PESTICIDE INDUCED EFFECTS IN SOME TISSUES OF *TILAPIA* (SARCOICHTHICUS) *ROSSABILICA* EXPOSED TO CARBARYL.

For this experiment, the fishes were brought from Kerala and then acclimatized in different aquaria in the Departmental Laboratory for about a week at a room temperature of $21 \pm 5^\circ C$ respectively. The fishes varied between 5-7 cm in length and 4-5 gm in weight. The bioassay methods used were as suggested by American Public Health Association (1975). The water in the aquaria was changed on alternate days and they were fed with fish food alternately. The test solutions of different concentrations were prepared from common stock solution. 1 gm of Carbaryl was dissolved in 1% Dimethyl formamide solution.

During the 1st phase of experiment, the fishes were kept in different concentrations of Carbaryl and their $L_{0}$, $L_{50}$, and $L_{100}$ values were obtained. The $L_{0}$ value was 1.5 ml/litre, $L_{50}$ value was 3.8 ml/litre and the $L_{100}$ value was 4.1 ml/litre. (The Lc values have been summed up in the table No 3 A & fig No. 1 A )

During the second phase of experiment about 100 fishes were exposed to a sub-lethal concentration of Carbaryl for a period of about 30 days. The sub-lethal
concentration of Carbaryl to the fish *Tilapia* (*Sarcotherodon*) *mosambica* was 1 ml/litre. Simultaneous controls were also maintained. The water and the pesticide was renewed on alternate days during the experimental period.

For the histopathological procedure, 15 fishes were taken out at 96 hrs, 10 days, 15 days and 30 days respectively. Each fish was removed from water and stunned with a blow on the head. The tissues were taken out and rinsed in saline solution to remove any debris. After this the tissues were fixed in Aqueous Bouin's solution for a night. After fixation, the material was thoroughly washed in water, dehydrated in different grades of alcohol, kept in Methyl benzoate for a night and then kept in Benzene for 15 minutes. After this the material was transferred to Paraffin wax in the oven with the temperature maintained at 62° C. After giving 3 changes in wax of 30 minutes each, paraffin blocks were prepared. Sections cut at 7 μ thickness were stained with Haemotomylin/Eosin and Heidenhain's Azan stains.

Histopathological changes observed in the liver, gills, kidney, intestine and skin of *Tilapia* (*Sarcotherodon*) *mosambica*, exposed to a sub-lethal concentration of Carbaryl.

1. **Liver** - The liver of a teleost fish is a yellowish
brown gland consisting of two main lobes that are subdivided into smaller lobes. Histologically, the liver is composed of a large number of polyhedral hepatic cells each of which contains cytoplasm and a nucleus. Numerous bile ductules and blood capillaries are embedded in it. The liver of *Tilapia (Sarcotherodon)* mossambica too is similar in structure like that of any other teleost and the structure of the control liver resembles the normal one. (Fig. 74.)

Morphologically the liver of *Tilapia (Sarcotherodon)* mossambica is yellowish brown in colour which on exposure to Carbaryl shows shrinkage and the colour changes to dark brown.

After 4 days of exposure to Carbaryl, the hepatic cells show shrinkage resulting in the formation of a compact cell mass as a result of which spaces are being formed. The hepatic vein and the hepatic artery show no evident change. (Fig. 75.)

After 10 days of exposure, the peripheral hepatic wall is broken at places and the cells become compact. The hepatic cells appear to loose their shape and the nuclei become disintegrated. Spaces are also being formed. However the hepatic artery and the hepatic
EXPLANATION TO THE FIGURES

Fig. No. 74. Photomicrograph of a section of Liver of Control *Tilapia mossambica*. Heidenhains Azan. 640x.

Fig. No. 75. Photomicrograph of a section of Liver of *Tilapia mossambica* after 4 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640x.
vein appeared normal. (Fig.)

After 15 days of exposure the hepatic cells loose their shape, and the hepatic tissue becomes spongy due to the formation of spaces in between the hepatic cells, the nuclei become insignificant and slight thickening was noticed in the hepatic vein and the hepatic artery. (Fig. 77.)

After 30 days of exposure to carbaryl the hepatic tissue shows a marked increase in the Spongiosis. Complete necrosis was observed in the hepatic cells. Only the insignificant nuclei are visible with some cytoplasm of the atrophic hepatic cells. The hepatic artery and the hepatic vein become more thickened and dilated and at this stage, the damage as a whole is more severe than the previous stage. Space formation was also observed at this stage. (Fig. 78.) The simultaneous controls show no change.

2- **GILLS** - The gills of a teleost are of 4 pairs and two pairs of primary gill lamillae are borne by the ceratobranchial and epibranchial segments of each gill arch. Each primary gill lamella bears a large number of Secondary lamellae on both sides. Each Secondary lamella consists of a central vascular layer
EXPLANATION TO THE FIGURES

Fig. No. 76. Photomicrograph of a section of Liver of *Tilapia mossambica* after 10 days of exposure to 1 ml/litre Carbaryl. Haemotoxylin Eosin. 640X.

Fig. No. 77. Photomicrograph of a section of Liver of *Tilapia mossambica* after 15 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.
EXPLANATION TO THE FIGURES

Fig. No. 78. Photomicrograph of a section of Liver of *Tilapia mossambica* after 30 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.

