local lake (altitude 1938 m) and acclimatized to laboratory conditions for a week under natural photoperiod (13L/11D approx.), water temperature (range 8.5–17.5°C, mean 12.8°C), and food ad libitum (Pant & Gill 1983). The fish were exposed to commercial formulations of carbaryl,

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1-naphthyl methyl carbamate ("Sevin", 50% W.P., Union Carbide India Ltd., Calcutta) and dimethoate, 0,0-dimethyl-S-(N-methyl-carbamoylmethyl) phosphorodithioate ("Rogor", 30% E.C., Rallis India Ltd, Bombay). The pesticides were first dissolved in 1 mL absolute alcohol and diluted with test water (pH 7.4; EDTA hardness 402.3 mg/L; D.O. 8.06 mg/L; temperature 13°C) to the desired concentration. The nominal concentrations tested were 0.194 and 0.306 mg/L for carbaryl and 0.434 and 0.683 mg/L for dimethoate, being the 1/11th and 1/7th fractions of the 96-h LC50, respectively.

The fish maintained in static indoor aquaria, were monitored for 60 days and given food ad libitum during the exposures. All control and experimental tanks were constantly aerated. Fish from both control and pesticide-treated groups were removed every 15 days, and sacrificed by decapitation for collection of tissue samples. The liver, gills, and kidneys were fixed for 24-30 h in freshly prepared aq. Bouin's fluid. The gill arches, after being treated with a decalcifier for 4 h, as well as small pieces of liver and kidneys, were dehydrated through graded ethanol series and cleared in xylene. Following infiltration with paraffin, the blocks were sectioned at 4-6 μm. The sections stained with Delafield's hematoxylin or Heidenhain's iron hematoxylin, with eosin as an optional counter stain, were examined light microscopically for histological lesions.

RESULTS AND DISCUSSION

Gill pathology. Structure of gills in P. conchonius is comparable to that described in most freshwater teleosts. The gill filaments bear a double row of thin, leaf-like secondary lamellae which consist of pillar cells and their flanges supporting the confluent blood spaces, and covered over by a thin epithelium (Figure 1). In the interlamellar crypts are found the acidophilic chloride cells and mucocytes.

Experimental carbaryl poisoning induced wilting of pillar cell system resulting in separation of lamellar epithelium after 15 days exposure to 0.194 mg/L (Figure 2).In some specimens, occurrence of lamellar thrombosis, curling and ballooning of secondary lamellae, and hypertrophy of chloride cells was also noticed (Figure 3). At higher carbaryl concentration, 0.306 mg/L, gills were so severely damaged that individual lamellae were indistinguishable due to extensive necrosis of epithelium, chloride cells, and a total collapse of the pillar cell system (Figure 4).

Dimethoate intoxication for 60 days at lower dose, 0.434 mg/L, caused degeneration of secondary lamellae due to edema and lifting of epithelium (Figure 5). With higher concentration, 0.683 mg/L, necrotic lesions were observed in the gills of fishes examined after 60 days. At this time, fragile, curled-up secondary lamellae revealed lysed blood cells in the sinuses and degenerated chloride cells in the interlamellar crypts (Figure 6).

