CHAPTER VIII

SUMMARY
In the present investigations, the effects of four widely used pesticides viz. BHC, Endosulfan, Malthion and Carbaryl have been evaluated on three fresh water teleost fishes, viz.- *Heteropneustes fossilis*, *Mystus tengara* and *Oreochromis mossambicus*. Histopathological changes induced by above mentioned pesticides in the brain of above mentioned fishes have been reported. Histopathological changes induced by BHC, malathion and carbaryl in liver, gills, intestine, kidney and skin of *M. tengara* are also reported. Accumulation of pesticide residues in the brain of *H. fossilis* and metabolism of these pesticides have been assessed by thin layer chromatography.

The bioassay methods used in the present study were in accordance with the standard methods as suggested by American Public Health Association (APHA) *et al.* 1985).

Experiments were conducted in two sets. In the first set of experiments, lethal concentration and the LC$_{50}$ values of the fishes were calculated. In the second set of experiment, fishes were kept in sublethal concentration of pesticide over a period of 96 hrs, 10 days, 15 days and 30 days to study the long term exposure. After the experiment, brain, liver, gills, intestine, kidney and skin were taken out and processed for histopathological procedure to study the histopathological effects.
Qualitative detection of these pesticides in the brain was done by thin layer chromatography (TLC) by the method of Walker and Beroza (1963). TLC has been particularly suitable for both detection and confirmation of organochlorinated, organophosphate and carbamate pesticides.

Toxicity assessment studies show that the toxicity of the pesticides used are in the order BHC > endosulfan > carbaryl > malathion.

For the fish *H. fossilis*, the lethal concentration was 7.5 mg/litre for BHC, 0.0037 mg/litre for endosulfan, 5.8 mg/litre for carbaryl and 3.8 mg/litre for malathion. For the fish *Mystus tengara* the lethal concentration for BHC was 1.1 mg/litre, for carbaryl 24 mg/litre and 12 mg/litre for malathion. For the fish *O. mossambicus* the lethal concentration for BHC was 0.5 mg/litre and for malathion 6 mg/litre. In *M. tengara* the Lc<sub>50</sub> for 96 hrs was 0.95 mg/litre for BHC, 18 mg/litre for carbaryl and 8 mg/litre for malathion. In *O. mossambicus* the Lc<sub>50</sub> values for 96 hrs was 0.65 mg/litre for BHC and 4 mg/litre for malathion. The sublethal concentration for *H. fossilis* in which the fishes survived for 30 days was BHC 0.8 mg/litre, endosulfan 0.001 mg/litre, carbaryl 2.0 mg/litre and malathion 8 mg/litre. The sublethal concentration in *M. tengara* was BHC 0.25 mg/litre, carbaryl 5 mg/litre and malathion 2 mg/litre. In *O. mossambicus* was 0.17 mg/litre for BHC and 0.9 mg/litre for malathion.

The behavioural responses of the fish varied in accordance
to the test concentration. Reduced respiratory activity as revealed by decreased opercular movements, difficulty in respiration, impairment of the sense of balance, secretion of mucus, loss of equilibrium and banging of head to the wall of the aquarium were noticed. The exposure to pesticides also induced external changes in *H. fossilis*, *M. tengara* and *O. mossambicus*. The response of these fishes towards different pesticides shows that they are more sensitive to chlorinated hydrocarbons than the organophosphorus pesticides.

The brain is relatively a large and important organ which is affected by pollutant. Although it has no direct contact with the pesticides dissolved in water, yet it is affected through the blood circulation and causes various alterations.

**HISTOPATHOLOGICAL CHANGES INDUCED BY PESTICIDES IN THE BRAIN OF *H. FOSSILIS* -**

The effects on the brain of *H. fossilis* were as follows.

The olfactory lobes were not much affected. The olfactory cortex was intact in all cases showing minimal damage. The non-nervous thin roof pallium was not affected. The meninx covering the cerebral hemisphere was broken down or shifted in all exposures.

In *H. fossilis* on BHC exposure, no changes were seen in 4 days but after 10, 15 and 30 days, the meninx was broken down, clumping, shrinkage, space formation, blood coagulum were observed. After 30 days, the entire structure showed haphazard structure, hypertrophy, clumping and vacuolization.
In *H. fossilis* after endosulfan exposure, the outer wall of the cerebral hemisphere was degenerated in all exposures. Fusion, clumping of cells, bi-multinucleated cells and shrinkage of cells were observed.