Fig. No. 79. Photomicrograph of a section of Control gills of *Tilapia mossambica*. Haemotoxylin Eosin. 640X.
surrounded by a thin layer of connective tissue and epithelium. The vascular layer consists of a net work of capillaries supported by the Pillay cells. The primary and the Secondary gill lamellae are the main seat of gaseous exchange.

The gills of *Tilapia* (*Sarcotherodon*) *mossambica* are alike in structure like that of any other teleost. They are a bit larger in size in comparison to the teleost *Heteromeustes fossilis*. They are pale cream in colour which on gradual exposure to carbaryl turn to reddish brown and seem to secrete excess of mucus.

The control section of the gill of *Tilapia* (*Sarcotherodon*) *mossambica* resembles the normal gill and there appear no marked changes. (Fig. 79.)

After 4 days of exposure to Carbaryl, vacuolization is seen in the primary gill lamellae. The Secondary gill lamellae appear shortened and degeneration is seen to start in them and haemorrhage starts in the primary gill lamellae. (Fig. 80.)

After 10 days of exposure marked changes were observed in the gills. Vacuolization was more prominent in the primary gill lamellae in comparison to the
EXPLANATION TO THE FIGURES

Fig. No. 80. Photomicrograph of a section of gills of *Tilapia mossambica* after 4 days of exposure to 1 ml/litre Carbaryl.
Heidenhains Azan. 640X.

Fig. No. 81. Photomicrograph of a section of gills of *Tilapia mossambica* after 10 days of exposure to 1 ml/litre Carbaryl.
Haemotoxylin Eosin. 640X.
previous stage, lamellar fusion was observed in the Secondary gill lamellae and the ends of Secondary gill lamellae becomes swollen and shorten up. The pillar cells show vacoulation and increase in their volume was also observed. (Fig. 81.)

After 15 days of exposure shrinkage was observed in the primary gill lamellae. There was a marked increase in the fusion and swelling of the Secondary gill lamellae. Some cells appear to loose their nuclei, while in some the nuclei shows shrinkage. Haemorrhage was observed in the pillar cells. The gill filaments on the whole exhibit atrophy and degeneration. (Fig. 82.)

After 30 days of exposure to Carbaryl some of the primary gill lamellae show swelling and shrinkage while recovery is seen in some of them. The secondary gill lamellae become elongated more in comparison to the previous exposure and appear to be fused with the pillar cells. The pillar cells appear scattered and vacoulation was seen in them. (Fig. 83.)

The simultaneous control sections were similar to the normal ones.
EXPLANATION TO THE FIGURES

Fig. No. 82. Photomicrograph of a section of gills of *Tilapia mossambica* after 15 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.

Fig. No. 83. Photomicrograph of a section of gills of *Tilapia mossambica* after 30 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.
3 - INTESTINE - The intestine of a teleost in general is short in case of carnivorous fishes and is cooled and elongated in case of herbiverous teleosts. The intestine is the main digestive organ and is concerned with the absorption. Histologically, the intestine of teleosts resembles the intestine of any other mammal made up of an outermost layer namely the Serosa. This is followed by the muscle layers - the longitudinal muscle layer and the circular muscle layer. This is followed by a Sub-mucosal zone. Next to this zone are seen the villi, which are thrown into long wavy folds, the outer border of the villi containing the mucus secreting cells and lined by columnar epithelial cells. Blood cells are also seen in the sub-mucosa.

The intestine of *Tilapia (Sarcotherodon) mossambica* is long, thin and coiled morphologically, and histologically it resembles the intestine of any other teleost. The control section of the intestine of *Tilapia (Sarcotherodon) mossambica* resembles that of its normal counterpart. (Fig. 84.)

After 4 days of exposure to Carbaryl, the outermost layer i.e., the Serosa appears broken at many places. The muscular layers appear normal with no peculiar change. The sub-mucosal tissue is seen withdrawn from the inner area of the villi, as a result of which spaces are being formed in this area.
EXPLANATION TO THE FIGURES

Fig. No. 84. Photomicrograph of a section of Control Intestine of *Tilapia mossambica*. Heidenhains Azan. 160X.

Fig. No. 85. Photomicrograph of a section of Intestine of *Tilapia mossambica* after 4 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 160X.
The villi appear with no change in their size and structure. The blood vessels too appear normal as in the case of controls. (Fig. 25.)

After 10 days - the intercellular spaces formed in between the cells of villi increases. This might be the reason for dilation in the villi. Withdrawal of the submucosa from the inside of the folds of villi on increase, and the villi becomes dense and come close together, the outer surface touching each other. The mucosa appears compact. (Fig. 26.)

After 15 days of exposure, the Serosa is detached at many places, slight necrosis is observed in the muscular layers. Space formation increases due to the withdrawal of the sub-mucosal tissue and the cells of the villi appear loosely arranged. The tips of the villi are also ruptured at places. (Fig. 27.)

After 30 days of exposure to Carbaryl the Serosa is detached at many places. The longitudinal muscle layer and the circular muscle layer shows acute necrosis. The villi show a sudden shrinkage as in evident by the large lumen cavity, the cells of the villi appear like that of a control. The
EXPLANATION TO THE FIGURES

Fig. No. 86. Photomicrograph of a section of Intestine of *Tilapia mossambica* after 10 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.

Fig. No. 87. Photomicrograph of a section of Intestine of *Tilapia mossambica* after 15 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.
submucosal tissue also appears like that of normal control. Clumping of blood cells and shortening of the villi was also observed. (Fig. 36, 26.)
The simultaneous controls show no evident changes and appear like the normal controls.

