CHAPTER IV:

HISTOPATHOLOGY OF GILLS AND HEPATOPANCREAS
INTRODUCTION

Although pesticides have been used for many years, our concern for their environmental effects is of recent origin. There are mainly two reasons for this, first the number of pesticides used was rather limited and, second the amount of pesticide used was not great prior to 1945. The new pesticides developed during the last four decades present an entirely different picture. Not only has the number of compounds increased drastically, but also spectrum for their use has become almost unlimited.

Most of the pesticides from agricultural fields find their entry into riverine system affecting the ecosystem of freshwater. The fauna inhabiting freshwater barely could escape and hence the effect is remarkable. Among the fauna, fishes and crustaceans had always received attention since both are economically important.

Gills and hepatopancreas in crustaceans are regarded as important organs because of the number of functions they perform. Hepatopancreas which is comparable to liver of vertebrates is also an
important gland which is responsible for maintaining normal physiological activities in Crustacea, whereas gills are respiratory as well as osmoregulatory in function. As the gills always come in contact with external media, are expected to be exposed to pollutants. Most of the pesticides will have one or the other type of effect on the inhabiting organism with variation in degree, the toxicant causes to the animal body i.e. on tissues. These effects are known as pathological effects and histological study of pathological tissues gives clue to evaluate the toxicity of a particular chemical on the target organisms.

The assimilation of pesticides within the organisms at lethal and sublethal levels induce a sequence of biological effects. These ranges from molecular interferences with cellular organelles through pathological changes at the tissue and organ level. (Connell and Miller, 1984).

Aiken and Byard (1969) observed histological changes in the gills of Lobster, Homarus americanus after exposure to yellow phosphorous. Vernberg et al. (1974) noticed cellular gill damage in fiddler crab,
_Uca pugilator_ exposed to mercury. Nimmo et al. (1977) reported damage to the gills after exposure to cadmium in _Penaeus duorarum_, _Palaemonetes pugio_ and _P. vulgaris_. Couch (1977) studied the effect of cadmium on marine shrimp. Ghate and Mulherker (1979) observed changes in the gill structure of two freshwater prawns, _Macrobrachium kistnensis_ and _Caridina_ species exposed to copper sulphate. Bodkhe (1983) revealed the changes in the gill structure of _Barotula phusa cumulicaris_ exposed to sevimal. Breakage in the gill lamellae and gill sacs of _Scylla serrata_ was observed by Rao (1984) when exposed to pesticides. Papathorassious (1985) studied the effect of cadmium on the ultra structure of gill epithelium in brown shrimp, _Cragon crangon_. Gangshettiwar (1986) noticed the effect of phenol on the gills of _Macrobrachium laterilii_. Sarojini et al. (1986) have studied the effect of fenitrothion on the gills of _Macrobrachium laterilii_.

Recently effect of pesticides on the histopathology of gills of _Caridina weberi_ was studied by Martin (1989) using methyleparathion and Yadav (1989) with endosulphon.
The study of histopathological lesions give a clue to understand the effects of toxicants on aquatic biotypes. Any cellular damage resulting in the disorganization of hepatopancreas would have serious consequences in the physiological functioning in the body of the organisms.

In crustaceans, the hepatopancreas has the function of both liver and pancreas of vertebrates (Vonk 1960) and therefore it is involved in the secretion of digestive enzymes and absorption and storage of lipid materials (Bunt, 1968, Smith et al., 1975). Various pollutants cause structural changes in the storage and secretion kinetics.

Aiken and Byard (1969) observed changes in hepatopancreas of lobster Homarus americanus exposed to yellow phosphorous. Doughtie and RangaRao (1984) studied the changes in the hepatopancreas of grass shrimp, Paleomonetes pugio when exposed to chromium. Gangshettivir (1986) noticed histopathological changes in the hepatopancreas of Macrobrachium lamerrii exposed to phenol. Effect of naphthalene on the hepatopancreas of prawn, Macrobrachium kistnensis was revealed by Jaiswal (1986). SrinivasaReddy (1988) and
Ashokkumar (1988) have reported the changes in the hepatopancreas of *Macrobrachium lamerrii* and *Caridina vazehi* when exposed to sea anemone and sea cucumber toxins respectively.

But there is no available literature in relation to synthetic pyrethroids and histopathology of gills and hepatopancreas of crustaceans. So an attempt has been made to study the effect of Fenvalerate, a pyrethroid on the histopathological changes in the gills and hepatopancreas of the prawn, *Macrobrachium lamerrii*. 
MATERIAL AND METHODS

The freshwater prawns *Macrobrachium laevis* were procured from river Godavari at Paithan near Aurangabad (M.S.) and transported to laboratory and maintained as described in Chapter 1(A).

