Histological and Histochemical Effects
6.1 Introduction

To understand the pathogenesis that a toxicant imposes on the cells, tissues and organs, it is imperative to probe into the histopathology of those structures. Determination of cause and tissues reaction, must be followed by interpretation of their significance in the degeneration process. To-day the pesticide entry into aquatic system of human use questions the survival of not only the dependents but also the inhabitants of that system. In this context it has been decided in the present study to examine the possible histopathological changes the tissues of the fish, *Cyprinus carpio* will develop if it has accidental exposure to the commonly used pesticide phosphamidon. As the edible fish *Cyprinus carpio* can be used as a candidate species to indicate pollution, an in-depth study on the histological damages the fish develops on exposure to pesticide will shed light on the possible threat to other non-target organisms and human beings.

Several studies have been made (Table 6.1) on the histopathological and histochemical changes in fishes enduring pesticides medium. However
nothing is much known about the histological and histochemical changes the fish *C. carpio* develop on their encounter with phosphamidon toxicity. Hence the present study aims to trace the changes in the histology and histochemistry of the fish *C. carpio* exposed to the pesticide phosphamidon.

**Summary of Histopathological effects of pesticides on fish**

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6.2 Material and Methods

C. carpio in different size ranges were selected from the previously acclimatized laboratory stock and exposed to two sublethal concentrations (3.93 and 7.86 ppm) of phosphamidon. After 30 and 60 days of exposure to sublethal concentrations of phosphamidon, the fish were taken out and the following organs in their body were dissected out [viz gills, liver, kidney, brain and reproductive organs] and fixed in fixatives for future studies.

The pesticide medium was supplied daily and the fish were fed on a mixture of groundnut oil cake and rice bran nodules every day. In addition to the phosphamidon exposed tissues, tissues from control fish (non exposed to pesticides) were also fixed for comparative study of tissue damages.

To fix the tissues, Zenker's fixative was used. The tissues were fixed in the fixative for 12 hrs and washed in running tap water overnight and dehydrated in ascending grades of iso-propyl alcohol. Two changes of absolute alcohol were followed by clearing in methyl benzoate. The tissues were then left in 1% celloidin dissolved in methyl benzoate. The celloidin infiltrated tissues were left in toluene till they became translucent. After removing toluene, the tissues were infiltrated with paraffin wax of melting point 56-58°C. Three changes of wax, totaling a period of 90 minutes were sufficient for infiltration. The tissues were embedded in wax. Thus double-embedding in celloidin and wax was employed according to the method of Peterfi as given in Pantin (1962). The sections were cut at the thickness of 6 μ in a rotary microtome. The ribbon strips were floated on water poured over albuminised slides. The sections were allowed to expand by gently warming the slide on a hot plate maintained around 50°C. When the sections became flat and expanded, the water was drained. The slides were left overnight on the hot plate maintained at constant temperature of 45°C.
The sections were stained by monochrome and dichrome methods. Heidenhain's iron haematoxylin (Baker 1955) method was employed to get cellular as well as subcellular structural details. In the dichrome method, Weigert's iron haematoxylin and Biebrich scarlet stains were used.

The sections were deparaffinized in xylene and rehydrated in descending grades of iso-propyl alcohol. As mercuric chloride was used in Zenkers fixative, the fixed tissues were passed through 70% alcohol with 0.5% iodine in order to remove mercury precipitate from the tissues (Murugesan, 1988). The tissues were further treated with 5% aqueous suspension of sodium thiosulphate to remove the iodine. The tissues were washed in water for 5 minutes and were left in distilled water till they were stained. The stained sections were rapidly dehydrated in ascending grades of iso-propyl alcohol or acetone. The latter proved to be a better dehydrating agent as it was gentler to most of the stains. After two changes of absolute alcohol or acetone, the sections were passed through 1:1 acetone xylene/alcohol-xylene and cleared in xylene. The sections were mounted with DPX mountant.

For histochemical staining, acrolein-Schiff reaction for proteins, periodic acid Schiff (PAS) for glycogen and toluidine blue for RNA were used. The sections were critically examined under oil-immersion objective and were photographed.

6.3. Results

6.3.1. Gills

Control Medium

In the control fish, the gills are unaltered in their structure. In normal fish there are two sets of 4 holobranchs forming the sides of the pharynx. Each holobranch consists of two hemibranchs projecting from the
Posterior edge of the branchial arch. The hemibranch consists of a row of long thin filaments, the primary gill filament. The surface of the primary gill filament is further increased by the formation of regular semilunar folds across its dorsal and ventral surfaces, the secondary gill filament. The secondary gill filament consists of an envelope of epithelial cells. The epithelial layers of two sides are separated by pillar cells or pilaster cells. The pillar cells are arranged in rows, occupying the whole area of the secondary gill filament. At the base of the secondary gill filament, the epithelial layer of one gill fold fuses with that of other to form a complete lining to enclose blood channels which connect the afferent and efferent lamellar vessels. Gaseous exchange takes place across the surface of secondary gill filaments (Fig 6.1-5).

**Exposure to 3.93ppm Phosphamidon**

The fish *C. carpio* exposed to the sublethal concentration (3.93ppm) of phosphamidon for 30 days, developed the following histological changes in the gill tissue. Extensive haemorrhage in blood vessels of the gill rachis and gill filament, swelling up of secondary gill filament, haeman-giectasis and disorganisation of the epithelial layer were observed (Fig 6.6 - 10).

In the same (3.93ppm) concentration, after 60 days of exposure, the degree of damage to the gill architecture was higher. Complete disorganisation of secondary gill filaments and the disintegration of their epithelial lining were noticed. The integrity of the connective tissues was affected. Epithelial corrosion was noticed. Hypertrophy of mucous cells, pyknotic nuclei in epithelial cells and necrosis were other common features in the gills. Edematous condition was noticed in many epithelial cells. The secondary gill filaments got fused with adjacent filaments and looked like bulbous structures (Fig. 6.11 - 16).
Fig (6.1)

Photomicrograph showing the structure of control gill, with perfect ra supported by core of cartilagenous skeleton (SK) and comb like arrangement of gill filament (F). The finger like gill filament is composed of epithelial c forming outer layer. In the epithelial layer the mucous cells are scattered. The tip of the filament has pillar cells (P).

Heidenhain's iron haematoxylin x Ca 400.

Fig (6.2)

A single gill filament (S) of control fish enlarged showing cellular organisation. The tip of the filament has pillar cells (P). The core of the filament (E) formed by blood vessels associated with arteriole (BV) and vein. The bran capillary extends up to the tip of the filament.

Heidenhain's iron haematoxylin x Ca 100.

Fig (6.3)

The tip of gill filament of control fish is enlarged in this micrograph. Blood capillaries and the intervening blocks of tissues are clearly visible. Mucocytes are seen on the outer border of the gill filament (M). See the presence of erythrocytes in the gill filament (E).

Heidenhain's iron haematoxylin x Ca 400.
Fig (6.4)

Section showing a portion of gill filament of control fish in which b1 sinuses, (BS) connective tissues (CT), cartilagenous support (C) & muscles(M) are seen in normal condition. A core of connective tissue form the axis (CT) and regular row of transversely cut branchial (BV) capillaries also seen.

Heidenhain's iron haematoxylin x Ca 400

Fig(6.5)

Section of gill of control fish showing normal presence of PAS positive material and uniformly distributed mucocytes (M) over the respiratory surface (SK) skeleton, (MU)-muscles. The mucos is mostly the neutro-polysaccharide.

PAS - Coelestine blue x Ca 40.

Fig 6.6

A section through the gill of fish exposed to 3.93 ppm for 30 days showing extensive haemorrhage in blood vessels (H). Gill filaments get swollen and look stumpy (SG). The muscle tissues are attenuated (MT). The skeleton support appears crumbled (SK).

Mallory's triple stain x Ca 400.
Fig. 6.7

Section showing the tip of gill filament of fish exposed to 3.93 ppm for 30 days showing disintegration of epithelial surface (−), vacuolation of epithelial cells (V), the mucous secretions are seen in the form of mass granule (MG), blood sinuses are dilated (S). The skeletal support to gill filament is weak and transparent (SK).

Heidenhain's iron haematoxylin x Ca 400

Fig. 6.8

Microphotograph showing few secondary gill filaments which have lost their elongated finger-like structure and changed into short, stumpy ovoid structures. See the changes in the skeleton of the gill filament (SK). The matrix of cells became hyaline and a loss of basiphilia is seen. The adjacent secondary gill filaments are fused and haematanglectasis is seen (−). The epithelial margin of the filaments shows blebbing (BLB) and vacuolation (V) in epithelial cells. Necrotic (N) cells are also seen.

3.93 ppm x 30 days.

Heidenhain's iron haematoxylin x Ca 100.

Fig. 6.9

Section through the gill of a fish exposed to 3.93 ppm for 30 days showing aneurism and clumping of blood cells (C). The red blood cells are hypertrophied (RBC). The nucleus in the RBC is highly swollen (N). The mitochondrial clouds are seen (MC). The thrombocytes (T) are club shaped. The macrophages are loaded with debris (M).

Colestine blue - Coles haematoxylin x Ca 100
Fig. 6.10

See the hypertrophied epithelial cells and haemorrhage in the gill of the fish exposed to 3.93 ppm for 30 days (H). The haemorrhage resulted in blood cells developing abnormal characteristics. The nuclei of (N) blood cells are swollen and lie in diagonal position. The red blood cells have (R) a crenated cell wall and loss of basophilia is seen in them. The thromboocyte nuclei are hypertrophied (T). Attenuation is seen in other blood cells (A). Epithelial cells hypertrophied (EH).

Ehrlich haematoxylin - Biebrisch scarlet x Ca 100.

Fig. 6.11

In the fish exposed to 3.93 ppm for 60 days, the gill filaments develop atrophy and many of them become detached from the rachis (SF). The skeletal (SK) support to the filament becomes necrotic and degenerated. The soft tissues surrounding the skeletal core has delaminated leaving gaps (G).

Heidenhain's iron haematoxylin x Ca 400

Fig. 6.12

A section passing through the fish exposed to 3.93 ppm for 60 days shows the degeneration of gill filaments to different magnitudes. Corrosion of the filament and necrosis of cells of the filaments had produced the shortening of their lengths (→). The filaments are short, stumpy and edematous. Adjacent filaments are fused (F). The soft tissues in the skeletal core (SK) of the rachis had delaminated leaving gaps.