4 - KIDNEY - The Kidneys are the main excretory and osmoregulatory organs of fishes. The kidneys play an important role in the excretion of nitrogenous wastes and in maintaining the watersalt balance (homeostasis). The kidneys of teleosts are paired, elongated structures placed above the alimentary canal and are close to the vertebral column. The kidney of a teleost is generally divided into two portions, the head kidney and the tail kidney, but in most species, these regions are not distinguishable by external examination.

Histologically, the kidney is made up of a large number of nephrons, each consisting of a renal corpuscle and the tubule. The intertubular space is made up of the haemopoietic tissue which is unevenly distributed. The glomerulus, blood vessels and the capillaries can also be seen in the haemopoietic tissue.
EXPLANATION TO THE FIGURES

Fig. No. 88. Photomicrograph of a section of Intestine of *Tilapia mossambica* after 30 days of exposure to 1 ml/litre Carbaryl. 
Haemotoxylin Eosin. 160X.

Fig. No. 89. Photomicrograph of a section of Intestine of *Tilapia mossambica* after 30 days of exposure to 1 ml/litre Carbaryl. 
Haemotoxylin Eosin. 640X.
The kidney of *Tilapia (Sarcothorodon)* mossambica is similar in morphological as well as histological structure like that of other teleosts. The control section of the kidney of *Tilapia (Sarco- therodon)* mossambica is similar to that of its normal counterpart and there appear no changes or alterations. (Fig. 9c.)

Morphologically, on gradual exposure to Carbaryl, the kidney show shrinkage and its colour changes from yellowish brown to brownish black.

After 4 days of exposure to Carbaryl, necrosis is seen in the haemopoietic tissue, as a result of which spaces are being formed. The uriniferous tubules appear ruptured and shrinkage is seen in the glomerulus. Blood cells are not visible at this stage. (Fig. 9f.)

After 10 days of exposure necrosis in the haemopoietic tissue is more prominent in comparison to the previous stage. The uriniferous tubule appears to shrink up and its lumen appears widened, more than the previous stage. The cells of the uriniferous tubules appear disturbed with displaced and insignificant
EXPLANATION TO THE FIGURES

Fig. No. 90. Photomicrograph of a section of Kidney of Control *Tilapia mossambica*.
Haematoxylin Eosin. 640X.

Fig. No. 91. Photomicrograph of a section of Kidney of *Tilapia mossambica* after 4 days of exposure to 1 ml/litre Carbaryl.
Haematoxylin Eosin. 640X.
nuclei. The glomerulus shows an overall shrinkage and the blood cells appear few in number and they show clumping nature. (Fig. 92.)

After 15 days of exposure, the haemopoietic tissue shows acute necrosis and appears dislocated. The uriniferous tubules show shrinkage, their walls are broken at places, but the lumen tends to show recovery. The outer wall of the glomerulus is broken at places and the cells appear disturbed and necrotic. Vacuoles are formed in the cells of the uriniferous tubules. The nuclei appear disturbed and the blood cells appear few in number and show clumping nature. (Fig. 93.)

After 30 days of exposure, necrosis in the haemopoietic tissue increases, which results in the formation of a spongy mass. The glomeruli and the uriniferous tubules show acute damage. However the blood cells appear normal and there is no clumping at this stage. (Fig. 94.)

The simultaneous controls however appear normal and there is no peculiar change in them.
EXPLANATION TO THE FIGURES

Fig. No. 92. Photomicrograph of a section of Kidney of *Tilapia mossambica* after 10 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.

Fig. No. 93. Photomicrograph of a section of Kidney of *Tilapia mossambica* after 15 days of exposure to 1 ml/litre Carbaryl. Haemotoxylin Eosin. 640X.
EXPLANATION TO THE FIGURES

**Fig. No. 94.** Photomicrograph of a section of Kidney of *Tilapia mossambica* after 30 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.

**Fig. No. 95.** Photomicrograph of a section of Skin of Control *Tilapia mossambica*. Heidenhains Azan. 160X.
5 - SKIN - The skin of animals forms the external covering of the body and is concerned with important functions. The skin is one of the largest of the organs, making 16% of the body weight in mammals and 6.4 to 10.7% in fishes. The skin performs a number of functions besides protecting the fish from injury and infection. It also performs respiratory, excretory and osmoregulatory functions. The skin of a fish is of great survival value, as it provides the first line of defence against infection by environmental toxicants present in water. The skin is a delicate structure, highly vulnerable to damage by pollutants present in water.

Histologically, the skin of teleosts is composed of two distinct layers - outer epidermis and the inner dermis. The epidermis consists of a few layers of flattened cells, the mucus cells and the columnar cells. The dermis is composed of connective tissue, blood vessels and a few chromatophores.

The skin of *Tilapia* (*Sarotherodon*) *mosambica* is similar to that of its normal counterpart, and there are no changes. (Fig. 95.)

After 4 days of exposure to Carbaryl, the
outer layer i.e. the Epidermis appears reduced in thickness. The outer layer of the Epidermis is broken at some places and is detached from the dermis. The connective tissue of the dermis shows splitting. The Mucus cells are however easily visible. (Fig. 96)

After 10 days of exposure, reduction in the thickness of epidermis was observed, more then that in case of the previous exposure. The dermis also appears reduced in thickness. (Fig. 99.)