Mature prawns of intermoult stage were selected and exposed to LC$_{50}$ values of fenvalerate for 24, 48, 72 and 96 hours for acute studies and for chronic treatment 1/10th of LC$_{50}$ of 48 hrs. was used for histopathological studies. The water media was changed daily and measured concentration of fenvalerate was added to maintain concentration of pesticide in the exposed troughs. Simultaneously control animals were also maintained. Prawns were not fed during experiment but prawns exposed to chronic study were fed twice a week.

At the end of exposure period 24, 48, 72 and 96 hrs. for acute study and 7, 15 and 21 days for chronic exposure, the tissues, gills and hepatopancreas were dissected from the control and also from experimental prawns and fixed in Bouin’s fluid, dehydrated, cleared in xylol and embedded in paraffin wax. Serial sections were cut at 6-7 µ and stained with Harris’s haematoxyline and eosin.
OBSERVATIONS AND RESULTS

Effect of fenvalerate on gills of Macrobrachium lamarrii:

Control gills:

The gills of freshwater prawn species are phyllobranchs, with thin plate-like gill filaments arranged in two rows on a narrow axis like the pages of a book as in Palaemon (Patwardhan, 1958). Each gill plate or lamella is made up of a double layer of cuticle enclosing within it a single layer of cells or gill epithelium (Fig. 1).

Effect of lethal concentration of fenvalerate on gills:

After exposure to 24 hrs. there was not much damage to the gills, but the cuticle lining the gill epithelium showed tendency of separation with vacuolization in the gill stem (Fig. 2). After exposure to 48, 72 and 96 hrs. multiple lesions were observed in the gills. The distal portion of the gill process was congested with haemocytes. Complete separation of cuticular lining from the gill epithelium and appearance of vacuoles in the hem were observed.
Effect of fenvalerate on the gills of prawn, *M. lemerrii*

**Fig. 1** T.S. of control gill showing gill stem, gill sac and gill lamellae along with haemocytes. 
Haematoxyline eosin X 100

**Fig. 2** T.S. of gill treated with fenvalerate for 24 hrs. showing gill lamellae. 
Haematoxyline eosin X 100

**Fig. 3** T.S. of gill treated with fenvalerate for 96 hrs. showing vacuolization, in the gill stem and rupturing gill sacs. 
Haematoxyline eosin X 100.

\[\begin{align*}
C &= \text{Cuticle} \\
GL &= \text{Gill lamellae} \\
GS &= \text{Gill sac} \\
GST &= \text{Gill stem} \\
HC &= \text{Haemocytes} \\
V &= \text{Vacuoles.}
\end{align*}\]
72 hrs. brought about swelling in the gill stem, enlarged gill sac and some lamellae totally degenerated. At 96 hrs. of exposure highly damaged tissue exhibiting disintegration of gill sac and gill lamellae was noticed (Fig. 3).

Effect of sublethal concentration of fenvalerate on gills:

On chronic exposure for 7 days the gill lamellae were swollen and highly affected gill sac with detached cuticle, and vacuolization in the gill stem was noticed (Fig. 4). 15 days exposure caused extensive damage to gill processes showing degeneration of gill lamellae, gill stem and affected haemocytes. At the end of 21 days exposure severe vacuolization in the gill lamellae and shrinkage of gill epithelium was observed (Fig. 5).

Effect of fenvalerate on hepatopancreas of Macrobrachium leherrii:

Control hepatopancreas:

Hepatopancreas in its histological structure shows a number of oval shaped tubules bound by connective tissue. Each tubule is covered with a
Effect of fenvalerate on the gills of prawn, *M. lemerrii*.

**Fig. 4**: T.S. of gill treated with fenvalerate for 7 days showing swollen gill lamellae with vacuoles in the gill stem. Haematoxyline eosin x 100.

**Fig. 5**: T.S. of gill treated with fenvalerate for 21 days showing increased vacuolisation in the gill stem with degenerating gill lamellae. Haematoxyline eosin x 100.

C = Cuticle  
GL = Gill lamellae  
GS = Gill sac  
GST = Gill stem  
HC = Haemocytes  
V = Vacuoles
thin epithelial layer enclosing a central cavity, the lumen (Fig. 6). Generally three types of cells are noticed in each tubule, the first are columnar with nucleus towards the base and appear vacuolated due to the presence of fat globules and are absorptive cells. Second type of cells are larger in size, the secretory cells, and these discharge their secretion into the lumen of the tubule. The third type are smaller compared to the other two types located beneath the secretory cells and are very few in number, known as embryonic cells.

