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The Effects of Monocrotophos to Different Tissues of Freshwater Fish *Cirrhinus mrigala*

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Abstract The histopathological effects of monocrotophos on the gill, kidney and intestine tissues of the *Cirrhinus mrigala* were determined by light microscopy. The changes in the gills were characterized by epithelial hyperplasia, aneurism, epithelial necrosis, desquamation, epithelial lifting, oedema, lamellar fusion and curling of secondary lamellae. Pycnotic nuclei in tubular epithelium, hypertrophied epithelial cells of renal tubules, contraction of the glomerulus and expansion of space inside the Bowman's capsule were observed in the kidney tissues of fish after exposure to monocrotophos. In the intestine tissues of fish exposed to monocrotophos, oedema, necrosis and atrophy of epithelial cells were observed.

Keywords Monocrotophos · *Cirrhinus mrigala* · Histopathology · Fish

The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. Pesticides are one of the major classes of toxic substances used in India for management of pest in agricultural lands and control of insect vectors of human disease. The runoff from treated areas enters the river and aquaculture ponds that are supplied by rivers. Such rivers and the adjacent aquaculture ponds are likely to be contaminated by pesticides (Begum 2004).

Monocrotophos is one of the organophosphorus insecticides extensively used in agriculture and animal husbandry (Rao 2004). Monocrotophos has been withdrawn from use in developed countries due to its high toxicity against beneficial and non-target insects such as honey bees, fish and birds (available from http://www.pan-uk.org/pestnews/actives/monocrot.htm). But its usage for the control of major insect pests in agriculture is being continued in developing countries like India primarily due to lack of alternative replacements (Banerjee et al. 2000).

The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on data on the toxicity and effects of pesticide preparations to non-target organisms. Fish are among the group of non-target aquatic organisms. The present paper is a contribution to the assesment of toxicity and effects of a monocrotophos-based pesticide to fish. Histological changes provide a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs (Bernet et al. 1999). Limited histopathological studies have been reported in treated with monocrotophos (Santhakumar et al. 2001). Therefore, it was decided to determine the histopathological effects of gill, kidney and intestine tissues in *Cirrhinus mrigala* exposed chronically to monocrotophos.

Materials and Methods

For this experiment, *C. mrigala*, were obtained from Bharath fish seed farm, poondi, Tamil Nadu. This source was selected for the supply of fish, since there is no agricultural land or industries nearby. They weighed 9.36 g ± 0.56 (mean ± SD) and their length was in the

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range 6–8 cm. *C. mrigala*, was utilized as the model organism in this study, because it is widely distributed Indian major carp that represents majority of the total Ichthyomass of Indian sub-continent and its fishery is strongly dependent on the commercial and recreational demand.

The fish were acclimated to the laboratory conditions for at least 20 days prior to the experiment in a glass aquarium (150 L) filled with dechlorinated water. Water quality characteristics were determined. The mean values for test water qualities were as follows: temperature 27.5 ± 1.5°C, pH 7.5 ± 0.03, dissolved oxygen 6.4 ± 0.2 mg/L, alkalinity 250 ± 2.8 mg/L as $CaC0_3$, total hardness 456 ± 3.5 mg/L. The fish were fed daily with commercially balanced fish food sticks. The fishes were maintained on a photoperiod period with 12 h light/12 h dark.

Commercial grade monocrotophos (36% soluble liquid (SL)) (Rallis India Ltd, Mumbai, India) was used in this study. The commercial formulation of monocrotophos was used because only commercial preparations are used in agriculture.

Renewal toxic test methods (APHA 1995) were done to find out the 96-hr LC_{50} concentration. The 96-hr LC_{50} for monocrotophos in *C. mrigala* was found to be 19.2 ppm. The fish were divided into three groups and placed in separate glass aquaria. A total of 15 fish were used for each group. Groups I and II were exposed to sublethal concentration of monocrotophos. Group III was maintained in pesticide-free water to serve as control. The nominal concentrations of monocrotophos tested were 1.92 and 3.84 ppm. These concentrations were chosen because they are lower than lethal concentrations for *C. mrigala*.

After treatment, both the experimental and control fishes were sacrificed at the end of 10th day. Gill, kidney and intestine tissues were removed and dropped in aqueous Bouins fluid. After fixation, tissues were dehydrated through a graded series of ethanol, cleared in xylene, and infiltrated in the paraffin. Sections of 4–6 μm were prepared from paraffin blocks by using a rotary microtome. These sections were then stained with Hematoxylin-Eosin. Histopathological lesions were examined and photographed, using Leica photomicroscope.