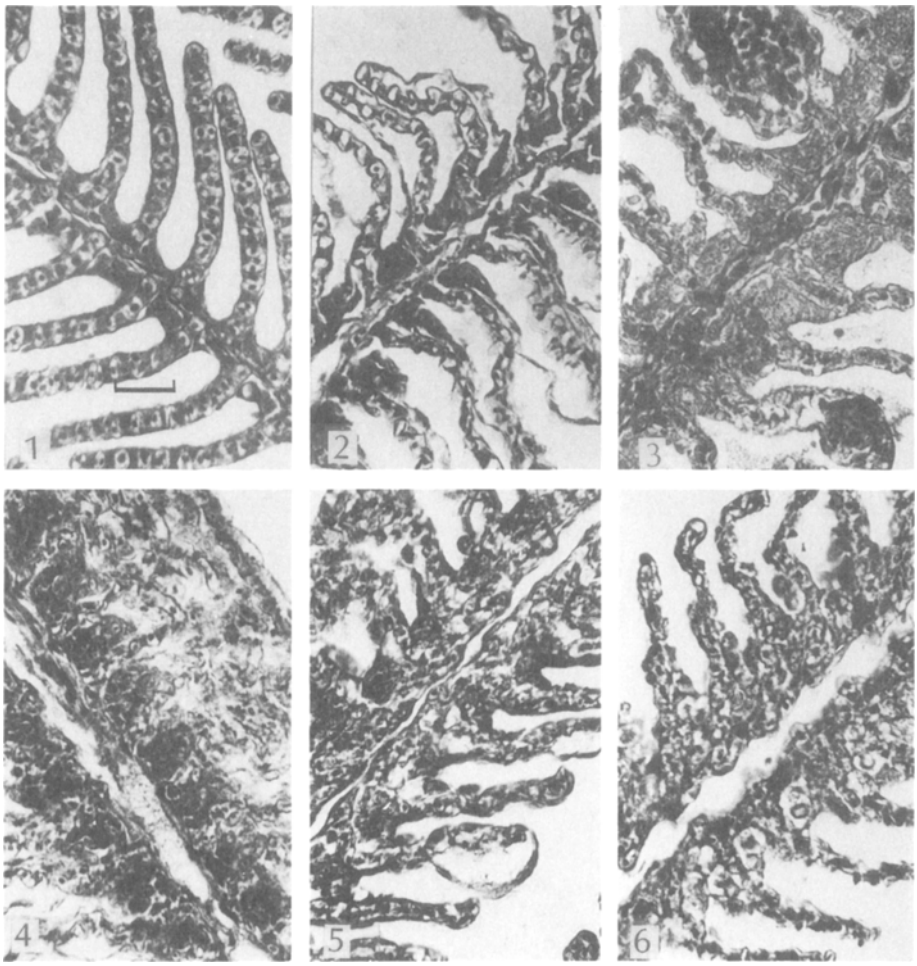

Figure 1. Secondary gill lamellae of normal, untreated Puntius conchonius. Hematoxylin and eosin stain. Scale bar denotes 20 μm and is common to all figures.
Figure 2. Wilting of pillar cell system and epithelium separation (15 days; 0.194 mg/L carbaryl).
Figure 3. Hypertrophy of chloride cells and thrombosed lamellae (15 days; 0.194 mg/L carbaryl).
Figure 4. Extensive necrosis and fusion of adjacent lamellae (15 days; 0.306 mg/L carbaryl).
Figure 5. Edematous spaces and sloughing of branchial epithelium (60 days; 0.434 mg/L dimethoate).
Figure 6. Degenerated secondary lamellae and chloride cells (60 days; 0.683 mg/L dimethoate).

The observed gill lesion in carbaryl-treated P. conchonius has its genesis in the collapse of pillar cell system causing constriction of lamellar blood channels with resultant dilation of sinuses and pooling of blood which eventually thromboses due to stagnation. Another important pathological alteration in the gills associated with carbaryl trauma was extensive hypertrophy and hyperplasia of lamellar epithelial cells and the chloride cells leading to almost complete fusion of adjacent lamellae, possibly to shut the gills in order to escape the toxicant onslaught. Hyperplasia of gill lamellar epithelium has been cited as a finding suggestive of toxicity in fishes following exposure to pesticides (Eller 1969). In the dimethoate-treated fish, the gill lesions were similar to those after carbaryl exposure, except for the formation of large edematous spaces at the bases of secondary lamellae, and gross degeneration of chloride cells as well as the lamellar epithelium and its supportive infrastructure. This agrees well with pathological effects noticed in the gills of cutthroat trout, Salmo clarki, exposed to 0.04 mg/L endrin for 12 months (Eller 1971). In an allied species, Puntius gonionotus, Sinhaseni & Tesprateep (1987) described marked swelling of secondary lamellae and hydropic vacuolation of epithelial cells following exposure to 1 and 4 mg/L paraquat for 12 days. The observed edematous separation of respiratory epithelium in P. conchonius results in increased diffusion distance, and combined with necrosis of lamellar epithelial cells, it might be the most likely cause of respiratory distress which may eventually lead to serious hypoxic conditions adversely affecting oxidative metabolism and ionoregulation.

