CHAPTER VI

TOXICOLOGICAL IMPACT OF THE
SUBLETHAL MALATHION EXPOSURE
ON THE VITAL ORGANS OF
MONOPTERUS CUCHIA (HAM-BUCH)
6.1 INTRODUCTION:

Air-breathing fishes in India form an economically important group of fishes. The availability of major (protein, fat and carbohydrates) and minor (vitamins, minerals, etc.) nutrients in fish make them a source of high nutritional value. Despite this, they are being continuously exposed to ever increasing number of contaminants, which adversely alter their health, hygiene, growth, maturity and metabolic pool (Jha, 1999). Malathion, an organophosphorus insecticide, has assumed the status of one of the major freshwater contaminants causing adverse effects on aquatic flora and fauna, including fishes (Mukherjee et al., 1992; Maruthanarayam et al., 1994).

Toxicological studies on fish are reviewed in several reports (Laws, 1993; Roesijadi and Robinson, 1994; Ray, 1994). These include metal toxicity through bioaccumulation of metals from aquatic environment, pesticides and pollutants in industrial affluent entering water bodies. Bhattacharya (2000) has suggested exnobiotic route of cadmium to fish. The impact of these chemicals on aquatic fauna, particularly the fish fauna has currently become a subject of concern. A range of pesticides in different concentrations can produce chronic histopathological and biological disorders in fish, leading to mass scale mortality. In particular those fishes inhabiting the natural habitat like paddy fields, swamps and canals linked to paddy field are severely affected as they get direct exposure to such chemicals.

A review of relevant literature has revealed that much research has been undertaken with respect to the impact of pesticides on
fish fauna. Hamilton and Mehrle (1986) have reported that cadmium has high potential of toxicity mainly in liver and kidney. Olsson et al. (1989) and Olsson and Kille (1997) have studied cadmium sensitivity in fish. Work performed in this direction is chiefly confined to bioassays (Lal et al., 1983), physicochemical characteristics of test water (Panigrahi and Konar, 1990) and growth and maturity of fish (Chattapadhyay and Konar, 1984). Only a few (Raju et al., 19; Konar, 1986), reports has been documented concerning the impact of pesticides on the internal organs or histology of fish.

The present study has been conducted on the impact of sublethal concentration of one of the most commonly used pesticides on the impact of sublethal concentration of malathion on histology of some vital organs of fish, viz., liver, stomach, intestine, kidney, skin and gonads. The test fish taken in the study is Mud-eel, *M. cuchia* inhabiting naturally the wetlands, swamps and paddy field of the state and this study was performed which attempts to diagnose the pathological lesions appearing in vital tissues of the body of *M. cuchia*.

6.2 OBJECTIVES :

1. To assess the effect of sublethal malathion exposure on the histology of the vital organs of *M. cuchia*.

2. To determine whether the sublethal concentration of the pesticide malathion 50EC is safe for application in respect to *M. cuchia*.

6.3 MATERIALS AND METHODS

*M. cuchia* of average length (60-68 cm) and weight (180-250 gm), procured from local wetlands of Bahona area, Jorhat, Assam were used during this study as the test animal. The fishes were acclimatized to the controlled condition for 7 days prior to treatment in the glass aquarium.
The concentration of malathion 50EC is kept at sublethal level (0.006 ml/l) determined on the basis of bioassay trial, conducted with different concentrations viz. 0.004 ml, 0.005 ml, 0.007 ml and 0.008 ml per litre. The test fishes (total no. 10) were kept in an aquarium with 10 liters water treated with 0.006 ml malathion 50EC/l water. A control set with equal numbers of fish maintained without treatment for comparison.

At the end of the exposure period (day 15), fishes of both groups were separately weighed and dissected in the laboratory. The desired tissues (stomach, intestine, liver, kidney, skin and gonads) were quickly taken out; the organs were weighed and fixed in aqueous Bowin's solution. After proper washing, dehydration and cleaning, tissues were embedded in paraffin where the serial section of 7 μm were cut and stained with haemotoxylin/eosin. The selected slides were subjected to routine histological examination.

6.4 RESULTS AND DISCUSSION

Various histopathological disorders were observed in the gut (digestive tissues) and skin, kidney and gonads etc...