Effect of lethal concentration of fenvalerate on hepatopancreas:

After exposure to 24 hrs. the secretory cells increased whereas absorptive cells decreased in their number. The secretory cells poured their secretion into the lumen of the tubule, so the lumen was filled with fluid (Fig. 7). At 48 hrs. exposure there was disturbance in the secretory activity and it was found to be negligible. The shape of the cells was most affected and 72 hrs. exposure brought about the decrease in the number of cells which
Effect of fenvalerate on the hepatopancrease of prawn, *M. lamarrii*

Fig. 6: T.S. of control hepatopancrease showing tubules with control lumen. Haematoxyylene eosin X 100.

Fig. 7: T.S. of hepatopancrease treated with fenvalerate for 24 hrs. showing lumen filled with secretion. Haematoxyylene eosin x 100.

Fig. 8: T.S. of hepatopancrease treated with fenvalerate for 96 hrs. showing desintegration of connective tissue with damage to cells. Haematoxyylene eosin x 100.

AC = Absorptive cells
EC = Embryonic cells
EPL = Epithelial layer
L = Lumen
SC = Secretory cells
M = Nucleus
T = Tubule
undergo shrinkage and as a result the size of the lumen was increased. In case of 96 hrs. exposure (Fig. 8) loosely arranged tubules due to disintegration of connective tissue and dissolution of boundary wall of tubule was observed. The shrinkage of absorptive and secretory cells lead to the appearance of wide lumen and lobules loose their normal appearance.

Effect of sublethal concentration of fenvalerate on hepatopancreas:

After 7 days of sublethal exposure, epithelial layer covering the tubules was ruptured. The enlarged absorptive cells and secreting cells became abnormal and hepatic tubules lost their shape and enlarged lumen was seen (Fig. 9).

15 days exposure caused disintegration of tubular epithelial layer. The shrinkage of absorptive cells, vacuolization in the cytoplasm followed by degeneration and enlargement of empty lumen was noticed. At the end of 21 days exposure highly destroyed tubular structure with enlarged lumen was seen (Fig. 10). The connective tissue was degenerated as a result of which scattered tubules were seen loosing their normal shape.
Effect of fenvalerate on the hepatopancreas of prawn, *M. immerrii*.

**Fig. 9** T.S. of hepatopancreas treated with fenvalerate for 7 days showing damaged tubules with degeneration of hepatic cells. Hæmatoxyline eosin x 100.

**Fig. 10** T.S. of hepatopancreas treated with fenvalerate for 21 days showing highly destroyed tubule, and complete degeneration of connective tissue. Hæmatoxyline eosin x 100.

**AC** = Absorptive cells  
**EP.L** = Epithelial layer.  
**L** = Lumen  
**N** = Nucleus  
**T** = Tubule
DISCUSSION

The lethal and sublethal concentration of fenvalerate, a synthetic pyrethroid caused various histological disorders in the hepatopancreas and gill of freshwater prawn, Macrobrachium lamarrii.

The lethal and sublethal exposure to the concentration of fenvalerate brought about changes in the gill organization affecting the process of respiration. Increase in the hemocytes, rupturing of gill sac, separation of cuticular lining and vacuolization in the gill stem are observed.

Herbert (1962) has pointed out that the toxicity is influenced by concentration of toxicants. As the toxicants accumulate in the gills, the respiratory rate is altered. He therefore, pointed out that the variance in the toxicity is also related to the histological structure of gill, surface area, consumption tolerance of tissue and control of permeability.
Himma et al. (1977) observed necrosis, vacuolization of gill tissues and distension of gill filament in shrimps exposed to cadmium. Similar changes were also observed by Ghate and Mulherkar (1979) in the gills of two freshwater prawns Macrobrachium and Caridina after exposure to copper sulphate. In the present investigation similar changes were observed in the prawn M. lamterii exposed to fenvalerate.

Studies on Macrobrachium kistnensis by Kishore et al. (1982) suggested that the severe separation of cuticle from the gill epithelium in acute doses of simethion, might be acute inflammatory response which is basically protective response to tissue damage due to pollutant. Our results with fenvalerate to Macrobrachium lamterii showed that the cytological architecture of the gill was completely damaged so we may conclude that cellular damage due to pollutant would have serious consequences. Bodkhe (1983) demonstrated similar changes like changes in gill structure, vacuolization, shrinkage and enlargement of gill sacs in Barytelphusa cunicularia exposed to sevimol. Damage to the gills observed in the present investigations also concide with the findings of
Sarojini et al. (1986) who revealed necrosis, vacuolation and breakage in the cuticular lining of gills in *Macrobrachium lamerrii* exposed to fenitrothion. The decrease in mitochondria has been observed by Bubel (1976) in the gills of Isopod, *Jaera nordmanni* after exposure to different concentrations of copper, mercury and cadmium. He suggested that the disruption of mitochondrial membrane reduced the ability of mitochondrial to synthesize ATP and this led to an increase in permeability which might account for their swollen appearance. The energy deficiency within the cells could be associated with inhibition of protein synthesis and this is supported by the reduced number of ribosomal particle, which shows that less ribonucleic acid is presented in the affected cells.