Ehrlich haematoxylin x Ca 100
Fig. 6.13

Micrograph showing the magnified part of some of the gill filaments. See corrosion and complete deorganisation in the gills. The epithelial layer of filaments have delaminated with a complete loss of skeletal core. Epithel balooning (EB) and edematous gill filaments (ε—) due to the accumulation fluid including blood are obviously seen.

3.93 ppm - 60 days.

Ehrlich, haematoxylin x Ca . 40.

Fig (6.14)

Section through the gill of fish exposed to 3.93ppm for 60 days showing decrease in PAS positive material (ε—). The length of the gill gets shorter with a dilation of central core of rachis (R). Some of the gill filaments curved in their terminus (C). Some other had become edematous. Some them had become corroded and only their basal stumps remained and they were projecting as a bladder-like expansions (B).

PAS Coelestine blue - Coles haematoxylin x Ca 100.

Fig (6.15)

Micrograph showing the epithelial cells of gill filament of a fish exposed 3.93 ppm for 60 days. See the complete loss of basiphilia indicating very low protein in the cells. The nuclei are hypertrophied (H). Karyorrhexis noticed (K) is some cells. The epithelial cells are edematous indicating loss cellular osmoregulatory mechanism (E).

Colestine blue-Coles haematoxylin x Ca 1000.
Fig (6.16)

A magnified portion of the terminus of the gill filament showing hypertrophied pillar cells (PC). The nucleoli in some cells are pyknotic (PY) and loss of basiphilia are seen. Loss of cytoplasmic matrix is quite obvious in many cells (C). Aneurism is seen in several sites (A). Mucocytes are hypertrophied (M) and phagocytic (P) invasion is also seen.

3.93 ppm - 60 days
Coelestin blue - Coles haematoxylin x Ca 400

Fig. 6.17

A section passing through a gill of fish exposed to 7.93 ppm for 30 days showing the disintegration of epithelial cells in the gill filament (→). Pillar cells are completely deorganised and vacuolation appear (PV). Blood vessels are dilated (BV). A collection of blood cells (BC) inside the sinuses (BS) develop hypertrophy. The nuclei of blood cells are extremely swollen (BCM). Mucocytes are hypertrophied. Haemorrhage and scattering of blood cells are seen (H). Mucous lining is thick in the outer margin of gill filament (M).

Ehrlich haematoxylin x Ca 100

Fig (6.18)

Another micrograph showing the terminal portion of gill filament. Epithelial cells are corroded and mucous granules (M) are released excessively. Vacuolation is seen amidst pillar cells (V). Loss of basiphilia is seen in many cells (B). Blood vessels (BV) get ruptured leading to haemorrhage in blood sinuses (BS). Desquamation of epithelial cells and ingestion of epithelial cells that were sloughed off from the lamellae are seen (IE).

Ehrlich haematoxylin - Biebrich scarlet x Ca 400
Fig. 6.19

See the separation of subepithelial layer from the epithelial layer in the gill fish exposed to 7.86 ppm for 60 days. Unusual extragrowths are seen in the subepithelial part (EG). Epithelial layer corroded (→). Macrophage invasion are seen (M). Blebbing is seen (B). The skeletal support is vacuolated (V). Pyknotic nuclei and loss of basiphilia are seen in pillar cells (P).

Colestine blue - Coles haematoxylin x Ca 1000.

Fig. 6.20

A portion of lamellae is magnified (7.86 ppm 60 days). See the heavy invasion of phagocytes in the (P) degenerated cellular debris site (D). The nucleus hypertrophied in RBC(RN). The blood sinuses are seen (BS) with few blood cells. Loss of basiphilia is quite obvious in many cells. Lymphocytes are set with atrophy (L). Blood cells are highly hypertrophied with swollen nuclei (BC).

Colestine blue - Coles haematoxylin x Ca 1000

Fig (6.21)

Another micrograph showing heavy infiltration of macrophages (M) to eat the cellular debris formed due to phosphamidon toxicity on the cells of the lamellae.

7.86 ppm - 60 days.

Colestine blue - Coles haematoxylin x Ca 1000.
Fig. 6.22

Photomicrograph of a portion of gill lamella showing cellular deformation of fish exposed to 7.86 ppm for 60 days. Cells have become desquamated and lost their shape (→). Highly hypertrophied and vacuolated cells are common (HV). Disintegration of nucleus is seen (N). The epithelial lining is corroded, exposing the subepithelial layer. Highly attenuated nuclei are seen (AN).

Coelestine blue - Coles haematoxylin x Ca 400.

Fig (6.23)

A photomicrograph showing extragrowth of respiratory structure from epithelial wall of the gill lamella (←). It is a mass of tissue without internal support.

7.86 ppm x 60 days.

Heidenhains iron haematoxylin x Ca 400.

Fig 6.24

A magnified view of extragrown structure in the gill lamellae. See the blebbi in the epithelial layer (BB). The cells in this structure are swollen.

7.86 ppm x 60 days.

Heidenhains iron haematoxylin x Ca 1000.
Fig 6.25

Photomicrograph showing the highly deorganised gill lamella and addition lamellar growth (→) of respiratory structures. Also disintegrated addition respiratory structures are focussed (←L).

7.86 ppm x 60 days.

Heidenhains iron haematoxylin x Ca 100.

Fig 6.26

Photomicrograph showing the extragrown lamellae in the gill of fish exposed 7.86 ppm for 60 days. The lamellae appears as a mass of cells with club shaped outline (→). Blood vessels are seen at the base of the lamellae (BV).

Heidenhains iron haematoxylin x Ca 400

Fig (6.27)

Photomicrograph showing the different phases of development of lamellae (from the gill filament (GF). The cells of the filaments are greatly swollen indicating the breakdown of osmoregulatory mechanism (S).

7.86 ppm x 60 days.

Heidenhains iron haematoxylin x Ca 400
Fig 6.28

Micrograph showing a magnified part of the above section in which lamel have swollen cells (S). See the binucleate condition and one nucleus hypertrophied (HN). The other is pyknotic (PN). Nuclear vacuolation (NV) seen. Macrophages are seen in the part of the gill filament where cells have degenerated (M). In a cell, the nucleus appears crescentic in outline (CN).

7.86 ppm x 60 days.

Heidenhains iron haematoxylin x Ca 1000

Fig 6.29

Photomicrograph showing poor protein content and disintegration in the gill (←).

7.86 ppm x 60 days

acrolein - Schiff x Ca 40.

Fig (6.30)

Micrograph showing the section of cartilagenous skeletal tissue of the gill fish exposed to 7.86 ppm for 60 days showing swollen cells with polymorphonuclear characters in the nuclei (N) of the different cells. Mucous content is poor in the cytoplasm (PR). But the cell wall and nuclear wall have rich mucous.

PAS - Coelestine blue x Ca 400
Exposure to 7.86 ppm Phosphamidon

In the fish exposed to 7.86 ppm of phosphamidon for 30 days the epithelial disorganisation was high. Desquamation of epithelial cells and ingression of epithelial cells that were sloughed off from the secondary lamellae were visible. The mucous cells were hypertrophied. The pillar cell nuclei became pyknotic. Subepithelial layer was exposed on account of the corrosion of the epithelial layer. Hypertrophy of blood cells, increase in erythrocyte number and haemorrhage were common in blood sinuses (Fig 6.17-19).

After 60 days of exposure to 7.86 ppm of phosphamidon, remarkable deterioration was noticed in the cellular organisation of secondary and primary gill filaments. The secondary gill filament got corroded. Epithelial layer and several such gill filaments became short, stumpy and fused with adjacent filaments. Also development of additional secondary filament like structures were noticed. Soft tissues surrounding the rachis became delaminated leaving gaps. Some gill filaments showed blebbing and aneurism. Mucocytes were increased in number. In several sites the primary and secondary gill filaments looked like a mass of debris. Degeneration of rachis and weakening of skeletal support to the gills were noticed. Vacuolations have developed in the places of cartilagenous skeletons (Fig 6.20-30).

6.3.2 Liver

Control Medium

In the normal fish the liver is found to have a bilobed structure. The liver architecture forms a labyrinth of double plated muralia. Pancreatic exocrine tissue is scattered throughout the liver with a more or less regular distribution. For this reason, the liver is called the hepatopancreas. Randomly distributed bile duct (ules) appear as tubules formed by cuboidal
epithelium surrounded by a flat to columnar sheath of connective tissue. Clusters of swollen melano-macrophages centre are present scattered throughout the liver.

In normal liver hepatocytes lobules, which are the parenchyma cells of the liver measuring 8.1 μ to 10.9 μ in length and 6 μ to 8.1 μ in width form a homogenous mass and are grouped into lobules in the liver. The hepatocytes are arranged radially around branches of the hepatic vein in the form of distinct cords. The hepatocytes are polygonal and isodiametric. Each hepatocyte contains a distinct, centrally placed nucleus with densely stained chromatin. The nucleoli are very conspicuous in the hepatocytes and are one or two in number. The cytoplasm of parenchymal cell is basiphilic and several ergastoplasmic plaques or trabaculae (the granular endoplasmic reticulum) are scattered in the cytoplasm. Numerous blood vessels called blood lacunae or sinusoids and intracellular narrow long bile canaliculi are scattered in the hepatocytes (Fig 6.31 - 35).

**Exposure to 3.93 ppm phosphamidon**

On exposure to 3.93 ppm concentration of phosphamidon for 30 days, the liver of the fish *C.carpio* developed extensive damage in peripheral hepatocytes resulting in loss of their polygonal appearance and rupture of cell membrane. Increased flow of blood and haemorrhage in the hepatic blood vessel were observed. When blood escapes into tissues it accumulates as blood filled space called haematoma. The hepatic blood vessels are hypertrophied. Hypertrophy of nucleus, loss of basiphilia edematous hepatocytes and development of vacuoles were observed in the hepatocytes. (Fig. 6.36 - 38).

After 60 days of exposure to 3.93ppm phosphamidon concentration, the liver of the fish *C.carpio* developed marked deformities in cellular construction of the hepatopancreas or liver. The hepatic cords lost their
Fig (6.31)

A section of hepatopancreas (liver) of control fish showing regular distributed pancreatic exocrine tissue with secretions (Large arrow head). Bile ductules appear as tubules formed by cuboidal (small arrow head) epithelium surrounded by columnar sheath of connective tissue.

Heidenhain's iron haematoxylin x Ca 40.

Fig (6.32)

A microphotograph of hepatopancreas of control fish showing bile duct (+), exocrine pancreatic tissue and muralial architecture without lobular structure. There is uniform slight vacuolation indicative of glycogen storage.

