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Histopathological Changes Induced by Malathion in the Liver of a Freshwater Catfish, *Heteropneustes fossilis* (Bloch)

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Pesticides reach aquatic systems by direct application, spray drift, aerial spraying, washing from the atmosphere by precipitation, erosion and runoff from agricultural land, by discharge of effluents from factories and in sewage (Edwards 1976). Malathion (0,0-dimethyl S-(1,2-dicarbethoxyethyl)phosphorodithioate) is an organophosphorus insecticide which is widely used all over the world. Entry of this pesticide into the aquatic ecosystem can cause severe damage to many nontarget species like fish.

The liver is a very important organ performing vital functions like detoxification, synthesis of several components of blood plasma, storage of glucose in the form of glycogen, and release of glucose. Pollutant related morphological, histological and histopathological alterations in the liver of fish have been studied by various scholars (Konar 1970; Eller 1971; Nestel and Budd 1975; Bhattacharya et al 1975; Verma et al 1975; Kendall 1977; Dubale and Shah 1979; Mandal and Kulshrestha 1980; Kulshrestha and Jauhar 1984; Ahmad and Srivastava 1985). Their studies showed that these pesticides cause severe damage to the liver cells. The present study was undertaken to evaluate the histopathological alterations caused by a sublethal dose of malathion in the liver of a common catfish, Heteropneustes fossilis, with a special emphasis on the changes in the size of the hepatocytes due to malathion exposure.

MATERIALS AND METHODS

Adult female fish, H. fossilis (average weight 20 g and average length 15 cm) were collected from a swamp near Katihar (Bihar), India. All the fish were collected simultaneously from the same source. They were treated with 2-6 mg/L $KMnO_4$ solution in order to disinfect them. Then the fish were acclimated in the laboratory for 2 wk in plexiglass aquaria containing

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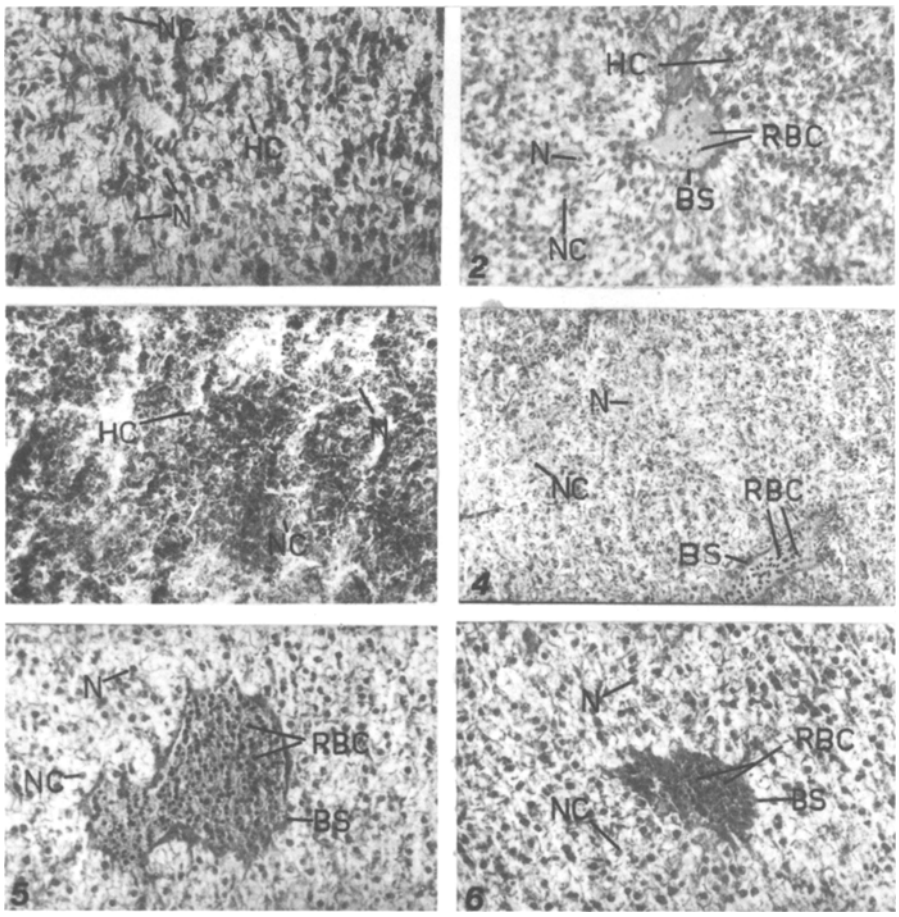

Fig. 1 T.S. of the liver of *Heteropneustes fossilis* showing hepatocytes (HC), nucleus (NC) 4 x 40. Fig. 2 T.S. of the liver of 24-hr malathion exposed *H. fossilis*. Fig. 3 T.S. of the liver of 48-hr malathion exposed *H. fossilis* showing compact hepatocytes (HC), nucleus (N), nucleolus (NC), blood sinusoids (BS) 4 x 40. Fig. 4 T.S. of the liver of 72-hr malathion exposed *H. fossilis* showing nucleus (N), nucleolus (NC) and blood sinusoids (BS) 4 x 40. Fig. 5 and Fig. 6 T.S. of the liver of 96-hr malathion exposed *H. fossilis* showing rupture of hepatocytes membrane, nucleus (N), nucleolus (NC) and blood sinusoid (BS) 4 x 40.

dechlorinated tap water (water temp. 20 $\pm$ 2^0 C). Fish were fed daily with chopped goat liver.