Liver pathology. In the fish exposed to low carbaryl concentration of 0.194 mg/L for 15 days, the hepatocytes, when compared with those of the normal, untreated fish (Figure 7), were found to be hypertrophied and vacuolated (Figure 8). At higher carbaryl concentration, 0.306 mg/L, hypertrophy of hepatocytes was still apparent after 30 days in addition to the widespread nuclear pycnosis and focal necrosis (Figures 9 & 10). Severe vacuolation and nuclear pycnosis occurred in the liver following 60 days exposure to 0.434 mg/L dimethoate (Figure 11). At higher concentration, 0.683 mg/L, there was extensive liver cord disarray, almost complete absence of nuclei, and signs of lytic necrosis (Figure 12).

Pesticide-induced hepatic cytopathology has been described in other piscine species also. Degenerative liver lesions in the blue gill, Lepomis macrochirus, occurred after 14 days exposure to 0.05 and 0.037 mg/L heptachlor (Andrews et al. 1966). In the widow-tetra, Gymnocorymbus ternetzi, chronic treatment with agallol (0.01-0.016 mg/L) induced focal necrosis of hepatocytes (Amminikutty & Rege 1977). Induction of prehepatomatous lesions was reported by Eller (1971) in the cutthroat trout, S. clarki, exposed to endrin in water or food. The pathological findings in this species included liver cord disarray, presence of mitotic cells, binucleate cells, pleomorphic cells, acidophilic, pigmented cells, and intrazonal and periportal inflammatory foci. Eller believed that degenerative changes in the liver suggested nutritional deficiency enhanced by endrin exposure. The hepatic lesions