After 15 days, the epidermis appears detached and cellular organization is lost in it. Splitting of connective tissue along with spaces observed in the dermis. The chromatophores are visible. Over all the damage is more than that of the previous stage. (Fig. 99.)

After 30 days of exposure to Carbaryl, the epidermis is completely detached from the dermis and the connective tissue of the dermis shows continuous splitting. (Fig. 100.)

The simultaneous controls appear normal with no changes.
EXPLANATION TO THE FIGURES

Fig. No. 96. Photomicrograph of a section of Skin of *Tilapia mossambica* after 4 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 160X.

Fig. No. 97. Photomicrograph of a section of Skin of *Tilapia mossambica* after 10 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 160X.

Fig. No. 98. Photomicrograph of a section of Skin of *Tilapia mossambica* after 10 days of exposure to 1 ml/litre Carbaryl. Heidenhains Azan. 640X.
EXPLANATION TO THE FIGURES

Fig. No. 99. Photomicrograph of a section of Skin of *Tilapia mossambica* after 15 days of exposure to 1 ml/litre Carbaryl. Haemotoxylin Eosin. 640X.

Fig. No. 100. Photomicrograph of a section of Skin of *Tilapia mossambica* after 30 days of exposure to 1 ml/litre Carbaryl. Haemotoxylin Eosin. 640X.
ABBREVIATIONS.

A  -  Atrophy.
BC - Binucleate cell.
BV - Blood vessel.
BCC - Basal columnar cells.
Ch - Chromatophores.
CL - Clumping.
JV - Compact vellii.
CMF - Circular muscle fibres.
COR - Cord formation.
D  -  Dermis.
DD - Degenerating Dermis.
DE - Degenerating epidermis.
VI - Dilatation.
DN - Degenerating nuclei.
DBM - Damaged basement membrane.
DBV - Degenerated blood vessel.
DEH - Displaced epithelial membrane.
DEP - Detached epidermis.
DG  - Degenerated gill axis.
DGL - Damaged glomerulus.
DHT - Damaged hepatic tissue.
DUT - Damaged uriniferous tubule.
DSM - Damaged sub mucosa.
<table>
<thead>
<tr>
<th>ABF</th>
<th>Epidermis.</th>
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<tbody>
<tr>
<td>ACF</td>
<td>Elongated gill filament.</td>
</tr>
<tr>
<td>FL</td>
<td>Fibrosis.</td>
</tr>
<tr>
<td>FU</td>
<td>Fusion.</td>
</tr>
<tr>
<td>GA</td>
<td>Gill axis.</td>
</tr>
<tr>
<td>GF</td>
<td>Gill filament.</td>
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<tr>
<td>GL</td>
<td>Glomerulus.</td>
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<tr>
<td>H</td>
<td>Haemorrhage.</td>
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<tr>
<td>HA</td>
<td>Hepatic artery.</td>
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<tr>
<td>HS</td>
<td>Horizontal splitting.</td>
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<tr>
<td>L</td>
<td>Lumen.</td>
</tr>
<tr>
<td>LP</td>
<td>Lamina propria.</td>
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<tr>
<td>LGC</td>
<td>Large granular cells.</td>
</tr>
<tr>
<td>LMF</td>
<td>Longitudinal muscle fibres.</td>
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<td>MC</td>
<td>Multinucleate cells.</td>
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<tr>
<td>NE</td>
<td>Necrosis.</td>
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<tr>
<td>PC</td>
<td>Pillar cells.</td>
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<tr>
<td>PN</td>
<td>Pyknotic nuclei.</td>
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<tr>
<td>PIC</td>
<td>Pillar cells.</td>
</tr>
<tr>
<td>RV</td>
<td>Ruptured villi.</td>
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<tr>
<td>RBC</td>
<td>Red blood cells.</td>
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<tr>
<td>RBL</td>
<td>Ruptured basement membrane.</td>
</tr>
<tr>
<td>RSP</td>
<td>Ruptured epidermis.</td>
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<tr>
<td>RGL</td>
<td>Ruptured glomerulus.</td>
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<tr>
<td>RSM</td>
<td>Ruptured sub mucosa.</td>
</tr>
</tbody>
</table>
RUT - Ruptured uriniferous tubule.
RCMF - Ruptured circular muscle fibres.
RLMF - Ruptured longitudinal muscle fibres.
S - Serosa.
SD - Shrunken dermis.
SM - Submucosa.
SN - Swollen nuclei.
SC - Scales.
SV - Shrunken villi.
SQ - Squamous cells.
SBC - Scattered blood cells.
SBV - Shrunken blood vessels.
SE - Seepage.
SE2 - Shrunken epidermis.
SGF - Swollen gill filaments.
SGL - Shrunken glomerulus.
SMU - Shrunken mucus cells.
SPG - Spongiosis.
SUT - Shrunken uriniferous tubule.
SCMF - Shrunken circular muscle fibres.
SLMF - Shrunken longitudinal muscle fibres.
TC - Trinucleate cell.
UT - Uriniferous tubule.
V - Villi.
VA - Vacuolization.
WL - Widened lumen.
WSM - Withdrawn submucosa.
In the present study toxicity assessment behaviour and histopathological changes in *Tilapia* (*Sarcotherodon* mousambica) exposed to Carbaryl were studied. The LC₅₀, LC₅₀ and LC₁₀₀ values, the sublethal concentration, behaviour and the histopathological changes have been studied in this fish.