The gut is the main entry route for toxic agents in teleost fish, and this makes it an important target fir such chemicals. The gut is also an important organ for the regulation of water and ions in fish, and this is the main reason why many toxic agents, either water-borne or present in the food, are known for disturbing the hydromineral regulation in fish.
6.5 STOMACH:

From the histopathological observations, it is evident that malathion induces remarkable pathological changes in the stomach of *M. cuchia*.

Plate VII shows the structure of the normal stomach and photo VIII. 2 depicts the changes appearing in the stomach after being exposed to pesticides. The stomach of treated *M. cuchia* became highly atrophied, deshaped, crumpled and shrunken. The atrophy was more pronounced in mucosa. The epithelium of the mucosal fold was almost completely damaged and disorganized. At the junction of muscularis and submucosa, the blood vessels were severely dilated. The columnar epithelial cells and mucous goblet cells were necrosed and liquidated leading to the accumulation of oedematous fluid within the tissue spaces. These pathological changes point towards hypertension caused by the malathion and may also, therefore, be a reason behind the loss of the usual shape of the stomach. The damage of the epithelium of the mucosal fold and atrophy of mucosa may be associated with hypersecretion of acids by stomach under toxic influence of malathion. Acid, so secreted, might have destroyed the surface epithelium of mucosa, leading to its erosion. As regards the disorganization of the columnar epithelial cells and liquefactive, necrotic and edematous changes in epithelial and mucous goblet cells, these appear to be governed by Starling’s law (Jha and Pandey, 1989), which states that hydrostatic pressure in vascular system moves fluid out of the system.

6.6 INTESTINE:

The intestine is one of the main targets of dietary and waterborne toxic agents, and given the important osmoregulatory function if the intestine, it directly affects the regulation of water and ion...
balance. The role of the intestine in hydromineral regulation is reflected by the structure of the epithelial cells.

As is evident from the comparison of Plate IX (Control intestine) with Plate X (Treated fish intestine), the intestine revealed severe atrophic degeneration. The mucosa lost its usual shape and normal architectural plan. Villi revealed altered orientation and were disorganized, greatly reduced, flattened and inflamed at the base. Their tips were found ruptured at places leading to the exudation of mucus in lumen. The nuclei were disintegrating and occupied the apical position contrary to the basal one in control. Lamina propria became reduced and the cells were autolysed leaving clear spaces suggesting oedema. Further, the connection of the mucosal cells with the underlying connective tissue was severed due to which mucosa hung freely in the lumen. The dominating cell type, the enterocyte, exhibits specially the appearance of cells involved in nutrient reabsorption, such as a well-developed brush border and a well-developed lysosomal system, in addition to smooth and granular endoplasmic reticulum and an occasional perioxisome. Along the entire length of the intestinal tract, however, particularly in its more rectal parts, the basal parts of these cells show the typical characteristics of cells involved in the transepithelial transport of water and ions, a basal labyrinth of folded membranous forming it – like lamina communicating by pores with the extracellular space at the serosa side of the epithelium, with many elongated mitochondria between these folds.

Moitra and Lal (1989) reported vacuolation of columnar epithelial cells, extremely reduced villi and exudation of mucus in lumen in the intestine of malathion and BHC treated fish *Puntius sarana*. Similar observations have also been made by Tembhre and Kumar (1994) under diemethoate exposure in *Cyprinus carpio* and by Anithakumari and Ram Kumar (1997) in *Channa punctatus* under influence of aquatic
pollutants. Rupture of tips of villi observed in the present case may be regarded as a mode of detoxification to get rid of the detergent as suggested by Luckey and Venugopal (1977). Other cellular injuries including oedema may be associated with the release of acid hydrolases from lysosomes leading to the autolysis of cell. Damages inflicted in mucosa, more especially the villi, are of special concern since the villus is the working unit of the intestine and it is on the villi that the inner ends of the absorptive cells come in contact with the blood and lymph which pick up the absorbed nutrients to carry them to other parts of the body.

The function of villi being so significant and the damages at this site in the present case being severe, the extent of disturbances in the intestinal physiology, including absorption, induced by pesticide, can easily be ascertained.