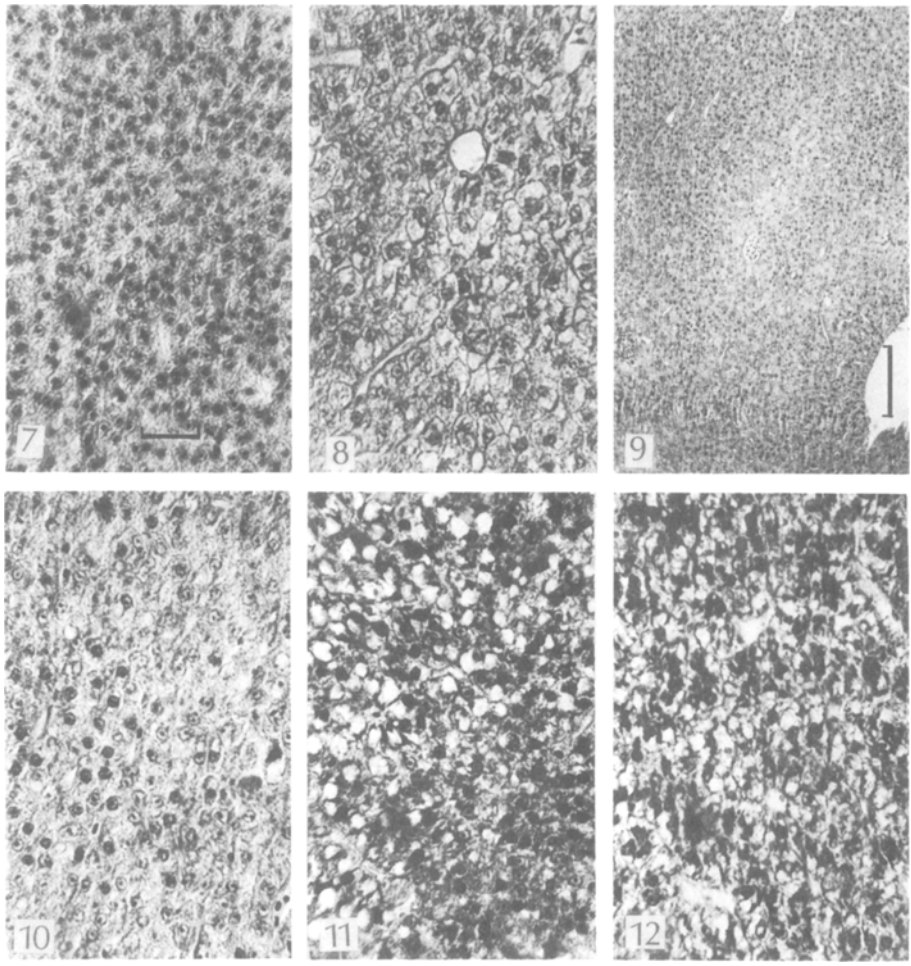

Figure 7. Part of the liver of normal, untreated Puntius conchonius. Hematoxylin and eosin stain. Scale bar denotes 20 µm and is common to all figures except figure 9.
Figure 8. Hypertrophy of hepatocytes (15 days; 0.194 mg/L carbaryl).
Figure 9. Widespread nuclear pycnosis (30 days; 0.306 mg/L carbaryl).
Figure 10. Magnified view of figure 9. Scale bar denotes 100 µm.
Figure 11. Vacuolated hepatocytes with pycnotic nuclei (60 days; 0.434 mg/L dimethoate).
Figure 12. Degenerated hepatocytes (60 days; 0.683 mg/L dimethoate).