Results and Discussion

No histopathological changes were observed in the gills of the control fish. The structural details of the gills of control *C. mrigala* are shown in Fig. 1 (1). Epithelial hyperplasia, aneurism, epithelial necrosis, desquamation, epithelial lifting, oedema and curling of secondary lamellae were noticed after exposure to 1.92 and 3.84 ppm monocrotophos (Fig. 1 [2–5, 7–9]). In addition to, lamellar fusion was observed after exposure to 3.84 ppm monocrotophos [Fig. 1 (6)]. The histological changes noticed in the pesticide exposed and control fish are shown in Table 1.

Gills are generally considered good indicator of water quality (Rankin et al. 1982), being models for studies of environmental impact (Wenderlaar Bonga and Lock 1992). Since the gills are the primary route for the entry of pesticide.

Several other studies have shown similar effects of pesticides on fish gills (Cengiz and Unlu 2002, 2003). Many investigators have reported the histopathological changes in gills of different fish species exposed to pesticides. Hemorrhage in the primary and secondary gill lamellae, degeneration and necrosis of epithelial cells, distortion of the secondary lamellae, disruption of epithelial cells from pillar cells were observed in gill tissues of *Anabas testudineus* exposed to monocrotophos (Santhakumar et al. 2001). Degenerative changes in gills, such as detachment and lifting of the epithelial linings from the surface of the gills, uncontrolled regeneration of the primary lamellae and secondary lamellae, hypertrophy, hyperplasia, necrosis of the epithelial cells, dilation of the blood sinuses of the secondary lamellae, lamellar aneurysm, hemorrhages were noticed after exposure of sublethal concentration of profenofos (Rao et al. 2006).

Histopathological changes were not observed in the kidney tissue of the control fish. The structural details of the kidneys of control *C. mrigala* are shown in Fig. 2 (10). Pycnotic nuclei in tubular epithelium, hypertrophied epithelial cells of renal tubules, contraction of the glomerulus and expansion of space inside the Bowman's capsule were observed in the kidney tissues of fish after exposure to 1.92 and 3.84 ppm monocrotophos [Fig. 2 (11–15)]. The histological changes noticed in the pesticide exposed and control fish are shown in Table 2.

The kidneys of fish receives much the largest proportion of postbranchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution (Ortiz et al. 2003).

Exposure of fish to toxic agents such as pesticides, histological alterations have been found at the level of the tubular epithelium and glomerulus. Das and Mukherjee (2000) reported dilation of tubules, necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of affected cells of *Labeo rohita* exposed to hexachlorocyclohexane. Tilak et al. (2001) observed severe necrosis, cloudy swelling in the renal tubules, cellular hypertrophy, granular cytoplasm, vacuolization in kidney tissues of *Ctenopharyngodon idellus* exposed to fenvalerate. Degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertro-