6.7 LIVER:

A range of contaminants including carcinogens, metals, biotoxins and persistent organic pollutants injure the livers of fishes, although some mechanisms of liver injury are unique to fish. Interest in the investigation of liver toxicity and injury in fishes stem from several motivations, including developing an understanding of vertebrate comparative physiology and anatomy: addressing problems in aquaculture associated with their pathologic conditions caused by nutrition-related factors or improper storage of dietary components, analysis of the pathogenesis of liver neoplasm in selected fish species and more recently, development of biomarkers of exposure and effect and use of toxic alterations/responses in risk effects assessment. The latter interest stems from the fact that the aquatic medium is a sink for many anthropogenic contaminants (Long and Buchman, 1990; Muckay, 1992a,
b; Tanabe et al., 2004). The time is now ripe for a critical assessment of the hepatic toxicology of fishes.

In part, the structure and function of the normal liver in fishes make it a target organ for toxic chemicals. Due to the dual blood supply of the liver; it is important for us to consider two principal routes of uptake that may lead to hepatic distribution of potentially toxic compounds. These are the intestinal uptake and the bronchial routes.

Fundamental aspects of fish liver anatomy, especially those important in the interpretation of toxicity, were recently reviewed (Hinton and Couch, 1998; Hinton et al., 2001). Despite of many structural similarities between the livers of fishes and those of mammals, certain features of hepatic gross and microscopic anatomy of fishes are recognized as different (Hampton et al., 1985, 1988, 1989).

The structural similarities and differences represent a comparative liver anatomy; rather, they are important in the interpretation of toxic responses within livers of fishes. Architectural and physiological differences in fish vs. rodent livers apparently lead to different histological patterns of toxicity.

Because of its central location in the circulatory system of vertebrates, the liver is a target for many toxicants, and it receives toxic substances both from intestinal and branchial routes.

Remarkable changes appeared in liver cells under the influence of malathion (Plate XII). Plate XI shows the status of the liver in control medium. The effect was a greater magnitude in the perilobular zone than in the central zone. The parenchymatous nature of liver was lacking and as a result the hepatic cords were disorganized or disarrayed.

The cell membrane were either dissolved, autolysed or disintegrating allowing the dead, darkened and necrosed nuclei to clump
together. There was almost complete vacuolar degeneration of cytoplasm and hepatocytic oedema was clear. Besides, splitting of hepatocytes, moderate dilation of sinusoidal spaces, hyperemia and liquefactive changes were also obvious. Findings akin to present one were earlier observed by Anithakumari and Ram Kumar (1995) in the liver of *Channa striatus* treated with aquatic pollutants, which have been attributed to the presence of toxic substances in the incoming blood. Goel and Garg (1980) correlated such changes with the disturbed enzyme pattern of the organ, suggesting that most of the enzymes, particularly the oxidative ones, decrease with the exposure period and lead to necrotic changes in tissues. Raju *et al.* (1994) based on their observations on oxidative enzymes under the influence of the detergent (Ariel powder), suggested tendency of the fish to shift from aerobic to anaerobic pathways.

The parenchymal disorganization, necrosis of hepatocytes, oedema, etc. observed in this study reflects exhaustion of liver cells and may be attributed to hypoxic conditions created by the detergent. Hypoxia lead to the release of lysosomal enzymes, causing focal to generalized necrosis resulting from weaker oxygenation of cells across hepatic lobules (Jha *et al.*, 1994). Further, vacuolar degeneration may be associated with the distortion and fragmentation of cellular organelles and the observed autolysis of cell-membrane depicts the point of irreversibility reached in hepatocytes.

The liver should therefore be regarded as an index for pollution studies as it is the active centre for all biochemical processes and a major site for synthesis, storage and turnover of the metabolites. It is affected more than any other tissue because it is the metabolic centre for detoxification and also the host in absorbing greater OP pesticide residues.
6.8 KIDNEY:

The multifunctional role of the fish kidney, which combines the functions of lungs, kidneys and intestines of terrestrial vertebrates, is reflected by its complicated structure involving many different cell types and an intricate vascular system.