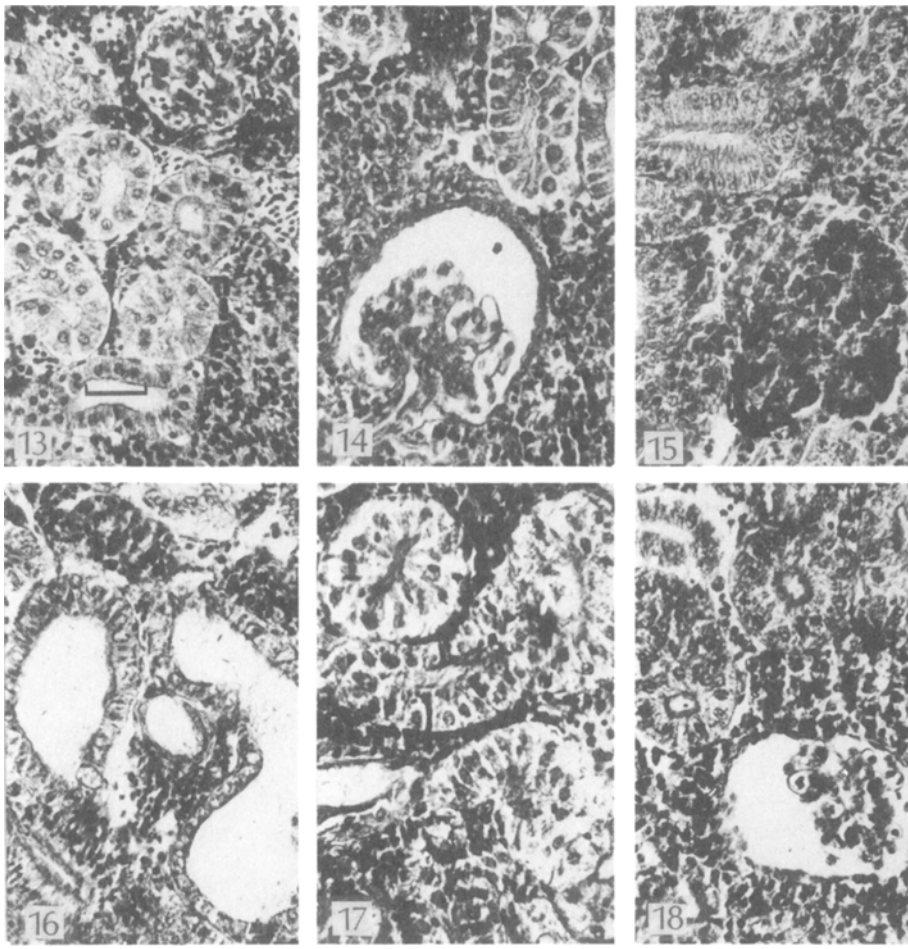

Figure 13. Part of the trunk kidney of normal, untreated Puntius conchonius. Hematoxylin and eosin stain. Scale bar denotes 20 μm and is common to all figures.
Figure 14. An affected tubule and a glomerulus inside the swollen Bowman's space (15 days; 0.304 mg/L carbaryl).
Figure 15. Deposit of unknown nature and lymphocyte infiltration (30 days; 0.304 mg/L carbaryl).
Figure 16. Distended renal tubules (15 days; 0.434 mg/L dimethoate).
Figure 17. Renal tubules with degenerated epithelium and pycnotic nuclei (60 days; 0.683 mg/L dimethoate).
Figure 18. Collapsed glomerulus (60 days; 0.683 mg/L dimethoate).

in most fishes, including P. conchonius, which have been exposed to pesticides, are non-specific. Nevertheless, manifestation of cytopathological changes in the liver clearly suggest impaired metabolic status of this vital organ.

Kidney pathology. The mesonephros in P. conchonius consists of several uriniferous tubules and glomeruli which are embedded in the lymphoidal tissue that fills the interstitium (Figure 13). Renal lesions associated with carbaryl poisoning included hypertrophy of tubular epithelial cells and their nuclei after 15 days exposure to lower dose, 0.304 mg/L carbaryl. In some cases, swelling of the Bowman's spaces was also noticed (Figure 14). Kidneys in the fish exposed for 30 days, revealed deposits of unidentified hyperchromatic substance amongst the renal tubules, in addition to a heavy infiltration of lymphocytes in the interstitium (Figure 15).

Dimethoate, 0.434 mg/L, induced degenerative changes in the renal tubules which appeared to be distended after 15 days (Figure 16). After 60 days exposure to 0.683 mg/L dimethoate, severe vacuolation of tubular epithelial cells accompanied by nuclear pycnosis, disruption of absorptive surface (Figure 17), and collapsed glomeruli were observed in the treated fish (Figure 18).

Degenerative changes in the renal tubular epithelium causally related to pesticide poisoning have been described by several workers. Pathological changes following exposure to DDT included degeneration and deposits in the distal tubules in coho salmon (Buhler 1969) and degeneration of tubular epithelium and debris in lumina in brown trout (King 1962). In the widowtetra, Gymnocorymbus ternetzi, renal lesions appeared following exposure to agallol and thiodan (Amminikutty & Rege 1978).

Descriptions of renal damage by different workers in the fish exposed to a variety of pesticides are generally in agreement with one another and with the present findings in P. conchonius subjected to experimental carbaryl and dimethoate poisoning. With both the pesticides tested, the pathological effects were most pronounced in the tubular epithelial cells which included hypertrophy, vacuolization, nuclear pycnosis, and disruption of the absorptive surface. In isolated cases, swollen Bowman's spaces and collapsed glomeruli were also encountered. The observed disorganization of tubular epithelial cells and glomeruli could be due to the damage to the cell's permeability barrier resulting in leakage of metabolites together with vital enzymes and coenzymes. Possibly, these events render the cell non-viable and autolysis manifests itself. However, presently a direct nephrotoxic action of the pesticides tested is difficult to demonstrate in this species. Possibly, the pesticide-induced damage to the gills, overburdens the kidneys with the task of water and electrolyte homeostasis.

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