Various methods were used in the present study to estimate the safe concentration of the pesticide used. Some of them were as described by Hart et al. (1945), Edwards and Brown (1966), Burdick (1967), Konar (1970, 1971) and APHA et al. (1975). The method used by APHA et al. (1975) gave higher values than any other method. Therefore the value obtained for estimated safe concentration of pesticides given by APHA et al. (1975) was used in the present study.

Acute toxicity data for the pesticides for a number of fishes have been reported by various authors viz. Quodoroff et al. (1953), Hayhew (1955), Parkhurst and Johnson (1955), Haynes et al. (1955), Rudd and Genelly (1956), Katz and Chadwick (1961), Lawallen and Wilder (1962), Songriyam et al. (1968), Joshi et al. (1975), Farrish et al. (1976).

According to Henderson et al. (1957), the 96 hr LC\textsubscript{50} of methyl parathion for fat head minnow was estimated to be 8.3 mg/litre and 7.5 mg/litre in hard and soft water respectively. Annees (1975) found that 96 hr LC\textsubscript{50} values in mg/litre for the fish Channa punctatus was 0.455, 0.920, 2.15 and 2.05 for diazinon, malathion, methyl parathion and dimethate respectively. Bengeri et al. (1984) found that two different size groups of Labeo rohita indicated two different toxic levels (6.34 and 11.0 mg/litre). The LC\textsubscript{50} values of methyl parathion for fry and adult male Lebistes reticulatus were 6.1, 7.6 and 11.5 mg/litre.

Acute toxicity is an important parameter according to Mount and Stephan (1967). Some authors (Cairns 1966; Alderice 1967) stress more on the sub-lethal toxicity than the acute toxicity. The 96 hr exposure time to any toxicant is more useful for calculating an application factor (AF), maximum acceptable toxicant concentration (MATC) and toxicity curve (APHA et al. 1975). The AF, MATC etc are useful
for deriving safe concentrations of a particular toxicant for particular test species for water. The $Lc_{50}$ data with reference to different species have been studied by many workers. In the present study, the 96 hr $Lc_{50}$ value for Carbaryl to the fish "Tilapia (Sarcotherodon mossambica)" was found out to be 3.8 ml/litre, which is higher in comparison to the cold water fishes like blue gill (Henderson et al 1960); trout and salmon (Post and Schroeder 1971); Carps (Arora et al. 1971 ab) Channa punctatus (Annees 1975); eastern mid minnow (Bender and Westman 1976); G. punctatus (Pandey et al. 1976); Clarias batrachus (Brown 1980; Abidi 1983, Bhatnagar and Bana 1987); H. fossilis and H. vittatus (Jagdeesh and Sahai 1987).

Arora et al. (1971 b) found that 96 hr $Lc_{50}$ values for malathion for the fish Cyprinidus carpio and Labeo rohita were 3.15 mg/litre and 5.05 mg/litre respectively. Verma et al. (1979) found the 96 hr $Lc_{50}$ values in H. fossilis to be 2.44 mg/litre for BHC, 0.537 mg/litre for Lindane and 15.00 mg/litre for malathion. Singh and Singh (1981) found that the 96 hr $Lc_{50}$ values for BHC to be 0.12 ppm for the fish Cyprinidus carpio. Choudhary et al. (1981) clearly indicated the toxic effect of long term exposure of H. fossilis to Malathion at a concentration which may
fail to produce any response of acute toxicity to fish. Verma et al. (1981) suggested that organochlorine group is relatively more toxic to fish than the organophosphate group. Basha et al. (1983) studied the toxicity of three different pesticides to *Tilapia mossambica*.

In the present study, toxicity has been assessed to a Carbamate pesticide - Carbaryl on a fresh water teleost - *Tilapia* (Sarcotherodon) mossambica. The LC$_{50}$ value for 96 hrs was found out to be 3.8 ml/litre and the sublethal dose at which the experiment was conducted was 2.0 ml/litre. Since the toxicity bioassay is influenced by a number of factors viz. temperature, environment etc and including the technique of bioassay employed, therefore a comparison of LC$_{50}$ values for several fish species by various authors was of no importance.

Behavioural responses of *Tilapia* (Sarcotherodon) mossambica after lethal and sublethal exposure to Carbaryl was in agreement with those seen in *H. fossilis* (Verma et al., 1978). The intense opercular movements, erratic movements, paralysis, loss of balance, banging of the head to the wall of the aquaria probably due to irritation exhibited by *Tilapia* (Sarcotherodon) mossambica after lethal
and sublethal exposure to Carbaryl may be attributed to a sort of hypoxic stress accompanied by a sequential inhibitory influence of the pesticides to the respiratory system. According to De.Candole et. al. (1953) death in mammals by acute organophosphorous intoxication, has been related to biochemical lesions leading to ventilation impairments. Eaton (1970) observed extreme extension of pectoral fins and reddish discolouration at the base of the dorsal fin of the gill. Burton et al. (1972) demonstrated that acute zinc poisoning in Salmo gairdneri involved a modification of gas exchange process at the gill level. Hughes (1976) observed that one of the likely effects of pollution affecting the respiratory system is that it limits the metabolic scope of activity. Gupta (1984) reported loss of locomotor activity in the Gold fish (Carassius auratus) when exposed to DDT.