Heidenhain's iron haematoxylin x Ca 40.

Fig (6.33)

A microphotograph showing the pancreatic secretions (→) in the pancreatic tissue of control fish. See the normal architecture of hepatocytes.

Heidenhain's iron haematoxylin x Ca 400.
Fig (6.34)

Another section showing the flow of pancreatic secretions into their duct (-)

Heidenhain's iron haematoxylin x Ca 100.

Fig (6.35)

A section showing the architecture of hepatopancreas in control fish. The hepatocytes are magnified (HC). The hepatocytes store fat (F) and glycogen (G). The glycogen is more lacy and more diffusely distributed (P) than fat (lipid), which is manifested as globular droplets. Note the normal shape of RBC in the lumen (R).

Heidenhain's iron haematoxylin - Biebrich scarlet x Ca 400.

Fig (6.36)

Section of liver of fish exposed to 3.93 ppm for 30 days showing haemorrhage and agglutination of RBC (HM) in a sinusoid (S) in the liver. RBC's are highly abnormal in shape (RBC). The hepatocytes are hypertrophied. Pancreatic cells are also hypertrophied (PH) with swollen nucleus (PC). Accumulation of globules distinct (F). Bile duct vacuolation (BV) and extreme swelling of bile duct epithelium (Bx–).

Heidenhain's iron haematoxylin - Biebrich scarlet x Ca 100.
Fig (6.37)

A section of liver of *C. carpio* exposed to 3.93 ppm of phosphamidon for days showing nodular lesion (left) diagnosed as hepatocellular adenoma showing obvious compression and clear demarkation from surrounding tissue but with near normal cytology. Note the presence of normal tissue not in neoplastic liver tissue, and pancreatic tissue (P). Pancreatic cells swell and are hypertrophied. Sinusoid enlarged (S).

Heidenhain's iron haematoxylin - Biebrich scarlet x Ca 100.

Fig (6.38)

See the detailed view of pancreatic tissue of *C. carpio* exposed to 3.93 ppm for 30 days showing hypertrophied pancreatic cells (HP). Haemorrhage (H), Hypertrophy of nucleus (N) and nucleolus. Vacuolation appeared in the hepatocytes (V), abnormal shape of RBC.

Heidenhain's iron haematoxylin - Biebrich scarlet x Ca 100.

Fig (6.39) a

Section showing the liver cells and intervening sinusoids of fish exposed to 3.93 ppm for 60 days in the damaged condition. Endothelial cells damaged with dilation of sinusoids is diffuse. Hepatocytes show various pattern of degeneration and necrosis: severe acute swelling of hepatocyte with cytolysis (C) ii, cell shrinkage and coagulative necrosis seen (N) iii, Vacuolar degeneration of hepatocytes that border sinusoids (V). Besides nuclear degeneration in hepatocytes, haemorrhage and accumulation of lipid globules (L), invasion of phagocytes (P) and Monocytes (M). In some hepatocytes pyknotic nucleus, loss of basophilia both in nucleus and cytoplasm are seen. Mitochondrial swelling (MS) is seen.

Ehrlich Haematoxylin - Biebrich scarlet x Ca 1000.
Fig (6.39) b

Section showing the liver cells and intervening sinusoids of fish exposed to 3.9 for 60 days in the damaged condition. Endothelial cells damaged with dilated sinusoids is diffuse. Hepatocytes show various pattern of degeneration and necrosis severe acute swelling of hepatocyte with cytolysis (C) ii, cell shrinkage coagulative necrosis seen (N) iii, Vacuolar degeneration of hepatocytes that be sinusoids (V). Besides nuclear degeneration in hepatocytes, haemorrhage accumulation of lipid globules (L), invasion of phagocytes (P) and Monocytes in some hepatocytes pyknotic nucleus, loss of basiphilia both in nucleus cytoplasm are seen. Mitochondrial swelling (MS) is seen.

Ehrlich Haematoxylin - Biebrich scarlet x Ca 1000.

Fig (6.40)

See the liver of fish exposed to 3.93 ppm for 60 days showing damaged parenchymatous tissues in liver. The nucleus become turgid (N) and perinuclear space dilated. Refraction of nucleoplasm occurs and is accompanied clumping or peripheralization of chromatin along the nuclear envelope Pyknotic nuclei are seen (P). Monocyte (M) invasion, and rich presence of macrophages in the necrotic site of liver cells. RBC hypertrophied (R). Lipid globules are seen (L). Karyolysis (K) is common. Haemorrhage is seen in several sites.

Ehrlich haematoxylin - Biebrich scarlet x Ca 1000

Fig (6.41)

Micrograph of fish exposed to 3.93 ppm for 60 days showing vacuol hepatocytes (V), reduction in glycogen content, hypertrophied (h) as well pyknotic nucleus (P), dilated sinusoids, and swollen mitochondria (M) seen.

PAS - Coeleistine blue - Cole's haematoxylin x Ca 1000
Fig (6.42)

Liver of control fish showing rich PAS (←) positive material indicating the presence of glycogen.

PAS x Ca 400

Fig (6.43)

Liver of fish exposed to 7.96 ppm for 30 days showing multiloculated hepatocellular hypervacuolation (←). Note the absence of fibrous sheet, irregular shape, slight compression, and condensed nuclei. Vacuolation ischaemic nuclei, hepatocytes with fibrillar cytoplasm are seen. Macrophage invasion is distinct (M). Mitochondrial swelling (MS) are also common.

Ehrlich haematoxylin - Biebrich scarlet x Ca 400

Fig (6.44)

Microphotograph of liver of fish exposed to 7.86 ppm for 30 days showing attenuated pancreatic tissue (←) with lacunae in the middle of exocrine tissues (V). The bile duct epithelial cells are hypertrophied.

Ehrlich haematoxylin - Biebrich scarlet x Ca 400.
Fig (6.45)

Histological changes in the liver of fish exposed to phosphamidon (7.96 for 30 days) showing the accumulation of fat globules (F), melanomacrophage invasion, mucous secretions (MU), glycogen rich cells (G), vacuolated cytoplasm (V) and hypertrophied hepatocytes and their nuclei (N). Hep (HT) like condition is seen.

Ehrlich haematoxylin x Ca 400.

Fig (6.46)

Micrograph showing attenuated pancreatic exocrine tissue with fat globules and glycogen (G) rich cells, melanomacrophage invasion (M) is seen. Vacuolation are seen in hepatocyctic pancreatic tissues. Haemorrhage are seen.

7.97ppm - 30 days
Ehrlich haematoxylin x Ca 400

Fig (6.47)

The liver of fish showing attenuated hepatocytes (H). Necrotic cells (N) karyorrhexis (KR) are seen. M- Macrophage

7.96 ppm x 60 days
PAS x Ca 400
Fig (6.48)

Liver of fish exposed to 7.86 ppm for 60 days showing deformed liver. No structures seen (→). Hepatocytes are hypertrophied (H). Nuclei are pyknotic (N), heavy vacuolations (V) are seen. Enlarged blood lacunae (RPN) are seen. M- Monocytes.

PAS x Ca 400

Fig (6.49)

Micrograph showing a highly disorganised liver tissue, in which the invasion of macrophages are prominent. The macrophages are loaded with debris and liberated into the sinusoids of hepatic tissue (M). Hepatocytes hypertrophied (H). Loss of basophilia, swollen mitochondria and vacuolation are seen. Karyorrhexis is prominently seen in many cells. Glycogen content reduced.

PAS x Ca 1000.

Fig 6.50

See the highly deorganised liver tissue showing vacuolation in hepatocytes (Accumulation of debris in macrophages(M), degenerated pyknotic nucleus (I abnormal RBC(R), poor presence of glycogen and loss of basophilia (→).

7.86ppm x 60 days

PAS x Ca 400.
Fig. 6.51

Liver of fish showing fibrotic condition (F), swollen cells, nuclear hypertrophy (N), karyolysis (K), heavy vacuolation (V), accumulation of cellular debris (--) and invasion of monocytes (M) are seen.

PAS x Ca 100.

Fig. 6.52

Photomicrograph showing a poor protein content (--) in the liver of fish exposed to 7.86 ppm for 60 days. Refractile amorphous granules are seen (RA)(R). The fat cells are seen in clusters (F).

Acrolein - Schiff x Ca 1000.
Fig 6.51

Fig 6.52
form and got completely disorganised. The cellular structure of the wall of blood sinusoids was lost. Haemorrhage of sinusoids resulting in spilling of blood cells into the surrounding tissues were noticed. The erythrocytes were completely scattered among the hepatocytes. The hepatocytes contained different amounts of cytoplasmic components as evident from different degree of staining compared to the surrounding parenchyma. Bile duct vacuolization, hepatocellular hyper vacuolation, necrosis, fibrillar hepatocytes and pre-neoplastic lesions were observed in the liver. Basiphilia were completely lost in the cytoplasm of hepatocytes. The nuclei became pyknotic and some of the nuclei were necrotic. The RBC showed vacuolation and it was loaded with some granules. In addition to these changes, there is fatty accumulation in hepatocytes. Degeneration of hepatocytes was noticed. (Fig. 6.39 - 42).

**Exposure to 7.86ppm Phosphamidon**

With 30 days of treatment at higher concentration of phosphamidon, there were marked alterations in the architecture of hepatopancreas. The hepatic cord lost their appearance along with the breakdown of sinusoids. Pronounced vacuolation with criss-cross blood vessels were seen in the liver tissue. Histolysis and appearance of cell debris as a lump of granules were observed. The phagocytic invasion of the hepatopancreas was prominent. The hepatic cells were hypertrophied and hyperplasia was also observed. The hepatocytic necrosis was common. The nucleus of the hepatocytes developed pyknosis. The nucleoli were also hypertrophied. The cytoplasm appeared confluent and showed amorphous inclusion. The pancreatic cells showed an increase in zymogen granules (Fig. 6.43 - 46).

After 60 days of exposure in the higher concentration of phosphamidon, the fish developed greater degenerative changes in their hepatopancreas. The liver cells and the blood cells in the hepatopancreas had lost their morphology and were degenerated. Empty spaces appeared in
the liver tissues and increased phagocytic activities were seen. Refractile amorphous granules were found in large clusters in the midst of the debris. Many cells were without nuclei and cytoplasm was granular. The nucleus became hypertrophied. Necrosis and hyperplasia were noticed in the hepatic parenchymatous cells. Nuclear vacuolation was distinct. Loss of basiphilia in the cytoplasm was seen. The mitochondria had become stumpy granules in the cytoplasm. Acinar cells were hypertrophied with an increase in zymogen granules.