Toxicological studies have revealed extensive histopathological alterations in the kidney as a result of the exposure of fish to heavy metals and organic chemicals. In general, the structural damage to the kidneys seems to show little toxicant specificity. The proximal tubules appear to be the first affected, but damage progresses to the posterior segments and glomeruli, following exposure of the fish for a longer period or to higher concentrations of the toxicant. Histopathological effects vary from slight disruption of the brush borders of the proximal tubular cells, increased numbers of phagosomes and lysosomes and structurally abnormal mitochondria to rupture of cellular membranes, swelling and vacuolization, increased cell death by necrosis and apoptosis and large lesions in the tubular epithelia (Gill et al., 1989; Oulmi and Braunbeck, 1996; Sovenyi and Szakolczay, 1993). Cadmium is well known for its preference for renal tissue as a site of accumulation, the highest concentrations of this metal, after water as well as dietary exposure, are usually found in the kidneys (Bentley, 1991; McCoy et al., 1995; Thomann et al., 1997). As a consequence, the cadmium is able to produce severe histopathological alterations (Gill et al., 1989; Ooi and Law, 1989; Oronsaye, 1989; Sovenyi and Szakolczoy, 1993). Although copper has no specific affinity for the kidneys, it can also produce significant structural changes to both glomeruli and renal tubules (Bakshi, 1991). Among the many organic chemicals producing similar effects are paraquat (Molek and Fries, 1997), 4-chloroaniline (Oulmi and
Braunbeck, 1996), TCDD (Henry et al., 1997), cyclosporine (Terrero and Coombs, 1989) and atrazine (Fischer-Scherl et al., 1991).

In Plate XIII (Treated) the toxic effect was accumulated chiefly in kidney.

In the treated condition, the capillaries of Bowman’s capsule were disorganized. As a result, the nephrons were not so active in function, because, from the viewpoint of the functional test, toxicants do not affect all organs to the same extent. Presumably, for some reasons, the kidney is more sensitive than the liver (Mazze and Lousins, 1973).

Nephrotoxins can exert adverse effects on various parts of the kidney, resulting in alterations of different functions.

The toxicants can affect the kidneys of the fish in many ways. Shrinkage of the glomerular tuft, reduction in the diameter of the kidney tubules, vacuolation of the cells of the tubules, necrosis and rupturing of glomerular wall and tubules are also on record.

6.9 SKIN:

The skin represents an important route for uptake, particularly for the early life stages of fish. From the skin, various return to the sinus venosus, passage through the heart, and return through the branchial circulation (to a varying degree dependent on development) would be necessary before the compounds reach the liver. The skin of fishes accumulates a greater amount of contaminants, due to its estimate contact with the environment and its importance as an effector of ionic and osmotic regulation.

In Plate XV (treated), the toxic effect was accumulated chiefly in skin than in the other tissues. The epidermis and dense dermis layers were irritated severely by the action of the pesticide, so the blood capillaries and the chromatophase were disorganized and disarrayed.
Plate VII: Stomach (N)
Columnar epithelial cells
Disorganized circular muscle fiber
Submucosa
Circumferential muscle fiber
Columnar epithelial cells

Plate VIII: Stomach (Treated)

Plate IX: Intestine (N)
Lumen
Lamina propria
Muscle
Blood vessels
Granular cells
Columnar epithelium

Plate X: Intestine (Treated)
Reduced muscle & submucosa
Disorganized cells
Dead cells

Plate X: Liver (N)
Exocrine cells of pancreas
Blood capillaries
Pancreas

Plate XI: Liver (Treated)
Autoimmune cell membrane with heaped nuclei clumped together
Disarrayed hepatic cords
Plate XII: Kidney (N)
Plate XIII: Kidney (Treated)
Plate XIV: Skin (N)
Plate XV: Skin (Treated)
Damages were inflicted in the connective tissues of the skin. Mucous cells were disturbed as a result; mucous secretions were not so significant. No Oedema was visible. Very slight erythema occurred after exposing it to the pesticides solution.

6.10 CONCLUSION:

The findings indicate that malathion 50EC, causes harmful effects on the non-target organisms like Mud eel at a very low concentration, leading to severe biological dysfunction. The Mud eel is more prone to such an impact as the fish inhabits the natural habitats that have greater chances of exposure to the OP pesticide. This is certainly one of the reasons for the drastic reduction of the population of *M cuchia* in its natural habitat. The findings of the study will help in determining the safe harmless level of the pesticide. In the case of *M. cuchia*, although the concentration 0.006 ml/l does not cause instant mortality to the test fish, yet prolonged exposure will lead to mortality at this level. Hence it's not considered safe for use.