It is generally observed that fish responds to toxic chemicals by increased opercular movements (Bieding 1929). Jones (1964) reported increased opercular movements in stickle-backs exposed to copper sulphate and lead nitrate solution at 17°C. Annees (1975) observed increased opercular activity in Channa punctatus exposed to Malathion, while Pandey et al. (1976) reported decrease in the opercular frequency for the same species exposed to Malathion. Nagendran
and Shakuntala (1979) observed increase in the opercular activity in *Puntius ticto* when exposed to sodium pentachlorophenate.

Therefore, one may conclude that the intense opercular movements exhibited by *Tilapia* (*Sarcootherodon*) mossambica after lethal and sublethal exposure to Carbaryl may be attributed to a sort of hypoxic stress accompanied by inhibitory influence of Carbaryl on the respiratory system. Pesticides may turn into pollutants if discharged into water irrationally causing wide spread disaster to aquatic life particularly to fish. It is now apparent that the pesticide pollution is toxic and brings histopathological changes in different tissues of fish.

The pesticides present in the water reach the fish body through water taken in with food, mucosa of the mouth or gills and they may reach the liver through blood circulation and to the intestine and kidney through food or blood.

The liver is an important organ which is affected by the pesticides. Several structural and functional changes in the liver are caused by pesticides. DDT produces hypertrophy of hepatic cells, hyaline
degeneration, liver cord disarray, vacuolization in cytoplasm, necrosis of cells, fatty liver and hepatoma, Mathur (1962 a, b) and Durham et al. (1965). King (1962) described various histopathological changes in guppies and brown trout after DDT intoxication. Mathur (1965) showed that Meldrin induced marked degeneration of liver in many species of teleosts. Sastry and Sharma (1979), Mandal and Kulshrestha (1980) showed that the nuclei become pyknotic, necrosis and vacuolization, breakdown of cell boundaries, loss of polygonal shape and formation of multinucleated giant cells. Mattheisen and Roberts (1982) observed histopathological changes in the liver of C. garpis exposed to Endosulfan. They observed lesions and focal necrosis. Shaifee et al. (1986) showed that Suquin caused hyperplasia of hepatocytes, pyknotic nuclei, denucleated hepatocytes in the liver of Barbus tiito and Rasbora daniconius.

Taking the present study into consideration marked changes were seen in the liver of Tilapia (Sarcopterodon) mossambica on exposure to Carbaryl. The changes induced were somewhat similar to those described by Sastry and Sharma (1979) and Mandal and Kulshrestha (1980). The changes were the shrinkage of hepatocytes resulting in formation of a compact
cell mass as a result of which spaces are being formed. Loss of polygonal shape and disintegration of nuclei were also observed. During the later stages formation of a spongy mass was observed, the nuclei became insignificant. The hepatic artery and the hepatic vein became more thickened and dilated after 30 days of exposure.

The spongy appearance in the tissue was in accordance with Crandall and Goodnight (1968) who have pointed out the 'coagulate' appearance in liver with Sodium penta-chlorophenate treatment in Lohistus reticulatus. The above mentioned changes were also mentioned by Singh (1985) and Kulshrestha and Jauhar (1984) which included rapid degeneration, hypertrophy, necrosis and vacuolization of the hepatocytes with splitting off of the tissue, formation of bi-nucleate cells and islet cells in P. ticto, R. dasyconius and G. strigatus respectively. However the author could not observe the binucleated cells as described by Kulshrestha and Jauhar (1984) and the islet cells as observed by Singh (1985). Rashatwar and Ilyas (1984) reported that the liver is an important organ of detoxification, where breakdown of toxic materials is carried out by the endoplasmic reticulum of
hepatocytes due to which the hepatic cells are damaged severely. Rani (1984) observed some of the identical changes in the liver of *Hystus vittatus* exposed to three organic compounds. Gupta and Dalela (1986) reported the formation of compact mass and the blockening of the Sinusoid capillaries due to the enlargement of hepatic cells in the liver of *Notopterus notopterus* when exposed to various combinations of phenolic compounds. Plaha and Sahai (1986) also reported such changes in *H. fossilis* exposed to Malathion. Bhatnagar and Bana (1987) also observed histopathological alterations in the liver of *Channa gachua* exposed to endosulfan. Similar histopathological changes were seen by Ghosh and Chaterjee (1987) in the liver of *Channa punctatus* during Dichlorvos intoxication.

The extent of damage to the liver was dependant on the duration of the exposure with Carbaryl.

The gill structure in case of teleost fishes is likely to be markedly altered on exposure to pesticides, resulting into pathological conditions in various ways. The gills of fishes are not only respiratory but are also excretory in function.

Most of the studies on the histopathology of
the gills in fishes are based on fish exposed to heavy
metals, fertilizers and detergents. The severity of
the damage is concentration and time dependant. Westfall
(1945) experimentally demonstrated coagulation film
anoxia with relatively high concentrations of lead and
suggested that the heavy metals can be lethal by their
rapid superficial action on the respiratory and excretory
function of the gills. Gupta and Rajbanshi (1979)
showed that during toxicity induced by copper in
_H. fossilis_, the gill filaments became completely
covered by a thick mucus layer. Haemorrhage in the
filaments of gill and degeneration or complete disruption
of cellular and tissue components are noticed. Similar
observations were noticed by Carpenter (1930) in case
of lead toxicity. Further Anderson (1960), Skidmore
(1970), Sisler (1971), Burton et al. (1972), Bilinski
and Jonas (1973), Wong et al. (1977) described the
deleterious effect of heavy metals and their salts and
suggested that the death of the fish in acute poisoning
by heavy metals was due to the disruption of the
respiratory process. Mahajan and Singh (1973) observed
atrophy and formation of bud like structures in the
central axis of gills of _H. fossilis_ exposed to
synthetic detergents. Verma et al. (1975) have
observed necrosis in some parts of respiratory cells
and atrophy of large glandular cells in *Colisa fasciatus* on Lindane exposure.