The protein content got decreased remarkably in the liver cells. The glycogen level also got decreased in the liver tissue of the fish on exposure to high concentration of phosphamidon for a longer period. There is a complete loss of basiphilia of cytoplasm in hepatocytes. The nuclei became pyknotic and some of the nuclei were necrotic. The necrotic cells were shrunken and their intracellular attachment were broken. Necrotic cells appeared distorted, smudged and homogenous. Nuclei were contracted. The nuclei were shrunken. Nuclear membrane had ruptured with fragmentation and release of nuclear contents (karyorrhectic) and complete dissolution of the nucleus with loss of chromatin material (Karyolytic) (Fig. 6.47 - 52).

6.3.3. Stomach

Normal Histology and Histochemistry

The stomach of C.carpio has an inner most mucosal lining consisting of epithelial cells and gastric glands. Underlying this layer is lamina propria consisting of connective tissue. The lamina propria is followed by muscularis mucosal layer consisting of smooth muscle fibres arranged in criss cross fashion. The mucous layer is made up of a continuous gastric epithelium composed of tall columnar cells. Underlying the gastric epithelium gastric glands are present (which appear circular in cross section). The glands are clustered together in groups. The glands are composed of cuboidal glandular epithelial cells.
External to the muscularis mucosal layer, is the submucosal layer consisting of dense connective tissues. The blood vessels and lymph vessels are present in this layer. Enveloping this layer is the muscularis externa and oblique muscles. The outermost layer is a thin layer of connective tissue called serosa (Fig. 6.53 - 57).

**Histopathological and Histochemical changes**

**Exposure to 3.93ppm Phosphamidon**

In the fish exposed to the low concentration of phosphamidon for 30 days, the most obvious feature was the enhanced blood supply to the stomach. The mucosal epithelial cells and the gastric glandular cells of the mucous wall registered hyper-secretion of mucous although they were hypertrophied in their cell structure. Phagocytic invasion into the intergladular region of the mucosal region was also observed (Fig. 6.58 - 63).

The stomach of fishes is treated in lower concentration for 60 days showed severe damage to mucosa. The epithelium and the mucous gland cells lost their characteristic organisation. Cell debris was found accumulated in the lumen of the stomach. Abundance of phagocytes in the lumen as well as on the wall of stomach was noticeable. Secretory activities in the gastric cells are retarded. Hypertrophy and different degree of necrosis and cytolysis were seen in the gastric gland cells. Cytoplasm was highly vacuolated. Most of the cells were moribund. A manifold increase of rugae are observed in the stomach wall. The connective tissues blood vessels have undergone haemorrhage. Unusual development of intestinal caecum with multifolded characteristics like scroll valve is seen. (Fig 6.64 - 69).
Stomach of control fish showing highly enlarged gastric glands (GG). The zymogenic cells (ZC) with zymogenic granules (ZG) are visible. Mucous epithelium (ML) and columnar submucosa (ME) with well defined connective tissues are seen. The lumen of stomach shows food particles (F), phagocytes (P) and RBC (R). Secretions are poured into the lumen in the form of granules. The gland cells are clustered into groups.

Mason’s trichrome x Ca 400.
Fig 6.53
Fig. 6.54

A section through the stomach of control fish showing normal histology. The mucosa is made up of columnar epithelial cells (CE). Amidst the columnar cells the secretions flows into the lumen. The lumen of stomach is engorged with food (F). Underlying the gastric epithelium the gastric glands are clusters (GG). The gastric gland cells are surrounded by connective tissue (C) followed by circular and longitudinal muscles (MU).

Mason’s trichrome x Ca 40.

Fig. 6.55

Stomach of control fish showing clusters of gastric gland cells (GG) beneath the columnar layer. Mucous cells secretions (MS) are poured into the lumen. Stomach is filled with digested food (DF) and phagocytes (P).

Ehrlich haematoxylin - Biebrich scarlet x Ca 400.

Fig. 6.56

A section through the gastric glands and nerve fibres of control fish showing circular gastric glands with rich secretions (→).

Heidenhains iron haematoxylin x Ca 400
Fig (6.57)

See the photomicrograph of stomach of control fish showing normal mucosa (MU), submucosal (SM), gland cells (G), connective tissue (C) and muscular coat (M).

Heidenhains iron haematoxylin x Ca 100

Fig (6.58)

Stomach of fish exposed to 3.93 ppm for 30 days showing attenuated necrotic gastric gland cells (GG). The connective tissues (C) are affected, Haemorrhage (H) is seen.

Mallory triple stain x Ca 400.

Fig (6.59)

Section through the stomach of fish exposed to 3.93 ppm for 30 days showing stomach wall and rugae. The mucosa is hypertrophied, vacuolated with swollen mucous cups (→). The gastric glands are hypertrophied (GG). Thickening of mucous layer due to increased output of gastric mucin observed (←M). Erosion of mucosa and hyperplasia (H) is observed in the place. Extensive haemorrhage as evident from RBCs (R) accumulation in mucosa and connective tissue layer. Connective tissue (CT) is broadened and showing ruptured blood vessels. The submucosal muscularis also show corrosion, discontinuity (D) and haemorrhage.

Mallory triple stain x Ca 400.
Fig (6.60)

Micrograph showing manifold increase of rugae (R) in stomach wall. See development of additional extensions from rugae (→).

Ehrlich haematoxylin x Ca 40.

Fig (6.61)

See the gastric glands in the gastric mucosa of fish exposed to 3.93 ppm for days. The gastric glands are hypertrophied and are found over the ent corpus. Of the different types of gastric gland cells, the zymogenic cells (z) and mucous cells (M) are seen with hypertrophied condition. The mucous granules are prominent and are heavily shed into the mucosal lining (→M). The zymogen granules are in the form for clusters (→Z).

PAS - Coelestine blue x Ca 100.

Fig (6.62)

Stomach of fish exposed to 3.93 ppm for 30 days showing hypertrophied gastric glands (GG) and enrichment of undigested food, cellular debris and phagocytes in the lumen (→).

PAS - Coelestine blue x Ca 40
Fig 6.60

Fig 6.61

Fig 6.62
Fig (6.63)

Another micrograph showing the stomach wall of fish exposed to 3.93 ppm for 30 days. The mucosal layer are interspersed with mucous cups and thick line of mucin bordering the mucosa is visible (→). Mucous cells and columnar cells are hypertrophied (H). The gastric glands are hypertrophied (GH) with rich presence of secretory granules and open into gastric pit or foveolae. The connective tissues are (CT) affected. Haemorrhage is seen (H) in blood vessels (BV). The submucousa is obliterated (SM).

PAS - Coelestine blue x Ca 40.

Fig (6.64)

Section showing the posterior part of stomach in which unusual development of intestinal caecum with multifolded characteristics like scroll valve (→). Gastric epithelial cells have blebbing in the outer margin in the caecum (B). The stomach wall from which the caecum arise also has hypertrophied mucosal cells (M). The gastric glands underneath the epithelium in caecum are swollen with loss of basiphilia (GE). Secretory activities are poor in these gland cells. The connective tissue are hyaline (C). The submucosa and serosa also show signs of degeneration (←S). Hyperplasia is seen in gastric mucosa (H).

3.93 ppm 60 days.

Ehrlich haematoxylin - Biebrich scarlet x Ca 40

Fig (6.65)

A portion of caecum magnified (←).

3.93 ppm, 60 days.

Ehrlich haematoxylin - Biebrich scarlet x Ca 100.
Fig (6.66)

Section of stomach of fish exposed to 3.93 ppm for 60 days. Extensive haemorrhage resulted in pools of blood at several places (←). The blebbing free end of mucosal cells (M), thickening of mucous lining (ML), rich presence of secretory granules in zymogen cells of gastric glands (Z), loss of basiphil in columnar cells (C), hypertrophied gastric glands (HG) are common.

Ehrlich haematoxylin - Biebrich scarlet x Ca 100

Fig (6.67)

Section of stomach of fish exposed to 3.93 ppm for 60 days showing hypertrophied mucous cells (MC), enlarged blood vessels (BV) in the connective tissues, widening of foveolae (F), attenuation of gastric glands (GG) and increased flow of mucous from mucous cells to the mucosal margin.

Heidenhain's iron haematoxylin x Ca 400

Fig (6.68)

A portion magnified in above micrograph showing hypertrophied mucous cells with (M) mucous granules. Phagocytic invasion is also seen (P).

Heidenhain's iron haematoxylin x Ca 400.
Fig (6.69)

A cross section of stomach of fish exposed to 3.93 ppm for 60 days showing poor presence of food in the lumen (−). The lumen has degenerated cellular debris (CD). The musculosa is hypertrophied (MU).

Ehrlich haematoxylin Biebrich scarlet x Ca 40.

Fig (6.70)

Micrograph of stomach of fish exposed to 7.86 ppm for 30 days showing attenuated, necrotic and hypertrophied gastric glands (−). The mucous cups are enlarged (MC). Extensive, haemorrhage is seen (HE).

Mallory's triple stain x Ca 100

Fig (6.71)

Section of rugae in which epithelial cells have hypertrophied (H). The mucous cups (M) were found to be large. Haemorrhage is seen in the lumen. The lumen is loaded with disintegrated cellular debris (−) and phagocytes (I). The connective tissue is constricted (C).

7.86 ppm x 30 days.

Mallory's triple stain x Ca 400.
Fig (6.72)

Section through the stomach of fish exposed to 7.86 ppm for 30 days showing "corrosion" in mucosal epithelium (←). Phagocytic invasion (P) is seen at that place. Constriction (C) of stomach wall can be seen from the restricted lumen (↔).

Mallory's triple stain x Ca 40.

Fig (6.73)

Section showing the highly disorganised mucosa. The mucous epithelium disintegrated (←). The gastric glands are directly exposed to the lumen. Blood vessels (BV) are hypertrophied in the connective tissue. The lumen full of RBC (R) due to hemorrhage. Muscle fibres are distinctly separated (↓)

7.86 pp x 30 days

Mallory's triple stain x Ca 400.

Fig (6.74)

Section showing the stomach of fish exposed to 7.86 ppm for 60 days showing necrotic, attenuated gastric gland (GG). Thickening of mucosal layer (M) and corrosion of mucous layer is also noticed. Highly dilated blood vessels in connective tissue (BV), the musculosa is disintegrated (MS). The lumen is seen with the accumulation of cellular debris (D), blood cells (BC) and phagocytes (P).

