



## Effect of Sublethal Doses of DDT on the Liver of *Apocryptes bato* (Bleeker, 1874)

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### ABSTRACT

Toxic effects of organochlorine pesticide, DDT on the liver of *Apocryptes bato* have been studied under chronic (sub-lethal) exposure in the laboratory. The major changes in the liver cell due to sub-lethal exposure of DDT were cytoplasmic vacuolation and degeneration of reticular tissue with scattered distribution of parenchymatous cells. The cells were severely damaged at the sites of heavy vascularisation.

**Keywords:** sub-lethal, cytoplasmic vacuolation, parenchymatous cells, vascularization.

### 1. INTRODUCTION

Widespread use of pesticides in recent times in agricultural lands and public health operations causes alarming degree of pollution in aquatic environment. Insect control measures adopted with insecticides frequently non selective and most often lead to heavy mortality of fishes and game birds [1]. Through food chain, pesticides may be accumulated in human bodies causing various diseases like heart attack, paralysis, kidney failure, brain damage, cancer and so on.

Information on the toxicity of pesticides in general is abundant but not seems to be known of the histopathological damage caused to the liver cell exposed to DDT. Several observations, however, on the toxicity of insecticides on fish are on record [2-7].

It is reported that DDT levels of 1 ng/L in Lake Michigan were sufficient to affect the hatching of coho salmon eggs [8]. DDT may be moderately toxic to some amphibian species and larval stages are probably more susceptible than adults [9]. In addition to acute toxic effects, DDT may bioaccumulate significantly in fish and other aquatic species,

leading to long-term exposure. This occurs mainly through uptake from sediment and water into aquatic flora and fauna, and also fish. Fish uptake of DDT from the water will be size-dependent with smaller fish taking up relatively more than larger fish. A half-time for elimination of DDT from rainbow trout was estimated to be 160 days [10]. The reported bioconcentration factor for DDT is 1,000 to 1,000,000 in various aquatic species [11].

Many pesticides are useful in controlling insects or pests but their effects on non-target organisms of aquatic habitat are becoming more hazardous. Among them, organochlorine pesticides are very harmful, persistent and transmit from generation to generation. The DDT, among the organochlorine pesticides, is the most harmful and is mostly insoluble in water. It is reported that it takes 1000 tons of water to dissolve few grams of commercial DDT. At high concentration, DDT effects range from mortality to retardation of growth, impairment of reproduction to fish and invertebrates, increase in fish thyroid activity and reduction of natural compensatory

reaction to stress and diseases. Use of 2-5 ppm DDT per gram body weight to shrimp, crab and estuarine fish in their food causes 30-100% mortality within 2-10 weeks [15].

*Apocryptes bato* is one of the common estuarine as well as the coastal fish species found in the south eastern coast of Bangladesh throughout the year, although it becomes abundant during the dry season between November and March. *A. bato* is mostly available in dry season in the culture farms located at Anwara, Banskhal, Chakaria, Kutubdia, Moheskhal, Cox's Bazar, Ukiya, Teknaf etc, and some parts of the surrounding of those areas. The rate of catch of the selected species is very high during full moon and new moon while the farmers exchange the water of the farm for several times.

*Apocryptes bato* was selected for this study because of its sturdy characteristics. Moreover toxicity of organochlorine compounds on coastal fish species, *A. bato* has not yet been studied though it is of prime importance to explore their effects on coastal biota for the better understanding of environmental threats resulting from them. Therefore, the present study was carried out keeping the view in mind. The study took place at the Institute of Marine Sciences, University of Chittagong from December 1999 to December 2000 to examine chronic and acute toxicities of organophosphorus and organochlorine pesticides on *A. bato*. This paper presents the findings of chronic effects from exposure to the highest concentration of DDT.