In *H. fossilis* on malathion exposure, space formation, clumping, and shrinkage of cells, vacuolization were noticed in 4, 10, 15 days but after 30 days of exposure the changes were more pronounced. Splitting, fibrosis, and net like appearance were also noticed.

In *H. fossilis* on carbaryl exposure, the outer wall of the cerebral hemisphere was degenerated in all exposures. Fusion, clumping of cells, bi-multinucleated cells, elongated nerve fibres and vacuolization were observed.

On exposure to BHC, the stratum fibrosum marginale was completely degenerated and detached in all exposure. Destruction, space formation, interzonal detachment, necrosis, clumping, deformation and vacuolization were observed in all components of the optic tectum in all exposures. Some dark pigment like structure also gets accumulated in 4 days exposures.

In *H. fossilis* exposed to endosulfan, the optic tectum shows detachment of outer most layer, damage of stratum plexiform et fibrosum externum, lesion, necrosis, vacuolization and acute haemorrhage in stratum griseum centrale and stratum fibrosum periventriculare.
In *H. fossilis* exposed to malathion, the stratum fibrosum marginale of the optic tectum is fused in all exposure except in 4 days. Cells of stratum plexiform et fibrosum externum and stratum griseum centrale have been destroyed. Lesion was prominent in 10 days exposure. Clumping and haemorrhage in all exposure was seen. Blood cells, blood corpuscles and cells of stratum griseum centrale become disorganized and clumped.

In *H. fossilis* exposed to carbaryl, the optic tectum showed degenerative changes in all layers except in 4 days exposure. There was separation of stratum fibrosum marginale in all exposure except 4 days. The cells of stratum, plexiform et externum & stratum griseum centrale have destroyed, necrosis, fibrosis and lesion was noticed.

On exposure to BHC, in cerebellum, separation of meninx, degeneration of outer wall, vacuolisation and net like structure was seen in 10 days. After 15 days, both the layers were separated from each other by a space, vertical splitting, fusion and clumping was observed in 30 days.

After exposure to endosulfan, cerebellum shows separation of meninx and degeneration of outer wall. There was separation between molecular and granular layer in all exposures. Lesion, splitting, shrinkage, vacuolization and clumping was present in all exposures.

On exposure to malathion, the meninx around the cerebellum shows separation and outer wall was degenerated in all exposures. Molecular and granular layer were separated by space in 4 and 30 days. Splitting and clumping was noticed in all exposures.
In carbaryl exposed, the outer wall was damaged in all cases except 4 days. Separation of layers, splitting, shrinkage, vacuolization, clumping and elongated blood cells were noticed.

The medulla oblongata was not affected in almost all cases by the pesticides.

In BHC exposed H. fossilis, cells of pituitary became destroyed, clumped. Loss of zonation, cellular disorganization, complete necrosis, vacuolization, fibrosis, space, cord like structure and cellular disintegration were noticed.

In endosulfan exposed, the pituitary shows rupture of outer wall, haemorrhage, cellular deformation, clumping and irregular arrangement of cells and zones.

The pituitary of H. fossilis exposed to malathion donot show changes in 4 days exposure. In 10, 15 and 30 days exposure, there were many changes viz. shrinkage, lesion, deformation of cells and irregular arrangement with loss of zonation was seen.

After treatment with carbaryl, no particular changes were seen in 4 days but on prolonged exposure, cellular disintegration, clumping and cord like structure were visible.

HISTOPATHOLOGICAL CHANGES INDUCED BY PESTICIDES IN THE BRAIN OF M. TENGARA

With BHC, the normal structure of cerebral hemisphere became destroyed in 4 days. The separation of meninx, degeneration
of outer wall, elongated nerve fibre and nerve cells and lesion were seen in all exposures.

With malathion, the olfactory lobes were not affected in all exposures. In cerebral hemisphere separation of meninx and degeneration of outer wall was seen in all exposures. Clumping and shrinkage of cells and vacuolization was seen in all exposures. In 15 days partial recovery was seen. In 30 days exposure, multinucleated cells, cord like nerve fibre and fusion of cells were seen.