Mallory's triple stain x Ca 40.
Fig 6.72

Fig 6.73

Fig 6.74
Fig. 6.75

A photomicrograph showing the constriction of lumen of stomach (→). The lumen is engorged with degenerated cellular debris and phagocyte. Disintegration of mucosal epithelium and oozing of blood from connective tissue part into the lumen through the disintegrated site of epithelial layer seen(←O). Vacuolation is seen in epithelial cells (V). Gastric glands (GG) are hypertrophied. Secretory granules get conglomerated into a single mass (SG). Blood vessels are hypertrophied (BV). Muscularis layer shows deorganisatic (MU).

Ehrlich hæmatoxylin - Biebrich scarlet x Ca 400.

Fig (6.76)

Micrograph showing the magnified portion of gastric glands. The mucous gland cells are highly hypertrophied (M). The zymogenic cells with granules at having vacuolation in the midst of their clusters (Z). The parietal cells (P) are hypertrophied. Pyknotic nuclei are seen in gland cells (PN).

7.86 ppm x 60 days

Mallory's triple stain x Ca 400

Fig. 6.77

A Photomicrograph showing highly disintegrated gastric epithelium in the stomach of fish exposed to 7.86 ppm for 60 days. Epithelial cells have lost the organisation (←) and sloughed off in to the lumen. The lumen of the stomach is invaded by different forms of phagocytes (P), Blood cells (BC) and lyse tissues.

Mallory's triple stain X Ca 400
Section Showing the stomach wall of fish exposed to 7.86 ppm for 60 days. The gastric mucosa is invaded by different forms of bacteria (←BA). Gland cells are hypertrophied (H). Vacuolation and poor secretory activity of zymogen cells (Z) and in other cells.

Acrolein Schiff x Ca 400.

Section through the stomach wall of fish exposed to 7.86 ppm for 60 days showing highly hypertrophied mucosa (M), submucosa (SM) and muscul (MU). The epithelial cells of rugae have lost their integrity. Blebbing epithelial cells is seen (B). Gastric glands (GG) are with highly vacuolated cells.

Acrolein Schiff x Ca 100.

A tangential section of stomach wall showing unusual developments (← Hyperplasia (HP), hypertrophied mucous cells (HM), hypertrophied nucleolus (HN). The protein content is poor (←) as glands cells are poorly coloured.

Acrolein Schiff x Ca 100.
Fig 6.78

Fig 6.79

Fig 6.80
Exposure to 7.86ppm Phosphamidon

After 30 days of exposure to the higher concentration, the fish developed marked degeneration in their histology. Disintegration of gland and disorganisation of gland cells were noticed. Secretory activity was poor in the stomach wall. Haemorrhage appeared at several places. The cell became moribund and chromatolysis of the nuclei could be seen. Phagocytic infiltration was heavy in the lumen and stomach wall. The lumen is loaded with disintegrated cellular debris and phagocytes. (Fig 6.70 - 73).

In the fish treated to higher concentration of phosphamidon for 60 days, there was a remarkable increase in the thickening of stomach wall and the lumen was obliterated. The lumen of the stomach was found to be filled with several types of degenerated cells. The mucosal layer was lost. Extensive haemorrhage was visible in the stomach wall. Blebbing of the free end of the mucosal cells was obvious at several regions. The extraordinary hypertrophy of the epithelium of mucosa and the mucous glands were observed. The gastric mucosa is invaded by different forms of bacteria.

As a result of decrease in secretory activity and its ultimate cessation, the protein content also is low in the gland cells coloured by acrolein Schiff reaction [Fig 6.74 - 80].

6.3.4. Intestine

Normal Histology and Histochemistry

The intestine of C.carpio is a long many folded tubular structure. In a cross section, it shows a central lumen surrounded by a single mucosal layer. The mucosal layer is made up of epithelial cells interspersed with goblet cells. The epithelial cells are columnar with a broad free end and a narrow base. Their nuclei are elliptical with dense chromatin and prominent nucleoli. Folds, villi and pitlike digestive glands occur in the
various parts of the canal as modifications of the mucosa. Next to mucosa is a submucosa, filled with fibrous tissue, blood and lymph vessels, a few smooth muscle cells and nerve endings.

A strong layer of circular muscle follows, and this becomes gradually thickened at the entrances to, and exits from, certain parts of the tract. Such ring like thick constrictors are called sphincter muscles. Longitudinal muscles cover the circular layer on the outside, its function being, obviously to shorten parts of the tract and resist stretching by large objects within. The serosa (peritoneum) covers the outside of the intestine. It is simply a connective tissue (Fig. 6.81 - 84).

Histopathological and Histochemical Changes
Exposure to 3.93ppm Phosphamidon

After 30 days of exposure in the low concentration of phosphamidon, the fish developed unusual changes in the intestinal histology. The most remarkable variation was the many fold increase in the intestinal villi and their encroachment in the lumen of the intestine. The epithelial cells and goblet cells of the mucosa developed hypertrophy. The goblet cells had increased in number and copious secretions of mucous was observed in these cells (Fig 6.85 - 87).

A long period of exposure (60 days) in the low dose of phosphamidon, revealed further alterations in the tissue architecture of the intestine of C.carpio. The foldings and microvilli development in the mucosa of the intestine were higher when compared to the low dose exposure for 30days. The microvilli elongation and their fusion with adjacent villi were the interesting development. The terminus of one microvillus fuses with other microvillus and in extreme cases the terminal cellular covering of the microvill was broken (Fig. 6.88 - 91).
Fig. 6.81

Transverse section of intestine of control fish showing normal histology. The mucosa consists of one layer of epithelial cells (M) forming the lining of lumen (L). The submucosa (SM) consists of connective tissue. The outer muscularis coat (MC). Lumen is loaded with digested food. Intestinal villi (V) are normal.

Ehrlich haematoxylin - Biebrich scarlet x Ca 400.

Fig. 6.82

Section showing normal histology of the intestine of control fish. The outer serosa (S), the longitudinal muscles (LM), Circular Muscles (CM), connect tissues (CT), mucosal layer (ML), the peritontial epithelium with mucous loaded goblet cells (GC) and columnar cells (CC) and villi (V) projecting into the lumen are in normal architecture.

Heidenhains iron haematoxylin x Ca 100

Fig. 6.83

A high power view of a section through the intestine of control fish shows serrated margin (SR), mucous loaded goblet cells (GC) and normal columnar cells (CC) in the peritontial epithelium. Phagocytes (P) are escaping into the lumen (L) and the nucleus of goblet cells are seen in normal structure. Mucosa (M) and connective tissues (CT) are supplied with blood vessels (B).

Heidenhains iron haematoxylin x Ca 1000
Fig 6.84

A micrograph showing the section of intestinal wall of control fish. Phagocytes (P) are escaping into the lumen (L). The different types of cells and the nuclear organisation are seen, Goblet cells (GC), columnar cells (CC) connective tissue (CT) Lymphocytes (LC) and blood vessels (BV).

Coelstine blue - Cole's haematoxylin x Ca 1000

Fig. 6.85

Transverse section of intestine of fish exposed to 3.83 ppm for 30 days showing histopathological changes. The mucosal epithelial cells are hypertrophied and the lumen is engorged with undigested food (L). The intestinal villi are abnormal and fusion of adjacent villi (FV) takes place. Lamina propria is distinctly seen (LP).

Ehrlich haematoxylin - Biebrich scarlet x Ca 40.

Fig. 6.86.

A high power view of previous section showing the fusion of intestinal villi (FV). Formation of new villi in between two villi (NV). Lamina propria distinct (LP). Rich capillary bed in villi (BV), haemorrhage (HG), erosion the tip of villi (EV), corrosion of serosa (CS), Intestinal lumen loaded with lysed villi, cellular debris and undigested food (L).

Ehrlich haematoxylin Biebrich scarlet, x Ca 100
Fig. 6.87a

A photomicrograph showing the longitudinal section of intestinal wall *C. carpio* exposed to 3.93 ppm for 30 days showing hypertrophy of goblet cells (G). Rich secretion of neutral (N) (NMP) and acidic (A) (AM) mucopolysaccharides are seen, number of goblet cells (G) increased.

PAS x Ca 100.

Fig. 6.87b

A photomicrograph showing the longitudinal section of intestinal wall *C. carpio* exposed to 3.93 ppm for 30 days showing hypertrophy of goblet cells (G). Rich secretion of neutral (N) (NMP) and acidic (A) (AM) mucopolysaccharides are seen, number of goblet cells (G) increased.

PAS x Ca 100.

Fig. 6.88

A midsagittal section of intestine of fish exposed to 3.93 ppm for 60 days showing complex fusion of villi (FV) and the labyrinthine structure (L).

Ehrlich haematoxylin Biebrich scarlet, x Ca 100
Fig 6.87a

Fig 6.87b

Fig 6.88
Fig. 6.89.

A high power view of above section showing occlusion of the lumen by hypertrophied villi and their fusion (OL). See the very narrow labyrinth system (L). The goblet cells (GL) are numerous in each villus.

Lillie / Aldehyde fuschin - Coelestine blue Cole's haematoxylin - x Ca 100

Fig 6.90

Intestine of fish exposed to 3.93 ppm for 60 days showing the disorganisation of the border in the lumen (DC) of the villi. A brush border is seen (BV) food vacuoles are poorly loaded with food (FV).

Ehrlich haematoxylin - Biebrich scarlet x Ca 40

Fig 6.91

High power view of absorbing cluster cells showing cavity corroded epithelium.

Ehrlich haematoxylin - Biebrich scarlet x Ca 400.
Fig 6.92

Micrograph showing the invasion of phagocytes in the lumen (P). Phagocytes are with food particles (F). Few lymphocytes, erythrocytes and microbial flora are seen amidst food particles. Polymorphism of phagocytes obvious.

Ehrlich haematoxylin - Biebrich scarlet x Ca 400

Fig 6.93a

Intestine of fish exposed to 7.86 ppm for 30 days showing unusual development of villi in the lumen (LA), fusion of villi (FV), serpentine villi (SV), hypertrophied goblet cells (HG), corrosion of serosa (CS obliterated musculosa (OM)). Villi growing towards one another and fusing their tips. Enlarged villi, hypertrophied mucosa and submucosa and lumen villi with food (F).