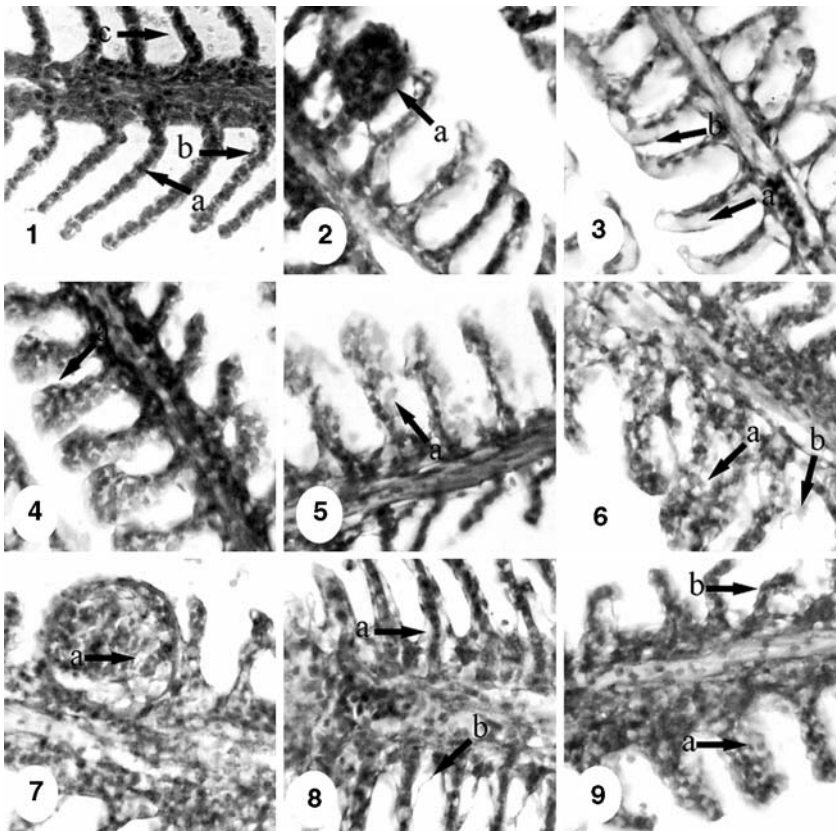

Fig. 1 Gill tissue of *C. mrigala*. (*1*) control – (a) pillar cell, (b) epithelial cell, (c) secondary lamellae. H&E, ×250; (*2*) exposed to 1.92 ppm monocrotophos – (a) aneurism. H&E, ×250; (*3*) exposed to 1.92 ppm monocrotophos – (a) oedema, (b) epithelial lifting. H&E, ×250; (*4*) exposed to 1.92 ppm monocrotophos – (a) epithelial hyperplasia. H&E, ×250; (*5*) exposed to 1.92 ppm monocrotophos – (a) epithelial necrosis and desquamation. H&E, ×250; (*6*) exposed to 3.84 ppm monocrotophos – (a) lamellar fusion, (b) epithelial necrosis and desquamation. H&E, ×250; (*7*) exposed to 3.84 ppm monocrotophos – (a) aneurism. H&E, ×250; (*8*) exposed to 3.84 ppm monocrotophos – (a) oedema, (b) epithelial lifting. H&E, ×250; (*9*) exposed to 3.84 ppm monocrotophos – (a) epithelial hyperplasia, (b) curling of secondary lamellae. H&E, ×250

Table 1 Summarized histopathological effects in the gills of *Cirhinus mrigala* exposed to monocrotophos and control fish

Concentration (ppm)	Epithelial hyperplasia	Aneurism	Epithelial necrosis and desquamation	Epithelial lifting and oedema	Lamellar fusion	Curling of secondary lamellae
Control	–	–	–	–	–	–
1.92	+	+	+	+	–	+
3.84	++	++	++	++	++	++

(–) none, (+) mild, (++) moderate, (+++) severe

phied cells and narrowing of the tubular lumen are observed in the kidney tissues of fish exposed to deltamethrin (Cengiz 2006).

Histopathological changes were not observed in the intestine tissue of the control fish. The structural details of the intestine of control *C. mrigala* are shown in Fig. 3 (16). In the intestine tissues of fish exposed to monocrotophos concentrations of 1.92 and 3.84 ppm, necrosis of epithelial cells and oedema were observed [Fig. 3 (17–20)]. Besides, atrophy of epithelial cells was seen after exposure to 3.84 ppm monocrotophos [Fig. 3 (21)]. The histological changes noticed in the pesticide exposed and control fish are shown in Table 3.

The intestine is a very important absorption place for the toxic compounds (Timbrell 1991).

Cengiz et al. (2001) reported oedema, degeneration, accumulation of lymphocytes in the lamina propria, pycnotic state of nuclei, and necrosis in the intestine of *Gambusia affinis* exposed to endosulfan. In other study, infiltration of mononuclear leucocytes and eosinophils towards lamina propria and necrosis were detected in intestine tissues of fish after exposure to deltamethrin (Cengiz and Unlu 2006). Velmurugan et al. (2007) observed atrophy of epithelial cells, necrosis of epithelial cells, desquamation of mucosal epithelium and infiltration of lymphocytes into the lamina propria in intestine tissues of fish after exposure to fenvalerate.

All the histopathological observation indicated that exposure to sublethal concentrations of monocrotophos caused destructive effect in the gill, kidney, and intestine tissues of *C. mrigala*. Histopathological alterations in the gill, kidney and intestine tissues, such as those observed in this study and findings from previous studies, may result in severe physiological problems, ultimately leading to the

