IMPACT OF PHOSPHAMIDON AND ITS METABOLITES ON HISTOPATHOLOGY OF THE LIVER, GILL AND INTESTINE OF LABEO ROHITA

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ABSTRACT

Impact of phosphamidon, an organophosphorus pesticide and its metabolites viz. dimethyl phosphoric acid and 2-chloro 2-diethyl carbamoylmethyl vinyl acid on histopathology of a common teleost, Labeo rohita was studied by exposing the fish to sub-lethal concentrations which were taken as 1/3rd of LC50 and were equal to 0.0123 ppm for phosphamidon, 0.0160 ppm for dimethyl phosphoric acid and 0.0167 ppm for 2-chloro 2-diethyl carbamoylmethyl vinyl acid respectively. The results revealed that hepatocytes in the liver were markedly swollen and exhibited hydropic degeneration. Fusion of primary lamellae and moderate congestion of blood vessels were evident in the gill. Intestine showed degeneration of mucosa and cellular infiltration in sub-mucosa. LC50 values and histopathological photomicrographs suggest that phosphamidon is more toxic as compared to dimethyl phosphoric and 2-chloro 2-diethyl carbamoylmethyl vinyl acid.

Key words: Phosphamidon, Metabolites, Histopathology, Labeo rohita.

INTRODUCTION:

As aquatic ecosystems acts as the ultimate sink for all anthropogenic and xenobiotic chemicals, there is a need to assess the relative hazards of these pollutants on non-target organisms like fish. In agricultural operations, extensive use of organophosphorus insecticides is recommended for reasons of their rapid degradability. Although the toxicity studies in warm water teleost exposed to organophosphorus pesticides have been reported earlier (Mukhopadhyay and Dehadrai, 1980; Anees, 1978; Coppage and Mathew 1974; Choudhary et al., 1993; Mandal and Kulshrestha, 1980) but much of the information about the severe effects of pesticides on aquatic biotypes has been obtained from mortality state of organisms. Besides mortality a lot remains to be understood regarding the damage to internal organs and the comparative impact of a pesticide and its metabolites because when a pesticide enters an aquatic system, it immediately undergoes hydrolysis, oxidization or isomerisation and the resulting metabolites may or may not have the similar impact as the original pesticide. Hence in our present investigation an attempt has been made to investigate the impact of phosphamidon, an organophosphorus pesticide and its metabolites viz. dimethyl phosphoric acid and 2-chloro 2-diethyl carbamoylmethyl vinyl acid on histology of a common teleost, Labeo rohita.

MATERIAL AND METHODS:

The test compound, phosphamidon (2-chloro 2-diethyl carbamoyl-1-methyl vinyl
dimethyl phosphate) was obtained from United Phosphorus Ltd, which is a non-systemic insecticide with toxicity class of 1a (WHO) and 1 (EPA). Two metabolites of phosphamidon viz. dimethyl phosphoric acid and 2-chloro 2-diethyl carbamoylmethyl vinyl acid were prepared in the laboratory as under.

Dimethyl phosphoric acid was prepared by taking 500g of phosphamidon (2-chloro 2-diethyl carbamoyl-1-methyl vinyl dimethyl phosphate) that was obtained from United Phosphorus and 25g of 1N KOH in methanol, refluxing for two hours on a water bath and cooling and putting into ice cold water, neutralizing with HCl (neutral to litmus paper). While solid thus obtained was filtered and recrystallised from methanol, M.P. 40-43°C, 2-chloro 2-diethyl carbamoylmethyl vinyl acid was prepared by taking the filtrate obtained above, concentrating it and extracting with ethyl acetate.

The fish Labeo rohita was selected for the present investigation, were procured from the Aarey Fish Farm, Mumbai. The fish were acclimatized to laboratory conditions for about 15 days before the commencement of the experiment and were fed ad libitum alternately with tubifids, earthwarms, egg-custard, goat liver and minced fish. The water was analysed for physico-chemical characteristics following APHA-AWWA-WEF (1998) and the values were; temp. 28 ±1.0°C; pH 7.2 ±0.2, dissolved oxygen ± 6.2 ± 0.01 mg/l, total alkalinity 50 ± 0.50 and total hardness 52 ±0.70.