With carbaryl, the separation of meninx and degeneration of outer wall has further increased with prolonged exposures. Clumping, shrinkage, cord like structure and multinucleated cells were common in all exposures. Hypertrophy in 10 days exposure and splitting was prominent in 15 days. After the 30 days, the entire cerebral hemisphere was shrunken and reduced, hypertropied and clumped condition.

With BHC treatment, the stratum fibrosum marginale of the optic tectum was detached in 4 days, normal in 10 days, only partially damaged in 15 and 30 days. There was fusion among layers in all exposures. Cells of stratum griseum periventriculare show shrinkage. Lesion was also noticed in all components of the optic tectum.

With malathion exposed *M. tengara*, the stratum fibrosum marginale of the optic tectum did not show any damage in 4 days
but got separated in 10 days and ruptured in 15 and 30 days. Cells of stratum griseum centrale showed splitting, space, necrosis, clumping and intensive vacuolization. Cells of stratum fibrosum profundum and stratum griseum periventriculare showed discontinuity and necrosis.

With carbaryl treatment, the stratum fibrosum marginale of the optic tectum donot show any damage in 4 days. Partial damage was seen in prolonged exposures. Cells of stratum griseum periventriculare show horizontal splitting and became destroyed in 4 and 10 days. But their appearance in 15 days was normal. After prolonged exposure again it became disorganised. Stratum fibrosum profundum and stratum griseum periventriculare were severely affected.

In BHC exposed, the outer wall of the cerebellum was degenerated but partial recovery was noticed on prolonged exposure. Splitting, vacuolization and necrosis was noticed in 10 and 15 days. Damage was less in 30 days exposed fish.

In malathion exposed Mystus, the meninx around the cerebellum was separated and outer wall was more degenerated with prolonged of exposure. There was no change in 4 days except spaces were formed at places. In 10 and 15 days of exposure cells became fused in the molecular region. There was intense vacuolization in both regions. After prolonged exposure, there was cellular disintegration in the molecular layer. Horizontal splitting was seen in the centre of granular layer.
In carbaryl exposed, the meninx and outer wall of the cerebellum did not show any damage in 4 and 10 days. But it was totally separated and degenerated in 15 and 30 days. Fused and elongated nerve fibre, multinucleated cells, vacuolization, splitting, net like structure shrinkage, clumping and hypertrophy was noticed.

With BHC, pituitary gland show shrinkage, vacuolization, necrosis, atrophy and clumping. The outer wall of pituitary was ruptured.

With malathion exposed Mystus, pituitary did not show any damage in 4 days. Shrinkage, clumping, splitting, lesion and loss of cellular organization with atrophy was noticed in 10, 15 and 30 days exposed fish.

With carbaryl exposed, the outer wall of pituitary was ruptured and the three zones become disorganised. Clumping was seen.

HISTOPATHOLOGICAL CHANGES INDUCED BY PESTICIDES IN THE OPTIC TECTUM OF O. MOSSAMBICUS

Histopathological changes in the optic tectum of O. mossambicus were as follows:

After BHC treatment, stratum fibrosum marginale of the optic tectum was degenerated with loss of normal appearance in all exposures. Cells of stratum plexiform et fibrosum externum were deformed in all exposures except in 15 days. Vacuolization and
shrinkage of cells was seen in 15 days. Cells of stratum griseum centrale, stratum fibrosum profundum and stratum griseum periventriculare were destroyed in all exposures. Vacuolization and fibrosis was seen in all layers of 10 days exposure. In 30 days exposures, the entire optic tectum decreased in width due to the fusion of different layer.

In malathion exposed Oreochromis, the stratum fibrosum marginale of optic tectum did not show damage in 4 days except degeneration and vacuolization of places. Degeneration of stratum fibrosum marginale increased with duration of exposure. Cells of stratum plexiform et fibrosum externum show fibrosis, vacuolization and deformation in all exposures. In stratum griseum centrale and stratum fibrosum profundum, there was irregularities with cellular organization and space was observed within this layer. Stratum griseum periventriculare was detached from stratum fibrosum profundum in longterm exposure.