## 2. MATERIALS AND METHODS

Three bioassay tests were conducted in order to determine the effect of organochlorine pesticide, DDT on the tissues of the fish *A. bato*. The specimens (length  $16.5 \pm 0.63$  cm and weight  $15.92 \pm 0.97$  g) were exposed to sub-lethal concentrations: 0.32 mg/l, 0.64 mg/l, 1.28 mg/l, 2.56 mg/l and 5.12 mg/l at five aquaria for 20 days. Each aquarium was filled with 40 liters of saline water. The recorded water parameters during the experiment were salinity 5 ppt, temperature  $26.8 \pm 1.5^\circ\text{C}$ , pH

$8.34 \pm 0.05$  and dissolved oxygen  $4.12 \pm 0.34$  ml/l. About 80 fishes were collected from local markets of Chittagong city and sorted out 60 healthy specimens for the experiment. In each aquarium 10 fishes were released for 24 hours acclimatization without food supply. Time to time bottom of each aquarium was cleaned off. Siphoning mechanism was applied for the renewal of water. To avoid bacterial and fungal infestation test solution was changed partially in every alternate day. Out of six aquaria, one aquarium (controlled) was not provided this pesticide but all other conditions remained same as that of the other aquaria.

At the end of the experiment abnormal fishes mostly affected by the highest concentration (5.12 mg) were collected and preserved in deep freeze for histopathological study to observe the effects of DDT on the cellular level of liver. The specimens in control group were also preserved at the same time for comparative study.

After 20 days of exposure the surviving fish from each aquarium (exposed & control) were removed and thereafter sacrificed.

For histopathological studies, the whole portion of the liver of the treated and controlled fish was removed, washed in normal saline water and subsequently fixed in Bouin's fluid for 12 to 24 hours.

Transverse paraffin sections at 4 to  $5\mu$  were made and stained with delafield's hematoxyline and eosin and permanent slide was made in order to find out the effect of DDT in the liver cell of *A. bato*.

## 3. RESULTS AND DISCUSSION

Fishes uptake various types of toxicants from the water. They extract oxygen from the medium by passing enormous volume of water over their gills. Water that is transported actively (marine fish) or passively (freshwater fish) into the body (because of the difference in the osmotic concentration between the external and internal environment) may also contribute to the uptake of toxicants from water during life time of a fish. The process

of uptake of toxicant either through food or from water, is influenced by several factors such as chemistry of the molecule, physical conditions of the medium and the fish itself- its lipid content, size and stage of development, physiological activity. However, DDT, because of its great affinity to lipid material, is taken up quickly from food and water, metabolized slowly and stored for extended period. DDT has more chronic effect rather than acute effect. Chronic effect differs from acute effect in the mechanism of its manifestation. It is due to the accumulative effect of the toxicant in different organs. Chronic poisoning of fish may proceed without visible symptoms.

No mortality was observed at different concentrations (0.32mg/l, 0.64mg/l, 1.28mg/l, 2.56mg/l and 5.12mg/l) during chronic exposure to DDT since the applied concentrations only represent sub-lethal/chronic doses for the experimental fish.

In the present study, changes of behavioural pattern at different concentrations and liver cell of *A. bato* at the highest concentration (5.12mg/l) was investigated.

Changes in behavioural pattern of fishes were observed at the end of 20 days exposure to DDT. At low concentrations of DDT (0.32mg/l and 0.64mg/l) lethargic movements were observed in some fishes, where at medium concentrations (1.28mg/l and 2.56mg/l) the fishes showed gulping of air, sluggishness and irregular swimming and at high concentration (5.12mg/l), fishes showed various agonistic behaviours (chasing, vacating and nipping) as well as comfort behaviours (flicks, thrusts and coughs). At the end of the experiment, most of the fishes were unable to maintain their position accompanied by partial extension of fins. Fin rays of some fishes became straight and seem to be paralyzed.