Aldehyde - fuscin - Coelestine blue x Ca 100

Fig 6.93b

Intestine of fish exposed to 7.86 ppm for 30 days showing unusual development of villi in the lumen (LA), fusion of villi (FV), serpentine villi (SV), hypertrophied goblet cells (HG), corrosion of serosa (CS obliterated musculosa (OM)). Villi growing towards one another and fusing their tips. Enlarged villi, hypertrophied mucosa and submucosa and lumen villi with food (F).

Aldehyde - fuscin - Coelestine blue x Ca 100.
Fig 6.94.

Intestine of fish exposed to 7.86 ppm for 30 days showing hypertrophy musculosa (HM) and serrations in serosa prominent (SS). Peritoneal epitheli showed blebbing (EB). The columnar cells are vacuolated (V), nuclei hypertrophied. Goblet cells enlarged, increase in size and number of goblet cells (GHC).

Coelestine blue-Coles haematoxylin x Ca 400.

Fig 6.95

T.S of Intestine showing histopathological changes. The lumen is filled with undigested food (F). Cellular debris, blood cells (B), phagocytes (P) load with food, food vacuoles (FV) amidstlysed tissue are seen.

Aldehyd-fuscin x Ca 400.

Fig 6.96

Micrograph showing proliferation of goblet cells (GL) and the hypertrophy (GC), changes in serosa, lacuna (L). Swollen muscularis coat (M), phagocytes (P), Hypertrophied epithelium (E) are seen.

Ehrlich haematoxylin x Ca 400.
Fig 6.97

Section showing the intestine of fish exposed to 7.86 ppm for 30 days show a thickening of mucous layer (M), tall narrow columnar cells (LL) which chiefly populating the epithelium and the extent to which they have encroached and squeezed the connective tissue. RBC and lymphocytes, leucocytes oozed out of the capillaries and entering into the cellular spaces. Noteworthy cells are not necrotic but nuclei are pyknotic (NP).

Ehrlich haematoxylin x Ca 400.

Fig 6.98

Intestine of *C. carpio* exposed to 7.86 ppm for 60 days show disorganisation of intestinal histology. The longitudinal muscles obliterated weak (LM). The submucosa eroded (SM). Goblet cells are hypertrophied, poor mucous (GL). Columnar cells lost their rigidity (CC). Corrosion disintegration of connective tissue. L - Lacuna.

Ehrlich haematoxylin x Ca 400.

Fig 6.99

A high power view of intestine of fish exposed to 7.86 ppm for 60 d showing hypertrophied columnar cells (HCC). Pyknotic nucleus (PN). Submucous layer (SL). GL - Goblet cells, L - Lacuna.

Ehrlich haematoxylin x Ca 100.
Fig 6.100

Photomicrograph of section through intestine of fish exposed to 7.86 ppm 60 days showing polymorphic phagocytes (P). Submucosa lost their integrity, Lacunae (L) in circular muscles, necrotic goblet cells are seen (NG).

Ehrlich haematoxylin x Ca 100.
Exposure to 7.86ppm Phosphamidon

In this higher dose of test medium, when the fish were exposed for 30 days, have registered marked abnormal changes in the intestinal wall. The lumen of the intestine was narrowed by heavy insurgence of serpentine microvilli from the mucosa. These long villi also fuse when they encounter each other. Such villi need not be exactly adjacent. Increase in height of the columnar epithelium was also observed. The number of goblet cells were also increased. The increase in number of goblet cells is accompanied by the hypertrophy of the same cells and increased output of mucous by them (Fig.6.92 - 96).

After exposure of (60 days) long duration in higher concentration of phosphamidon medium, the degree of pathogenesis in the intestinal histology was comparatively higher. Prolific increase in number and length of the villi enhances the area of absorption and narrows luminal passage.

In the lumen of the intestine, myriads of phagocytic cells were seen. The phagocytes were found loaded with debris. Heavy presence of both cocci and bacilli bacteria were seen amidst the luminal contents. The phagocytes were found foraging the contents of the intestine. Epithelial erosion was more prominent in the apex of microvilli. The goblet cells were hypertrophied. The nucleus of epithelial cells were pyknotic. The epithelial layer too was corroded at certain regions exposing the submucosal layer in to the lumen. The heavy insurgence of microvilli divided the lumen into compartments and the luminal cavity was reduced. The cells of the microvilli too showed necrosis. The proliferative growth of microvilli indicates the hyperplasia in the mucosal layer.

PAS preparations showed increased output of mucous in the intestine of phosphamidon exposed fishes. (Fig. 6.97 - 100).
6.3.5. Testis

Normal Histology and Histochemistry

The testis contains seminiferous tubules. The walls of the seminiferous tubules are spermatogonic. Sertolicells which are also called nurse cells are seen. Lumen of the seminiferous tubules are filled with sperms. The spermatozoa were normal in shape. The interstitial cells are prominent (Fig 6.101 - 102).

Histopathological and Histochemical Changes

Exposure to 3.93ppm Phosphamidon

In the fish exposed to the lower doses of phosphamidon for 30 days, abortive spermatogenesis was seen. The number of spermatocytes in the seminiferous tubules were low. The interstitial cells were on the decline. [Fig. 6.103 - 104].

In the testis of fish, exposed for 60 days, the spermatogenic activity was relatively poor. The boundaries of the individual seminiferous tubules were lost. The sperms started clumping. The spermatogenic tissues showed different degrees of disintegration, (Fig. 6.105 - 106).

Exposure to 7.86ppm Phosphamidon

At higher concentration of phosphamidon, a greater degree of cellular damages were noticed in the testis. The spermatogenic activity remained stand still. The seminiferous tubules had a meagre collection of sperms in their lumen. The interstitial cells underwent necrosis and degenerated, leaving spaces. Individual boundaries of the seminiferous tubules could not be traced. Abortive spermatogenesis was seen. The degenerated sperms appeared as a collection of debris, (Fig. 6.107 - 109).
Fig 6.101

Section through the testis of control fish showing seminiferous tubules with spermatogenic activity (ST). The lumen of the tubules are filled with spermatozoa (S). The interstitial cells (IC) are normal.

Mallory x Ca 100

Fig 6.102

Photomicrograph showing the section of testis of control fish in which the spermatozoa (S) are moving in the lumen of the seminiferous tubules. The sperms are normal in shape.

Mallory x Ca 100
Fig 6.103

Section through the testis of fish exposed to 3.93 ppm for 30 days showing abruptive spermatogenesis. The number of spermatocytes in the seminiferous tubules (ST) are low. In the interstitial cells sites the interstities appear empty spaces. The sperms (S) are atrophied and head of sperms look condense and pyknotic.

Mallory x Ca 100

Fig 6.104

Section showing a magnified portion of lumen of seminiferous tubules in the testis of fish exposed to 3.93 ppm for 30 days showing poor spermatogenesis activity. Spermatozoa (S) are few in number and are sparsely distributed in the lumen. The heads of spermatozoa are alone visible aspyknotic mass.

Mallory x Ca 1000

Fig. 6.105

See the highly disorganised nature of testicular organisation in the fish exposed to 3.93 ppm for 60 days. The spermatogenic activities are poor. The boundaries of the individual seminiferous tubules (ST) are lost. The spermatozoa are clumped (SC).

Mallory x ca 400
Fig 6.103

Fig 6.104

Fig 6.105
Fig 6.106

A high power view of the clumped sperms (SC) and poor spermatogenic activity in the seminiferous tubules. The sperm (S) heads are attenuated with pyknotic nuclear mass. Spermatogenesis is poor.

3.93 ppm 60 days

Mallory X Ca 400

Fig 6.107

Section through the testis of fish exposed to 7.86 ppm for 30 days showing greater degree of damage to testicular architecture. Only in some seminiferous tubules (ST) a meagre collection of sperms are seen. Abortive spermatogenesis is seen. The sperms are degenerated and appeared as a collection of detritus (SD).

Mallory x Ca 1000

Fig 6.108

Section through testis showing poor spermatogenic activity. Sperms clumped into a mass (SC).

7.86 ppm x 30 days

Mallory x Ca 1000
Fig 6.109

A high power view of a portion of testis of fish exposed to 7.86 ppm for 6 days. See the highly atrophied sperms (S). Spermatozoa heads are swollen into a mass. The middle piece and tail parts of testis too are attenuated. Note the highly disorganised seminiferous tubules and associated cells (ST).

Mallory x Ca 1000.
6.3.5 Ovary

Normal Histology and Histo-chemistry

In the ovary of normal fish different stages of oogenesis in progress were noticed. In the primordial germ cells, small oogonia and primary oocytes are seen. The primary oocytes look spherical or sub spherical in outline. The oocytes are sheathed by follicular epithelium.

In the developing oocytes the cytoplasm is homogenous with a large nucleus in the centre. Inside the nucleus the nucleolus and chromatin materials are seen. The chromosome appear distinct and they are lampbrush in nature. In addition to the distinct nucleolus, several small nucleolar extrusions are seen in the nucleus. The nucleolar extrusions or extrachromosomal nucleoli are refractile masses, sloughed off from the loops of lampbrush chromosomes and have a role in protein synthesis (De Robertis 1980). As the oocytes grow, the nucleolar extrusions move into the cytoplasm through the nuclear membrane.

In the cytoplasm of the oocyte one or more than one basiphilic yolk nuclei with spherical outline are present. RNA is rich in the nucleolus, extra-nucleolar extrusions and yolk nucleus (Fig. 6.110 - 112).

Histopathiological and Histochemical changes

Exposure to 3.93ppm Phosphamidon

In the fish exposed to the low concentration of phosphamidon for 30 days, the ovary registered malformation in vitellogenesis and malformed oocytes are seen. Oocytes developed abnormal structural outline like bizarre shape, pot-shape, double neck flask shape, pear shape, hatchet shape, multipronged forms and other cytomorphological changes. In most of the deformed oocytes vitellogenesis was defective. In some of the oocytes
vacuolations were present in the cytoplasm. The nucleus was shrunken in oocytes (Fig. 6. 113- 117).

After 60 days of exposure to low concentration, the deformities developed in the ovary of the fish were greater. Abortive oogenesis and abnormal developments were seen in oocytes.

The cytoplasm of oocytes showed a vacuolated zone in between the outer cortex and inner medulla. The vacuolated zone contained myriads of tiny unstained globules. Vacuolations were prominent in the cytoplasm. Synthesis of yolk granules was absent. The nucleus developed unusual shapes. The nucleolus and nucleolar extrusions too had lost their normal morphology. The nucleoplasm had coarse granules in the deformed oocytes. Vacuolations at different degrees were seen in nucleolus, nucleus and cytoplasm. The yolk nuclei were also deformed and developed vacuolation (Fig 6.118 - 123).