Replicates of five fish (wt. 20 $\pm$ 1 g) were exposed to a sublethal concentration of malathion, 50% active ingredient, 33% organic solvent, and 17% inert ingredients, North Minerals Ltd., Haryana, India (1.2 mg/L) for 24-hr, 48-hr, 72-hr, and 96-hr in different aquaria containing 200 L of water.

Whenever the exposure period was extended beyond 24-hr the test water was renewed every 24-hr. The LC_{50} value of malathion for-96 hr for this fish is 11,676 mg/L (Dutta et. al., 1992). Controls were maintained without pesticide for the same duration. The water had a temperature of 20 ± 2°C; pH 7.2 - 7.4; DO_2 7.1 -7.8 mg/L; FCO_2 3.8 mgL; alkalinity 114 mg/L as $CaCO_3$ and total hardness 90 mg/L as $CaCO_3$. The exposed and control fish were sacrificed after 24-hr, 48-hr, 72-hr, and 96-hr. Small pieces of liver were dissected out and fixed in alcoholic Bouin's fluid. Then they were dehydrated by passing through a graded series of alcohol and embedded in paraffin wax. Sections of 5μ thickness were cut and stained with hematoxylin and eosin. Detailed study of the sections was made with the help of a compound microscope, and measurements were performed using an ocular micrometer. Means and SDs were calculated for the control and different exposure duration of malathion (24-hr, 48-hr, 72-hr and 96-hr of Table 1). T-values with levels of significance were determined between the control and four different durations of malathion exposure (Table 1).

RESULTS AND DISCUSSION

The liver of H. fossilis is a large bilobed, orange colored organ having a homogenous mass of polygonal hepatic cells or hepatocytes with centrally placed nuclei and granular cytoplasm. The hepatocytes enclose the bile canaliculi which open into the hepatic ducts. These cells are supported by a fine reticular network of connective tissue (Fig. 1).

After 24-hr of exposure, the hepatic cells appeared compactly arranged with prominent nuclei and nucleolus. The average diameter of the hepatic cells decreased to 0.26 μ compared with control (0.33 μ) cells (Fig. 2, Table 1). The decrease in cell diameter is highly significant.

After 48-hr exposure, the shrinkage of the hepatic cells continued. The average diameter of these cells decreased (0.22 μ) compared to 24-hr (0.26 μ) exposed cells (Fig. 3, Table 1). The decrease in the diameter was due to the shrinkage of the cell. There was some degeneration of the cell membrane, vacuolation in the cytoplasm, and the nuclei became pyknotic and eccentric.

After 72-hr of exposure vacuoles were seen in the cytoplasm and the nuclei continued to be pyknotic and eccentric. As a result, the cytoplasm was moved to the inner surface of the cell membrane. The average diameter of the hepatic cell was 0.24 μ, indicating that the 72 hr exposure was the beginning of the increase in hepatocyte's diameter. The difference in cell size compared with control ones was highly significant (Table 1). Disintegration of some of the cell membranes continued at this stage. After

Table 1. Average diameter of hepatocytes of control and malathion exposed fish (in μm). T-tests: Control vs. Four Different Durations of Malathion (1.2 mg/L) Exposures. Number of Valid Observations = 50

Variable	Mean	Std. Dev.	T-Value	2-Tail Prob.
Control	.33	.04		
24-hr	.26	.05	7.66	.00
48-hr	.22	.04	12.39	.00
72-hr	.24	.04	10.11	.00
96-hr	.33	.05	.24	.81

96-hr exposure, the cellular organization was damaged to a greater extent. The hepatocytes lost their typical polygonal shape with nuclei continued to be displaced to the periphery. The average diameter of the hepatic cell was 0.33 μ. The cell membrane showed ruptures and fusion between two or more cells occurred, exhibiting binucleate or multinucleate cells at several areas. Some cells became necrotic and complete extrusion of nuclei was noticed (Fig. 5 and 6). Hemorrhage and widening of blood sinusoids were also observed. Compared with the normal cells, no significant difference was recorded in the hepatic cell diameter at 96-hr exposure. The hepatic cells became extensively dilated and vacuolated after 96-hr exposure. Similar observations have also been reported by Mathur (1965), Bhattacharya et. al. (1975), Walsh and Ribelin (1975), Sastry and Sharma (1979), Matthiessen and Roberts (1982), Srivastava & Srivastava (1984), Kulshrestha and Jauhar (1984) in liver cells of fish exposed to different pesticides. However, none of them measured the size of the liver cells or gave a statistical evaluation. Results from t-test indicated a high level of significance between the control and 24-hr, 48-hr and 72-hr of exposed ones. But it was not significant at 96-hr of exposure. Although there was an extensive cellular damage at 96-hr of exposure, the mean diameter (.33 μ) of the hepatocytes returned to the normal size.

The histopathological alterations resulting from an exposure to malathion may lead to a reduction in the functional efficiency of the liver, leading to malfunctioning of several organ systems of the fish. This in turn may cause the death of the fish which will eventually lead to a change in the population structure.

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