Fish with an average weight of 5.5 g and length 6-7 cm. were selected for the experiment. The LC50 value of phosphomordon, to fish was 0.018 ppm and its metabolites tichloropyridinol and diethyl thiophosphoric acid were 0.023ppm and 0.015ppm respectively (Finney, 1952). One third of these values, i.e., 0.0067 ppm for chloropyriphos, 0.0077 ppm for trichloropyridinol and 0.0050 ppm for diethyl thiophosphoric acid were taken for sublethal studies. A common control was maintained. Sublethal toxicity studies were conducted for 96 hours where fishes were exposed to sublethal concentrations and samples were drawn at 24, 48, 72 and 96 hours interval and compared with control. Each concentration was run in triplicate (APHA AWWA WEF, 1998). The data obtained from the experiment were processed by method of Buikemia et al., 1982.

The tissue sample of kidney, liver and gill were procured from the fish specimens subjected to sub-chronic test of 7 days duration. The fish in live condition were dissected on eighth day after pithing and samples of liver, gill and kidney were procured and stored in isotonic normal saline. The tissues were processed and 6 µ thin sections were cut with the help of a rotary microtome, stained with haematoxyline and eosin (Robert, 1989) and viewed under a research binocular compound microscope for histopathological examination (Zeiss, Axiophot, West Germany) and photomicrographs were taken using a computerized photomicrographs system.

RESULTS AND DISCUSSION

Histopathological alterations in kidney, liver and gill of the fish exposed to chlorophyriphos, trichloropyridinol and diethyl thiophosphoric acid revealed discernible damage to the tissues as compared to control.

Kidney

Although kidney has no direct contact with the pollutants, blood vascular system is responsible for carrying the toxicant and its metabolites to kidney for excretion purpose and thereby coming in contact with
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Plate 1. Kidney of *Labro rohita* (control) showing normal renal corpuscles and renal tubules. Haemopoietic tissue were abundant surrounding the convoluted tubules.

Plate 2. Kidney of *Labro rohita* exposed to phosphamidon showing hyperplasia of the interstitial tissue.

Plate 3. Kidney of *Labro rohita* exposed to trichloropyridinol showing mild to moderate cytoplasmic change in renal tubules.

Plate 4. Kidney of *Labro rohita* exposed to diethylthio phosphoric acid showing degeneration and cellular infiltration in the interstitium.

Plate 5. Liver of *L. rohita* (control) showing normal architecture with hepatocytes arranged in cord like fashion.

Plate 6. Liver of *L. rohita* exposed to Chloropyridinols showing moderately swollen hepatocytes exhibiting hydropic degeneration with vacuolation in the cytoplasm.

Plate 7. Liver of *Labro rohita* exposed to trichloropyridinol showing moderately swollen hepatocytes exhibiting hydropic degeneration with vacuolation in the cytoplasm.

Plate 8. Liver of *Labro rohita* exposed to diethylthio phosphoric acid exhibiting disorganisation of hepatic cord structure and fatty degeneration in the cytoplasm.
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Plate 3. Gill of Labeo rohita (control) showing extended and evenly spaced gill filaments. Gill arch appears normal. Both efferent and afferent filament arteries were intact and contained free blood cells within.

Plate 10. Gill of Labeo rohita extended to Chloropyridine showing complete loss of secondary filament in one lamella and severe thickening due to cellular infiltration in the other lamella of the gill tissue.

Plate 11. Gill of Labeo rohita exposed to trichloropyridinol showed prominent cartilaginous cells due to severe necrosis. Note the leftover cartilaginous tissue in the primary lamellae.

Plate 12. Gill of Labeo rohita exposed to diethyl thio phosphoric acid showing complete loss of the secondary filaments and marked cellular reaction in the primary gill filaments.

the toxic substance and undergoing secondary alterations. Plate 1 is the photomicrograph of control kidney tissue of healthy Labeo rohita showing normal renal capsules and renal tubules. Haemopoietic tissues were abundant surrounding the convoluted tubules and interstitium was normal looking and densely comprised of renal parenchymal cells.