**Exposure to 7.86ppm Phosphamidon**

The fish reared in this higher concentration of phosphamidon for 30 days developed aberrant cytomorphological changes and abortive oogenesis. Hypertrophy of nucleus and nucleolus was vividly seen. Arrested growth phase was noticed in oogenesis. The nucleolar extrusions become diminutive surrounded by vacuoles (Fig 6.124 - 126).

In fish treated for 60 days in higher concentration of phosphamidon the aberrations in vitellogenesis, atrectic and abnormalities in oocytes, abortive oogenesis, absence of nucleolus, anomalies in nucleus and abruptive changes in nucleolus and nucleoplasm were observed. The organelle of the oocytes were riddled with vacuoles. Oolemma was thrown into irregular shapes in many oocytes. Karyolysis and karyorrhexis were
Section through ovary of control fish showing developing oocytes (do) and very young oocytes (yo), nuclei (N) and yolk nuclei (yn) are normal.

Mallory's triple stain X Ca 100

A primary oocyte of control fish showing normal structure. The lampbrush chromosomes (LC) bud off nucleolar extrusions (NE). The nucleolar extrusions border the periphery of the nucleus. The cytoplasm is dense (C) and rich in RNA. The ootheca is intact with cytoplasm (OT). The nuclei appear to be bloated with fluid. The mitochondrial cloud ("Yolk Nucleus") (MC) are prominent.

Toluedene blue x Ca 400.
Fig 6.112

A mature oocyte of control fish showing enlarged nucleus and surrounding cytoplasm. The lambrush chromosomes (LC), bud off nucleolar extrusion (NC), Nucleolus (NL) is prominent. Yolk granules are uniform in the cytoplasm (YG). Many small droplets of fat (FD) are seen amidst the mass yolk.

Mallory x Ca 400.

Fig. 6.113

Ovary of fish exposed to 3.93 ppm of phosphamidon showing deform oocytes (DF), abortive oogenesis (AO), distinct pyknotic mass of yolk nucleus (YN), prominent nucleolus (NL), increased perinuclear space, thickened oolemma (OL) and vacuolation in nucleus (VN). Protein content is reduced little (LP).

acrolein Schiff x Ca 100

Fig 6.114

A mature ovary of fish exposed to 3.93 ppm for 30 days showing abnormal shaped oocytes. The nuclear sap reduced and the nuclear size decrease. Nucleoli (NL) are present close to nuclear membrane. Yolk platlets in the periphery of the oocytes waned. The cytoplasmic processes of the oocytes at the follicle cells are withdrawn. The egg membrane and chorion (Ch) are undulated.

Ehrlich haematoxylin x Ca 1000.
Section through the ovary of fish exposed to 3.93 ppm for 30 days showing changes in oocytes. The nuclear membrane breaks down and the nuclear sap merges with cytoplasm of the oocyte. Nucleolar extrusions are pyknotic (NE). Oolemma is corroded in an young oocyte (←). A highly necrotic oocyte also seen (→). Ehrlich haematoxylin - Biebrich scarlet x Ca 100

An oocyte from the ovary of fish exposed to 3.93 ppm for 30 days showing the localisation of nuclear extrusions in the peripheral region of the cytoplasm (NE). Vacuolation appears (V). Yolk nucleus (YN) becomes pyknotic. Yolk platelets are less dense (YP). Ehrlich haematoxylin - Biebrich scarlet x Ca 100

Photomicrograph of ovary of fish exposed to 3.93 ppm for 30 days showing a multipronged deformed oocyte (DO). Vacuolations are seen obliterating the cytoplasm (V). The nucleolar extrusions are pyknotic (NE). Abortive oogenesis is seen (AO). Mallory's triple stain x Ca 100.
Section through the ovary of fish exposed to 3.93 ppm for 60 days showing abortive oogenesis (AO), a shrunken oocyte in which follicle wall is wide separated from oocytes (FW) and heavy vacuolation in the cytoplasmic yol platlets. The nucleolar extrusions (NE) are close to nuclear membran. Vacuolation is also seen in the nucleus (V).

Heidenhains iron haematoxylin x Ca 100

Photomicrograph showing polymorphic oocytes in the ovary of fish exposed to 3.93 ppm for 60 days. Oocytes are hatchet (DO) shaped. Pear shaped (TO) crescentic shaped (CO). In the deformed oocytes (AO), vitellogenesis is affected.

Heidenhains iron haematoxylin x Ca 40

Another photomicrograph showing oocytes with structural deformities. Flask shaped (PO), crescentic (CO) and aborted (AO) oocytes are seen.

3.93 ppm x 60 days.

Heidenhains iron haematoxylin x Ca 40
Fig 6.121

Section through the ovary of fish exposed to 3.93 ppm for 60 days showing several highly deformed oocytes (DO). The acrolein - Schiff's reactive proteins are less as seen from the less intensity of staining. Vacuolation in the nucleus is prominent (V). The nucleolar protein content is rich (NL). Arrested vitellogenesis (AV) is seen in an oocyte. The nucleolar (NL) abnormalities are also common. Yolk nucleus (yk) is seen.

acrolein - Schiff x Ca 40

Fig 6.122

Another photomicrograph showing deformed oocytes as in the previous photomicrograph. NE - Nucleolar extrusions, nl- Nucleus.

acrolein - Schiff x Ca 40

Fig 6.123

Photomicrograph showing a pair of closely attached oocytes showing severe vacuolations (V). In one oocyte the yolk nucleus (YN) is highly enlarged and the nucleoplasm remains empty. Nuclear extrusion (NE) is seen in the periphery. In another oocyte shrunken and perinuclear space is seen (PN).

3.93 ppm X 60 days

acrolein - Schiff x Ca 100
Section through the ovary of fish exposed to 7.86 ppm for 30 days show highly affected oogenesis. The oocytes are abnormal. Nuclear (N) deformities are more prominent. The boundary of oocytes are even lost (OW). Abort oogenesis seen (AO). Cytoplasm is faintly stained (C).

Mallory's triple stain x Ca 40.

Photomicrograph showing highly deformed oocytes (do). Vacuolation of oocytes (V) and thickening of follicular wall (FW).

7.86 ppm X 30 days.

Heidenhains iron haematoxylin x Ca 40

An oocyte under higher magnification showing vacuolation (V) amidst yolk platelets and in the nucleus. The lampbrush chromosomes are broken in fragments (CF). Nucleolar extrusion structures are also fragmented and N seen at the two poles of the nucleus.

7.86 ppm x 30 Days

Mallory's triple stain x Ca 400
Fig. 6.127

Section through the ovary of fish exposed to 7.86 ppm for 60 days showing heavy fall in RNA content in the nucleus (N) and cytoplasm of oocytes except the nucleolar region (NL) and yolk nucleus (YN). See the abnormally shape oocytes (DO) and loss of oolemma (OL). Vacuolation is heavy (V).

Toluedene blue x Ca 100

Fig. 6.128

Photomicrograph showing a deshaped oocyte in which RNA content is poor. Only the nucleolus and extranucleolar bodies (NE) have RNA.

7.86 ppm x 60 days.

Toluedene blue x Ca 100

Fig. 6.129.

A highly magnified nuclear portion showing the lampbrush (L) chromosomes with bead like structures. The nucleoplasm is less rich in RNA content (→)

7.86 ppm X 60 days

Toluedene blue x Ca 100
Fig 6.127

Fig 6.128

Fig 6.129
Fig. 6.130

Photomicrograph showing severely damaged ovary in the fish exposed to 7.86 ppm for 60 days. Oocytes are abnormal (O).

Mallory's triple stain x Ca 40

Fig. 6.131

Photomicrograph showing deformed ovary with highly damaged oocytes. Oogenesis is retarded (→)

7.86 ppm x 60 days

Mallory's triple stain x Ca 40

Fig. 6.132

Micrograph showing highly enlarged oocyte in which RNA content is reduced in all portions except the nucleolus (NL). See the abnormally shaped nucle (←)

7.86 ppm x 60 days.

Toluidene blue x Ca 400.
common in the oocytes. RNA content in the extra nucleolar region of oocytes was much reduced. [Fig 6.127 - 132]

6.4 Discussion

6.4.1 Gills

The gills, primarily the respiratory devices, are additionally endowed with absorption and elimination of ions. As the gills are continuously bathed in the ambient medium, they are susceptible to contact and systemic poisoning of phosphamidon. In different phosphamidon concentrations and exposure durations, the damages to the gills are more or less same but the intensity of the pathology is greater as the concentration and exposure duration increases.

The gross pathological changes observed in the gills include dilation of blood vessels, haemorrhage, shortening and thickening of secondary gill filament, fusion of the bases of secondary gill filaments, erosion of the epithelial lining of the gill filaments, additional developments of semilunar foldings forming secondary gill filaments etc. (This suggests the enhancement of the respiratory surface in the fish).

The skeletal cartilages in the rachis of the gill became transparent and even absent in some sections. Degenerative changes like hypertrophy as well as necrosis of epithelial cells, pyknosis in nuclei, bulging of tips of gill filaments and atrophy of lamellae and disintegration of gill filaments and respiratory epithelial cells are noticed. Hyperplasia of inter-lamellar cells, enlargement of pillar cells and increase in blood spaces in and around the pillar cells, lamellar fusion and edematous separation of epithelial cells from pillar cells are also observed. Similar pathological changes have been reported due to physical and chemical trauma the fish experience on exposure to different aquatic pollutants [Eller, 1971; Shrivastava and Shri
vastava, 1974; Mallat, 1985; Murugesan, 1988; Othuman 1994; and Anitha and Sree Ram, 1997].

Dwivedi and Sarin (1996b) showed that the degenerative changes in the gills tend to be largely physiological adaptation to stress. Anitha and Sree Ram (1997) reported that in the fish *Channa punctatus*, the aquatic pollutants induce several degenerative changes in the gills as observed in the present study and these changes reduce the respiratory area there by reducing the respiratory and osmoregulatory potential. The histopathological changes in the gills further indicate a decrease in energy metabolism. The degeneration of respiratory epithelium culminates in tissue hypoxia (Anitha and Sree Ram 1997) or tissue anoxia (Dwivedi and Sarin 1996b) or asphyxiation (Tamse 1995). Gardner and Yevich (1970) suggested that the degenerative changes in the respiratory epithelium in the fish *Fundulus heteroclitus* resulted in a shift from aerobic to anaerobic pathway of metabolism in the tissues of fish. In the present study it is observed that the respiratory stress in the fish *C. carpio* has induced the development of additional respiratory area by the extra development of semilunar foldings in the gill filaments, and fusion of secondary gill filaments. The increase in the respiratory area by way of lamellar fusion is a defensive response against prolonged exposure to irritant and such changes are also suggested as a protective measure by decreasing the valuable surface area of the gill to maintain its osmoregulatory functions while sustaining progressive loss of its basic function (Abel, 1976).