Ansari and Shrivastava (1984) have reported hypertrophy in the respiratory lamellae and degeneration of epithelial cells of *Mylophorus vittatus* exposed to Sodium nitrite. Shrivastava and Shrivastava (1984) reported morphological deformities, shrinkage in secondary gill lamellae as well as fusion and clumping at the tip in *Cyprinus carpio* after exposure to malathion and chlordane.

In the present study, the changes observed on exposure of *Filaia* (*Sarcotherodon* moasambica) to carbaryl were vacuolization in the primary gill lamellae, lamellar fusion in the secondary gill lamellae, swelling in the ends of secondary gill lamellae, haemorrhage was observed in the pillar cells. After 30 days of exposure however, the secondary gill lamellae become elongated. The pillar cells appear scattered and vacuolization is seen in them. These changes were in accordance with the studies of Singh (1985) who observed similar swellings of gill filaments in *Rasbora daniconius* and *Puntius ticto*, but the sudden elongation of secondary gill lamellae after 30 days of exposure was not seen by any of the authors.
Fromm Paul O et al. (1971) observed changes in the gills of trout on exposure to some insecticides and MS 222. Histopathological alterations were also noted in the gills of Rainbow trout on exposure to Zinc Sulphate by Skidmore and Tovell (1972). Similarly histopathological changes have been reported by Cook et al. (1976). Geoffrey (1976) has seen the effect of ammonia exposure on the gills of Rainbow trout. Johnson (1981) observed marked histopathological changes in the gills of Rainbow trout. Rao et al. (1983) studied the effect of malathion in the gills of *Hilairia* (Sarcothorodon) mossambica, observing blackening of gill filaments, bulging of the tips of primary gill lamellae. Rani (1984) also observed some of the above mentioned changes in the gills of *Hystua vittata* on exposure to three organic compounds. Singh et al. (1986) showed the effect of malathion on the mucus producing cells in the gills of *Clarias batrachus*. Chauhan and Pandey (1987) also observed changes in the gills of *Channa gachua*. Similarly Plaha and Sahai (1987) reported histomorphological changes in the gills of *H. fossilis* exposed to Carbaryl.

From the above mentioned observations it is seen that the changes in the gills are time dependant
and increases after gradual exposure to the pesticide, i.e. Carbaryl in the present study.

The extent of damage caused by the pesticides is also visible in the intestine of fishes. The histopathological symptoms seen in the intestine of fishes after exposure to pesticides are the shrinkage and atrophy in the villi, detachment and vacuolization in the Serosa, and the muscle layers and damage in the Sub-mucosal layer. Previously Aminikutty and Rege (1978) studied the effect of Thiodan and Agallol in the intestine of the Widow tetra Gymnooxymus ternetzi. Mandal and Kulshrestha (1980) observed rupture in the villi and detachment of Serosa in the intestine of certain fishes induced by DDT. Naidu et al. (1983) also reported histological alterations in the intestine of Sarcotherodon mossambica. Bakthavasalam et al. (1984) observed marked changes in the intestine of Anabas testudineus exposed to Furadon. Similar changes were observed by Jauhar and Kulshrestha (1984) who reported damage in villi, detachment of serosa, vacuolization in the muscle layers in the intestine of Channa striatus after exposure to Endosulfan and Carbaryl. Wagh and Khillare (1987) have also reported histopathological changes in the alimentary tract of Barbus stigma.
The changes in the present study in the intestine of *Tilapia* (*Sarcotherodon* * mossambica* after exposure to Carbaryl were the complete detachment of Serosa, necrosis in the longitudinal as well as the circular muscle layer. The villi show a sudden shrinkage during the later stages as evident by the large lumen cavity. There was an increase in the space formation and the withdrawal of the sub-mucosal tissue. The tips of the villi are also ruptured at places. However the villi become dense and come close together after the initial exposure.

The sudden shrinkage in the villi may be correlated with the blood supply available to the intestine.

The diversity in toxic action of pesticides is also exhibited in the damage, they cause to the kidneys. The histopathological symptoms seen in the kidney after pesticide exposure are damage in the renal tubules by expansion, necrosis and swelling which cause the impairment in the functioning of the kidneys.