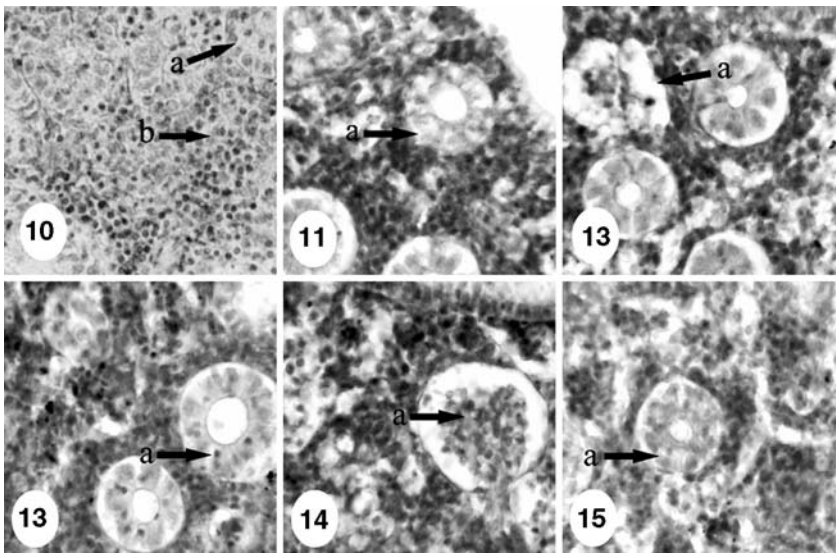

Fig. 2 Kidney tissue of *C. mrigala*. (*10*) control – (a) renal tubule, (b) hematopoietic tissue. H&E, ×250; (*11*) exposed to exposed to 1.92 ppm monocrotophos – (a) hypertrophied epithelial cells of renal tubules. H&E, ×400; (*12*) exposed to 1.92 ppm monocrotophos – (a) contraction of the glomerulus and expansion of space inside the Bowman's capsule. H&E, ×400; (*13*) exposed to 1.92 ppm monocrotophos – (a) pycnotic nuclei in tubular epithelium. H&E, ×400; (*14*) exposed to 3.84 ppm monocrotophos – (a) contraction of the glomerulus renal tubule and expansion of space inside the Bowman's capsule. H&E, ×400; (*15*) exposed to 3.84 ppm monocrotophos – (a) hypertrophied epithelial cells of renal tubules. H&E, ×400

Table 2 Summarized histopathological effects in the kidneys of *Cirhinus mrigala* exposed to monocrotophos and control fish

Concentration (ppm)	Pycnotic nuclei in tubular epithelium	Hypertrophied epithelial cells of renal tubules	Expansion of space inside the Bowman's capsule	Contraction of the glomerulus
Control	–	–	–	–
1.92	+	+	++	++
3.84	++	++	++	++

(–) none, (+) mild, (++) moderate, (+++) severe

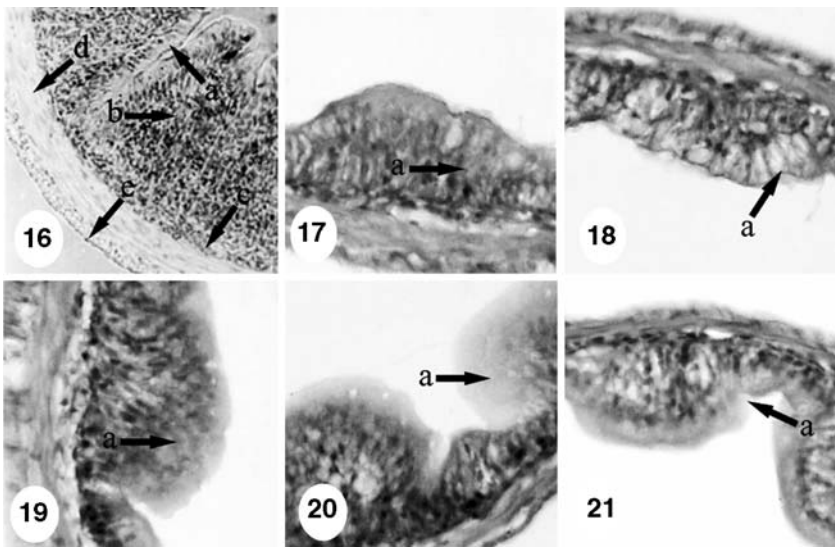

Fig. 3 Intestine tissue of *C. mrigala*. (*16*) control – (a) epithelium, (b) lamina propria, (c) stratum compactum, (d) muscularis layers and (e) serous membrane. H&E, ×250; (*17*) exposed to 1.92 ppm monocrotophos – (a) oedema. H&E, ×400; (*18*) exposed to 1.92 ppm monocrotophos – (a) necrosis of epithelial cells. H&E, ×400; (*19*) exposed to 3.84 ppm monocrotophos – (a) oedema. H&E, ×400; (*20*) exposed to 3.84 ppm monocrotophos – (a) necrosis of epithelial cells. H&E, ×400; (*21*) exposed to 3.84 ppm monocrotophos – (a) atrophy of epithelial cells. H&E, ×400

Table 3 Summarized histopathological effects in the intestine of *Cirhinus mrigala* exposed to monocrotophos and control fish

Concentration (ppm)	Atrophy of epithelial cells	Necrosis of epithelial cells	Oedema
Control	–	–	–
1.92	–	+	+
3.84	+	++	++

(–) none, (+) mild, (++) moderate, (+++) severe

death of fish. As a conclusion, the findings of the present histological investigations demonstrate a direct correlation between pesticide exposure and histopathological disorders observed in several tissues.

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