The bursting of gill sac and shrinkage of gill lamellae may be due to the reduction in size of gill cells or due to oozing out of fluid from the bursting of gill sac. The bursting of gill sac and swelling of gill lamellae were also observed after fenvalerate exposure. Swelling of gill lamellae and gill sac may
be due to reduction in the thickness of the epithelium lining. The severity of damage varies according to concentration and duration of fenvalerate exposure. The damage to the cytological inclusions probably is responsible for the vacuolisation.

Crustacean hepatopancreas originally considered only as a digestive gland is now being suspected to function also as a centre of intermediary metabolism and as an important storage depot like insect fat body and vertebrate liver and adipose tissue.

The lethal and sublethal concentration of fenvalerate causes various histological changes in the hepatopancreas of *Macrobrachium lamarrii*. Enlargement of absorptive cells and secretory cells, vacuolization and decrease in the size of the lumen, disintegration of cell membrane and degeneration of various cells of hepatopancreas were observed. The reduction in the size of lumen may be due to enlargement of absorptive and secretory cells. These changes may inhibit the secretory activity of the cells. Different cell types with diverse
functions like absorption, storage and enzyme secretion have been reported in the hepatopancreas (Homia and Rangnekar 1975, Laissi, 1971) of crustaceans.

Aiken and Byard (1969) observed the degenerative changes and cellular damage in the hepatopancreas of lobster, Homarus americanus when exposed to yellow phosphorous. They noticed increase in size and number of secretory and absorptive cells causing the obliterating of the lumen of the tubule. Bodkhe (1983) found pycnosis of nuclei which has darkly stained in the absorptive cells of hepatopancreas and tissue damage in the crab, Barytelphusa cunicularis after exposure to pesticide sevimol. Nagabhushanan et al. (1984) reported that hepatopancreas of Caridina weberi functions as a storage organ and the resources stored within are used in part for reproductive purposes.

Doughtie and RangaRao (1984) studied histopathological and ultra structural changes in the hepatopancreas of grass shrimp, Palaemonetes pugio when exposed to chromium. In Macrobrachium lamerrii. Gangshettivar (1986) has observed decrease in the size of the lumen as a result of enlargement of absorptive cells when
exposed to phenol. In the present investigation the results are in agreement with the findings of Gangshettiwar (1986). In the control hepatopancreas cells exhibited normal secretory activity and when exposed to lethal concentration, accumulation of secretion in the lumen was seen which indicates that due to pollutant stress the secretory cells become more secretory. Hence lumen appears to be reduced and fully filled with secretion. During chronic exposure the accumulation of secretion gradually reduced which indicates the effect of pollutants. Gradual disturbance in the normal appearance of hepatic lobule and elongation is seen. Cells became reduced in their size as a result lumen appears large. Signs of degeneration were also seen. Damage to the hepatopancreas depends upon the concentration of pesticide and duration of exposure i.e. in the chronic exposure to 21 days the cells of hepatopancreas almost degenerate and lose their function. Sreenivas Reddy (1986) reported the decrease in the size of the lumen of hepatopancreas in *Macrobrachium lamarcki* when exposed to sea anemone toxin. Ashokkumar (1988)
noticed structural changes in the hepatopancreas of *Caridina weberi* when exposed to sea cucumber toxin. Recently histopathological changes in the form of damage to the hepatopancreas were also studied by Martin (1989) and Mahatma (1989) due to pollutant stress in *Caridina weberi* and *Macrobrachium lamarcki*.

Two types of toxic reactions have been distinguished (Ariens *et al.*, 1976) which involve the parent compound or a metabolite as the active agent. The reaction mechanisms associated with toxic reactions such as cellular necrosis has resulted in the postulation of several possible mechanisms of toxicity (Gillette, 1980; Farber, 1980). The cell injury depends on the size and shape of reactive molecule and/or the macromolecule involved (McLeese and Nuttal 1976). However the basic mechanism associated with the cell necrosis have not yet been elucidated (McLean *et al.*, 1980) although the site of initial reactions is reasonably well established for some toxic substances. Farber (1980) has stressed the difficulties in attempting to develop chemical reaction in the mechanisms of cell injury. The impairment of cellular structure reflects the severe effects of the pesticides.
The present histopathological study on gills and hepatopancreas reveals progressive damage and degeneration clearly based on exposure period, i.e., extent of tissue damage is proportional to the period of exposure of *Macrobrachium lamarrei* to the pollutant. Altered gill structure affects the process of respiration and the damaged and degenerated hepatopancreas results in the disturbance of overall metabolism and several physiological processes.