Pfugifelder (1953) suggested that histopathological change in kidney was mainly due to physiological response to increased demands. Rasquin
and Rosenbloom (1954) interpreted that the kidney damage was attributed to hormonal imbalance. Mount and Putnicki (1956) observed a large number of vacuolated cells in the kidney after investigating a fish kill due to endrin poisoning. Rudd and Genelly (1956) reported that DDT produced chronic nephritis in Gold fish. Mathur (1962 a, b) reported that DDT produced degeneration of tubular epithelial cells and loss of parenchymatous cells of renal tubules in Ophioscopalus punxatus. Konar (1970) reported the rupture of renal epithelium, collapse of renal tubules, swelling and nuclear changes in Labeo rohiita treated with heptachlor. Gupta and Rajbanshi (1979) reported pathological changes in the interrenal exhaustion that change the metabolic ions to renal tissue through blood resulting in disorder of the excretory system. Kulshrestha et al. (1984) observed rupture of peritoneum lining, flattening of renal epithelium, widening of the tubules, migration of the epithelial nuclei, necrosis of haemopoietic tissue, shrinkage and degeneration of glomeruli, haemorrhage of blood vessels, scattering of erythrocytes in the kidney of Channa striatus after Carbamate and endosulfan poisoning. Rashidwar and Ilyas (1984) observed a marked loss of haemopoietic tissue and degeneration of glomeruli in the kidney of Hemochelius denisonii due to phosphomidon poisoning. Singh (1985)
observed internal haemorrhage, expansion of renal tubules and degenerated haemopoietic tissue. Similar changes were observed by Rani (1981) in Mystus vittatus on exposure to organic compounds. Gupta and Dalela (1987) also observed such changes in the kidney of Notopterus notopterus after exposure to phenolic compounds.

In the present study, the results obtained are in accordance with Kulshrestha et al. (1984). The changes seen after exposure to Carbaryl were necrosis in the haemopoietic tissue which resulted in the formation of a spongy mass after 30 days of exposure. The glomeruli and the uriniferous tubules show acute damage. The blood cells show clumping nature during the early stages but tend to appear normal after 30 days of exposure.

The recovery in the haemopoietic tissue has been pointed out by Kulshrestha et al. (1984) in Channa striatus and by Singh (1984) in Rasbora daniconius and Puntius ticto with exposure to Endosulfan. This prolonged exposure as put by Ingram (1980) and Kulshrestha et al. (1984) probably produced natural immunity substances in the body of these fishes which helped them against xenobiotic substances in their food and mucus.
According to the author, another reason for the kidney showing recovery might be due to the filtration properties of the kidney.

Shareef et al. (1986) observed marked histopathological changes in the kidney of *Barbus ticto* and *Rosbora daniconius* on exposure to Suquin, but in this case recovery of the haemopoietic tissue was not observed. Lurve and Dugar (1986), Ghoash and Chaterjee (1987) have also reported marked changes in the kidney of *Lebistes reticulatus* and *Channa punctatus*. Khillare and Wagh (1987) too reported changes in the kidney of *Barbus stigma* on exposure to Malathion, Sevin and Endosulfan. Similarly Plaha and Sahai (1987) reported damage in the kidney of *H. fossilis* exposed to Malathion.

Another organ which is attacked by the pesticides is the skin. The skin is in direct contact with water and so the pesticides present in water act directly upon it.

The damage caused to the skin by the pesticide are the reduction in the thickness of the epidermis and the dermis and the detachment of the epidermis from the dermis. The works on the effect of pesticides
to the skin are very much limited. Previously Saxena and Kulshrestha (1982) studied the effect of DDT on regeneration of cutaneous wounds in *Nystus vittatus*. Kulshrestha and Arora (1984) observed damage to both, the epidermis and the dermis. They also observed spongiosis in the dermis, increase in chromatophores and scattering of the muscle bundles in the dermis. The complete detachment of the epidermis was also observed by them on exposure of *Channa striatus* to sublethal doses of carbaryl and Endosulfan.

Kulshrestha and Saxena (1984) studied the effect of sublethal doses of DDT on the skin of *Nystus vittatus*. They reported suppression of epidermis, damage and loss of anchorage on the dermis in the initial stages. However during the later stages they observed reduction in the damage and epidermis reestablishing its anchorage to the dermis. Intensive mucus secretion was seen by them in the initial stages. Clumping of blood cells was also observed. Plaha and Sahai (1987) reported histomorphological changes in the skin of *Heteronineustes fossilis* exposed to Carbaryl.

The results obtained in the present study are in accordance with the studies made by Kulshrestha and Arora (1984). The changes observed after the exposure of *Tilapia* (*Sarcotherodon* ) *moasambica* to
Carbaryl are the reduction in the thickness of the epidermis and the dermis. The detachment of epidermis at some places in the initial stages followed by complete detachment after 30 days of exposure was also observed, alongwith complete loss of cellular organization in it. After 30 days of exposure, the connective tissue of the dermis shows continuous atrophy. The chromatophores however were visible clearly.

From the above results, one may conclude that in *Tilapia (Sarotherodon) mossambica* exposed to Carbaryl the changes induced in the above mentioned tissues were gradual, as such the necrosis starts gradually and there was no recovery in any of the tissue, as described by Kulshrestha et al. (1984) in *Ctenopharyngodon idella* and by Singh (1985) in *Hilsa mforum* and *Puntius tetora* with endosulfan and BHC. However the blood cells in the kidney appeared as normal after 30 days of exposure in the present study. The initial damage followed by recovery has also been studied by me in the previous chapter after exposure of *H. fossilis* to BHC.

Thus it is clear that in the case of organophosphate and Carbamate pesticides, the damage to the tissue is gradual, where as in the case of organochlorine pesticides
there is an initial damage to the tissue followed by recovery which might be due to immunity developed by the animal towards the pesticide. According to Ingram (1980) and Kulshrestha et al. (1984) the prolonged exposure of fish to the pesticide probably produced natural immunity in the body of fishes which helped them against xenobiotic substances in their food and mucus.