HISTOPATHOLOGICAL CHANGES INDUCED BY PESTICIDES IN SOME TISSUES OF MYSTUS TENGARA -

LIVER- In BHC exposed fish, shrinkage of hepatocytes, clumping, space formation, multinucleated cells, cord like structure and vacuolization were observed in the liver of Mystus.

In the liver of malathion exposed Mystus, multinucleated giant cells were seen. The entire tissue appeared like a spongy mass. Dialation in the hepatic vein and hepatic artery was seen in all exposures.
In carbaryl exposed fish, the changes were the loss of cellular organization (loss of polygonal shape of hepatocytes), necrosis, shrinkage, clumping and formation of spongy mass like structure.

**GILLS** - In BHC exposed fish, the gill lamellae lost their shape and fused with the pillar cells. There was loss of cellular organization in all exposure. After 30 days, the complete structure of gill was lost and accumulation of cartilage cells was also seen.

In malathion exposed fish, showed unusual inflammatory reaction in the gills, degeneration in primary and secondary gill lamellae, swelling and a gradual increase in the cellular disorganization.

In carbaryl exposed fish, shortening and swelling of the filament takes place. Gill lamellae again become elongated in 15 days. Clumping, cellular disturbances, damage in epithelial lining and space formation were noticed.

**INTESTINE**- After BHC exposure, the intestine showed severe damage in the initial stages. The serosa was crumpled with breaks and villi were more damaged. The entire intestine showed complete damage in all exposures except after 30 days.

After malathion exposure, intestine showed detachment of serosa, withdrawal of submucosa from the folds of the villi, shrinkage of villi and its walls were broken. Splitting in muscle layer was seen in 10 days exposure.
After carbaryl exposure, the changes were damage in serosa, necrosis in muscle layer, splitting, vacuolization, compactness and fusion of villi.

**KIDNEY**- After BHC exposure, there were severe damage. The uriniferous tubules appeared with widened lumen, breaks on the outer wall of the uriniferous tubules, necrosis, clumping, spongy like appearances, vacuolization and pyknosis were noticed.

After malathion exposure, there was shrinkage and breakage of uriniferous tubules, necrosis, clumping, haemorrhage, cellular disorganization, pyknotic nuclei, widened lumen and space formation.

After carbaryl exposure, the uriniferous tubules appeared ruptured. Spongiosis and vacuolization was seen. In 10 days there was partial recovery. In 15 and 30 days clumping, necrosis, cellular disorganization was noticed.

**SKIN**- The changes after exposure to malathion were, damage in the outer surface of epidermis. Horizontal splitting and deformities were seen in the dermis. Visibility of chromatophores was more in the late stages after treatment.

In malathion exposed fish, the changes were degeneration and reduction of the epidermis. The dermis showed horizontal splitting, cellular disorganization and cells showed shrinkage. Chromatophores were also visible in prolonged exposure.

In carbaryl exposed fish, the changes were vacuolization,
cellular deformities, necrosis, rupture in epidermis and dermis, space formation, splitting and cellular disintegration.

In the present study, metabolism of pesticides was done in order to assess their residues in the brain of H. fossilis.

The results obtained after the process of thin layer chromatography of the brain of H. fossilis are as follows:

On exposure of H. fossilis to BHC, no spots were detected in any of the exposure except a single spot after the 10 days exposure.

On exposure to endosulfan a single spot was obtained after all exposures.

Malathion breaks up into two metabolites. One to two spots were detected on exposure to 10, 15 and 30 days.

The present investigation give us an information regarding histopathological changes induced by different types of pesticide in the brain and some tissues of fresh water teleost fishes. These changes induced must have changed the normal physiology of the fish which will affect in their longevity, growth, reproductive physiology and normal life span with normal health. Naturally these diseased / affected fishes are not of economic importance as, they will be harmful to the consumers. The harmful effect on the consumers is more alarming when we see the accumulation of pesticides and their metabolites in the brain.
It is proposed that much more researches be done to assess the effect of pesticides in entire fish, taking into account with all the vital organs viz. - brain, gills, muscle, skin, alimentary canal and gonads to see the damage done and all this be correlated to see the longterm effects. A qualitative and quantitative detection of pesticide and their metabolites will be of much importance which also be done by future researches.