Plates 2, 3 and 4 exhibit alterations in the kidneys upon exposure to chloropyrophos and its metabolites trichloropyridinol and diethyl thio phosphoric acid respectively. In plate 2, interstitial tissue showed moderate hyperplasia, however, epithelium and glomeruli did not show any change. There was necrosis in tubular cells and vacuolated spaces were discernible in the parenchyma. Plate 3 exhibited mild edema in glomeruli, renal tubules and the interstitium due to accumulation of mononuclear cells. Plate 4 exhibited massive disorganization of renal structures along with diffused hyaline necrosis in its tuft. Epithelium was necrotic and interstitium also exhibited patches of necrotic tissue, edema and heavy infiltration of mononuclear cells. Tissue changes in liver are closely associated with kidney. The toxicants come to liver for detoxification, degradation and storage and if transformed they are excreted through bile and urine (kidney). Severely necrosised renal tubules with infiltration of mononuclear cells were observed in Labeo rohita upon endosulfan exposure (Das, 1997). Das and Mukherjee (2000) observed alterations in the kidney tubules upon HCH exposure where as glomeruli were spared.

Liver

Liver is the primary organ for detoxification of organic xenobiotics. H and E sections of liver of control Labeo rohita showed normal architecture with hepatocytes
arranged in irregular cords. The heptatic cells were large and polygonal in shape with almost centrally placed nucleus. The cytoplasm of hepatocytes stained homogeneously and sinusoidal spaces were narrow and of normal appearance (plate 5).

Sub-lethal doses of chloropyriphos and its metabolites produced dysfunction in liver of Labeo rohita. Histopathological structure of liver of Labeo rohita exposed to sub-lethal concentration of chloropyriphos revealed markedly swollen hepatocytes and exhibited hydropic degeneration with vacuolation in the cytoplasm. A good number of nuclei exhibited necrocised condensation of chromatin (Plate 6). Liver of Labeo rohita exposed to trichloropyridinol showed prominent cartilaginous cells due to severe necrosis. Left over cartilaginous tissue was visible in the primary lamella (plate 11). In fish exposed to diethyl thiophosphoric acid, the gill was severely damaged and gill lamellae were fused due to necrosis of epithelial cells. Well marked swelling and hyperplasia in gill filaments were also noticed (Plate 12). Histopathological alterations of gill due to aquatic stressors are an important diagnostic tool for aquatic toxicological studies as it comes in direct contact with the toxicant. Jain and Sahai (1999) observed severe necrosis in the gill filament and degenerated gill structure and inter-lamellar region upon malathion exposure. Mild to moderate congestion of the primary lamellae and hyperplasia of branchial plate upon HCH exposure was reported by Das and Mukherjee (2000) who commented that the changes were indicative of diminished oxygen supply to the test fish, resulting in hypoxic respiratory response. The damage of the gill tissue detected by histopathological examination was due to toxic effect of the pollutant leading to hypoxia and respiratory failure.

Gill

Gills are considered to be most appropriate indicators of water pollution (Alazemi et al., 1996) and hence all the pollution studies in fish, gill is an organ of primary importance to access the quantum of damage. H & E sections of gill of control Labeo rohita showed extended and evenly spaced gill filaments. Gill arch appeared normal and both efferent and afferent filament arteries were intact and contained free blood cells within. Gill lamellae were short and slightly curved and extended from apex to little before the trailing end of each filament (Plate 9).

In gill of Labeo rohita exposed to chloropyriphos, branchial arch was inflamed due to accumulation of inflammatory cells. There was complete loss of secondary lamellae leaving the thickened primary filaments alone (Plate 10). Gill sections of Labeo rohita exposed to trichloropyridinol showed prominent cartilaginous cells due to severe necrosis. Left over cartilaginous tissue was visible in the primary lamella (plate 11). In fish exposed to diethyl thiophosphoric acid, the gill was severely damaged and gill lamellae were fused due to necrosis of epithelial cells. Well marked swelling and hyperplasia in gill filaments were also noticed (Plate 12). Histopathological alterations of gill due to aquatic stressors are an important diagnostic tool for aquatic toxicological studies as it comes in direct contact with the toxicant. Jain and Sahai (1999) observed severe necrosis in the gill filament and degenerated gill structure and inter-lamellar region upon malathion exposure. Mild to moderate congestion of the primary lamellae and hyperplasia of branchial plate upon HCH exposure was reported by Das and Mukherjee (2000) who commented that the changes were indicative of diminished oxygen supply to the test fish, resulting in hypoxic respiratory response. The damage of the gill tissue detected by histopathological examination was due to toxic effect of the pollutant leading to hypoxia and respiratory failure.
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