During chronic exposure to DDT, it was observed that the long term physiological disorder revealed as the loss of equilibrium and thus the test fishes were unable to maintain their position and to continue

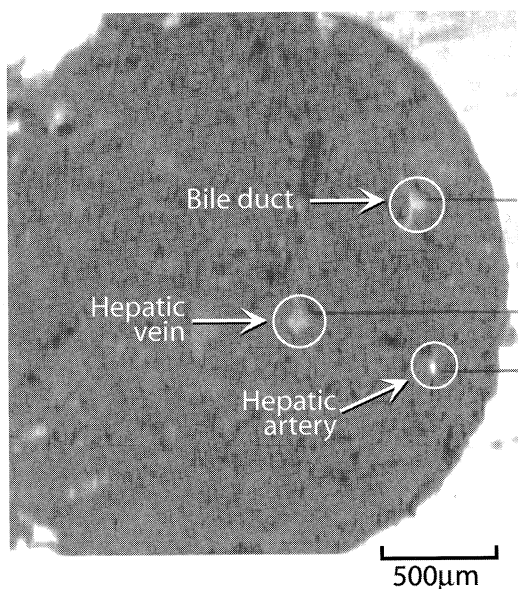
locomotion in the aquaria. The imbalance condition of fishes was observed remarkably on the exposure to the highest concentration.

The liver of *A. bato* consists of large number of hepatic cells. Each hepatic cell contains a prominent nucleus and granular cytoplasm which indicates its secretory nature. Portal veins, hepatic arteries, hepatic veins and bile ducts are spread throughout the liver. Arrangement of hepatic cells does not maintain a regular pattern. The connective tissue raticulum spread throughout the hepatic materials. Irregular interconnecting sheets or plates of hepatic cells radiate outward from the central vein and constitute the parenchyma. The parenchymatous cells are separated by blood sinusoids. Figure-1 shows a healthy liver section of *A. bato*.

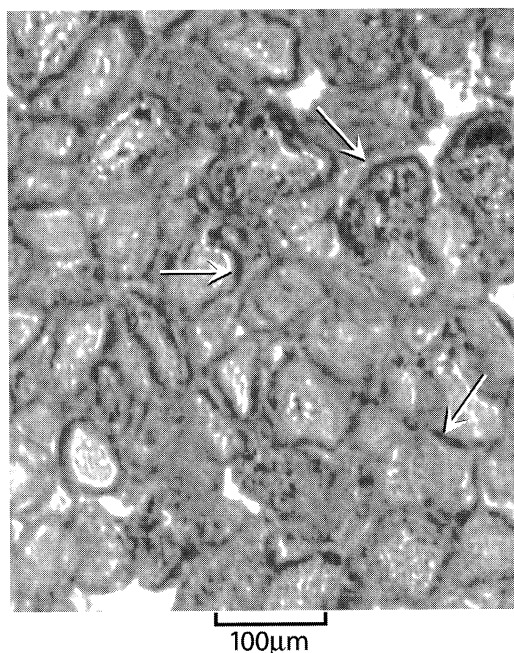
In the present study the toxic effect at sub-lethal concentrations of DDT on the liver of *A. bato* was investigated. Cytoplasmic vacuolation (Figure-2) and granular cytoplasm were detected in the DDT treated liver. Scattered distribution of parenchymatous cells (Figure-3) and black spots (Figure-4) were observed in the liver tissue. The cells were severely damaged at the sites of heavy vascularisation. Degeneration of raticular tissue and blood vessel (Figure-5) was noticed in the DDT treated liver. Swollen hepatic cells (Figure-6) were also found. Sastry *et al.* [12] found swollen hepatic cells, liver chord disarray, necrosis and vacuolation of cytoplasm in the liver of endrin treated *Channa punctata*. Anees [2] stated that the sublethal concentrations of diazinon, methyl parathion and dimethoate produced histopathological changes in the liver. Moitra *et al.* [13] found severely damaging condition at the sites of heavy vascularisation, swollen hepatic cells, vacuolation, surface necrosis in the liver of phosamidon treated *C. striatus*. Research findings of Areechon [14] revealed that the Channel catfish exposed to 4.5mg/l malathion for 96 hours showed pathological changes with hematological changes. The histopathological changes were necrosis and separation of the epithelial cells in the gill. In

addition, liver developed vacuolation and necrosis. In the present study more or less similar signs were observed as stated by the above mentioned authors. Histopathological signs observed in the present research would

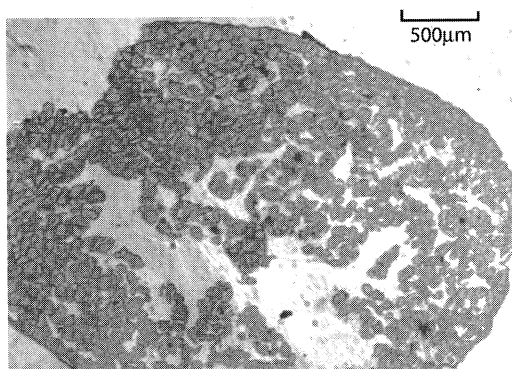
be the resultant of the affinity of DDT to the fatty parts of the liver. The air breathing test fish may accumulate DDT through gill exposure.



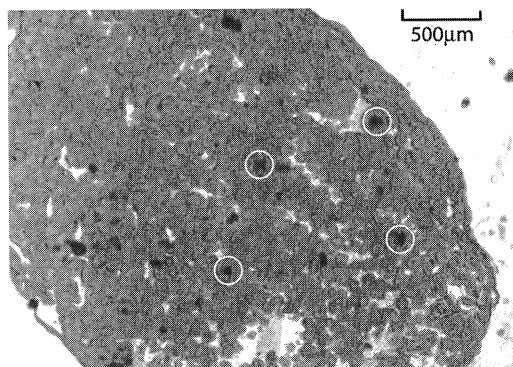
**Figure 1.** Photomicrograph of transverse section (T. S) of liver of *Apocryptes bato* (control) showing Hepatic Vein, Hepatic Artery and Bile Duct (10x10).



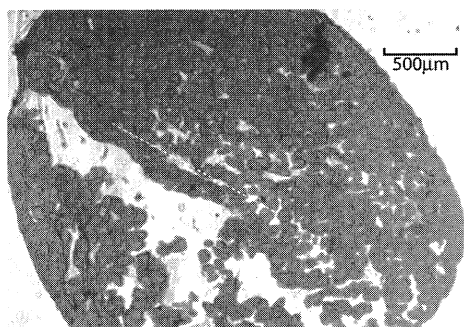
**Figure 2.** Photomicrograph of transverse section (T. S) of DDT treated liver of *Apocryptes bato* showing cytoplasmic vacuolation, damaging condition at the sites of heavy vascularization (10x40).



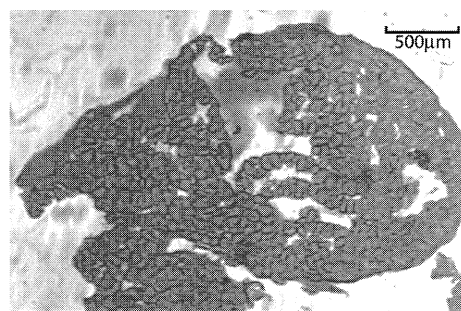
**Figure 3.** Photomicrograph of transverse section (T. S) of DDT treated liver of *Apocryptes bato* showing scattered distribution of parenchymatous cells (10 x 10).



**Figure 4.** Photomicrograph of transverse section (T. S) of DDT treated liver of *Apocryptes bato* showing black spots in the liver tissue (10x10).



**Figure 5.** Photomicrograph of transverse section (T. S) of DDT treated liver of *Apocryptes bato* showing de-generation of reticular tissue (10x10).



**Figure 6.** Photomicrograph of transverse section (T. S) of DDT treated liver of *Apocryptes bato* showing swollen hepatic cell (10x10).

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