Dwivedi and Sarin (1996) suggests that such changes help to slow down toxicant uptake in dysfunctional and even non functional gills and eventually asphyxiate the fish. Severe gill lesions at longer period of exposure can impair respiration and extra renal function in the gill (Gardner and Yevich, 1970, Mitchel *et al.*, 1978, Shrivastava and Shrivastava 1984) and could eventually lead to the death of the fish (Eller 1975). Furthermore, recovery is not possible at longer period of exposure as
evidenced by Tamse (1995). Fish mortalities observed in such situation may then be related to asphyxiation, partial or complete loss of gill physiological function or loss of cellular proteins from exposed gill lesions (Eller 1975).

An increase in the number of mucous secreting goblet cells and excessive secretion of mucous in *C. carpio* can be accounted by the fact that the fish tries to get rid of the molecules of poisonous chemical substance by combining them with mucous and eliminating them by copious secretion of mucous as reported by Murugesan, (1988) and Othuman, (1994)

The dilation of blood vessels, increase in RBC, haemorrhage of blood vessels at several regions and aneurysm or lamellar telengectasis observed in the present investigation might be due to the enhanced blood flow to the lesioned gill surface to sustain the respiratory functioning of the affected gills as reported in the fish *Oreochromis mossambicus* exposed to pollutants (Othuman, 1994)

6.4.2 Liver

The liver of teleost is relatively a large organ performing several vital functions such as absorption of digested food-stuff, detoxification, secretion of bile, excretion of detoxified and harmful substances, synthesis of several components of blood plasma, storage of glycogen, release of glucose and control over general metabolism, [Kulsherstha and Lakshmi, 1984 ]. As the fish *Cyprinus carpio* is exposed to the pesticide phosphamidon, there is every chance for the phosphamidon present in the water to enter the body of fish along with food, through mucous of the mouth or gills and it may also reach the liver through blood circulation. Several structural and functional changes are induced in the liver by phosphamidon treatment. The severity of the damages is more as the concentration and exposure durations are increased.
The effects of phosphamidon on liver includes, fatty change resulting in vacuolation of hepatocytes, pyknotic nuclei, infiltration by phagocytes, desquamation of hepatic cells, haemorrhage, dilation of blood vessels and necrotised pancreatic part etc. The sinusoids also undergo degeneration. Obviously the changes observed have resulted from the response of the liver to the toxins and its efforts to offset the harmful effects of the pesticide by detoxification (Othuman 1994).

Hypertrophy of hepatic cells, necrosis, vacuolation, karyolysis disturbances in the arrangement of hepatic cords, cirrhosis, fat accumulation etc have been already reported in fishes exposed to pesticides [Mathur, 1962, 1965, 1976; Konar, 1970; Chakrabarty, 1974; Bhattacharya et al., 1975; Amminikutty and Rege 1977; Mandal and Kulshrestha, 1984; Kulshrestha and Lakshmi, 1984; Sahai, 1990, Areechon and Plumb, 1993; Dutta et al., 1993; Gopal and Ram, 1994; Banerjee and Bhattacharya, 1997; Usha and Murthy, 1997; and Banerjee and Bhattacharya, 1997.

As reported by Usha Ananthi and Murthy (1997), in the fish Glossogobius giuris exposed to malathion, the chosen test fish C. carpio have also developed, considerable decrease in glycogen, protein and RNA content as evident from histochemical localizations. Kulshrestha and Lakshmi (1984) reported that the pesticide thiodan and sevin induced the loss of characteristic hexagonal shape of the hepatocytes, rupture of nuclei and cell membrane, formation of binucleate hepatocyte, higher degree of atrophy at the centre and disorganisation of hepatic cords on the fish Channa striatus. Such changes are also observed in C. carpio after their exposure to higher concentration of phosphamidon for longer durations. Many reports dealing with hepatic pathology were the results of experiments for a maximum period of 30 days of exposure to sublethal concentration of organophosphorus pesticides. The onset and severity of liver lesions did not exhibit a definite pattern. But in the present study, the exposure duration was extended to 60 days and more significant level of
histopathological changes were observed. Thus it may be surmised that the continuous exposure to low doses of organophosphorus pesticides may in the long run cause more harmful effect than a single exposure to higher doses for a shorter period in the fish *Channa punctatus* exposed to elsan, an organophosphate pesticide (Banerjee and Bhattacharya, 1997).

Fatty accumulation is the characteristic feature of the liver damages (Rouller 1964, Anitha and Sreeram 1997). The changes in fat observed in the present study may be due to increased mobilization and transport of fat to the liver. As the detoxification function of the liver was not in proportion to the influx of the pesticide, the detoxification process as well as the secretory activity of liver were hampered as evident from low RNA and protein level. In this context of poor enzyme secretion and protein synthesis, the functioning of liver is affected and the liver ultimately degenerates as reported earlier in the fish *H.fossilis* [Murugesan 1988].

**6.4.3 Stomach**

The gastric mucosa was affected by the pesticide, phosphamidon. Hypertrophy of the cells and deepening of the mucous cups of the cell in order to produce greater amounts of mucous were commonly seen in the *C.carpio* exposed to different concentrations of phosphamidon. Copious secretion of mucous was also observed in the gastric lumen. The gastric glands were also hypertrophied and their secretory activities had declined. The reduction in the secretory activity is evident from poor RNA level.

The hypertrophied submucosa shows increased vascularization, large blood vessels engorged with blood corpuscles occurring at several places, changes in the shape of folds, ulceration in the surface epithelium and lamina propria, pronounced desquamation and damages in the submucosal layers in the stomach of *C.carpio* have been reported in other fishes also exposed to pollutants [Isai arasu and Haniffa, 1987 and Othuman, 1994]
6.4.4 Intestine

As detailed under the heading results, the intestine shows incredible structural changes correlated with the functional demand as a result of the effect of phosphamidon. The mucosa of the intestine developed several caecum like projections in the lumen of the intestine. These projections are similar to the intestinal villi. The sequential development of the villi, their hypertrophy and the degeneration of columnar epithelial cells situated at the bases and tips of few villi forming a syncytial mass were observed. Similar changes have been reported by Anitha and Sree Ram (1997) in the fish Channa punctatus collected from polluted lakes. The additional development of villi in the intestinal mucosal layer enables the fish to live with partially damaged intestinal epithelial cells that have reduced their efficiency of absorption.

The manifold increase of mucous secreting goblet cells, their hypertrophy and copious secretions of mucous in response to the irritational sensation of phosphamidon on the intestinal mucosal layer were the manifestation of protective measures the intestine has taken to protect itself from the corrosive power of phosphamidon. Similar changes had been reported in other fishes exposed to different aquatic pollutants [Gopal and Ram 1994 and Anita and Sree Ram 1997]. Local necrosis, proliferation of villi, desquammation of the superficial parts of villi and necrosis of the tips of the villi were observed. Edematous epithelial cells and connective tissues, dilated blood vessel and lymphocyte migration were also seen. Anitha and Sree Ram (1997) reported that the increase in the blood vessels of the intestine might be due not only to allergic responses but also to anaemia triggered by exposure to phosphamidon. Murugesan (1988) attributes that the infiltration of leococytes (phagocytic lymphocytes) into the lumen was a compensatory measure to absorb the food by phagocytosis when the absorptive parts of the intestine gets damaged.
The reported necrosis of submucosa, the detachment of the portion of the villi and the disruption of intestinal brush border may result in impaired intestinal absorption [Crespo et al., 1986] and this has been confirmed by the significant reduction in the absorption efficiency as explained in the bio-energetics chapter [Chapter 4]

6.4.5 Testis

Under the phosphamidon stress at sublethal levels, the fish *C. carpio* could not carry out their reproductive activity to their full extent. As the fish suffers with poor food intake and reduction of energy level, the testicular activity remained reduced. This is evident from poor spermatogenesis act in the phosphamidon exposed fishes. In the lumen of the seminiferous tubules, clusters of sperms that were formed prior to the exposure of fish to phosphamidon were observed. But the sperms were not normal in their structure and showed deformities. The sperms had got clumped at several places. The pattern of pathological changes brought about by the phosphamidon was a steady decline in spermatogenic activity proportionate to the quantum of total exposure. The proliferation of cells of the germinal epithelium leading to the production of spermatogonia, primary spermatocytes and secondary spermatocytes were affected by the pesticide exposure.

As the organophosphorus pesticides are neuropoisons and detrimental to hormonal secretions, the gamatogenesis and development of fully mature sperms could not be attained in the test fish *C. carpio*.

6.4.6 Ovary

In the fish *C. carpio* exposed to the different concentrations of phosphamidon, considerable pathological changes have been noticed in the
ovary, oogenesis and oocytes, depending upon the concentrations and exposure durations. The ovarian degeneration may be accounted for contact as well as systemic toxic impact of phosphamidon and because of the breakdown in the secretion of the reproductive hormones due to the neuronal poisoning.

In very early stages of oogenesis the nuclei of the oogonia underwent complete lysis. Karyolysis resulted in the complete disappearance of the chromatin reticulum and the nucleoli and such oogonial cells were bound to disintegrate. Nucleolar vacuolation had been consistently observed in some nucleoli. Defective nucleoli were common. Nucleolar blebbing was also observed. Instead of a large nucleolus, two or more small nucleoli with lesser basiphilia were present. The basiphilia of the nucleolus depend on its RNA content. As the phosphamidon exposure had made nucleolar aberrations, it hampered the assembly of ribosomes in the nucleolus and protein synthesis in the cell [Flickinger et al., 1979] and the oogenesis is affected.

Attenuation of oocyte, vacuolation in cytoplasm, pyknosis, necrosis, cytolysis, karyolysis, ploymorphism in nucleus, karyorrhexis, chromatin condensation, nucleolar deformity and abortive oogenesis have been reported in other animals exposed to various pesticides. [Dubale and Awasthi, 1982, Ram and Sathyanesan, 1987, Ahi, 1987, Victor 1989, Ranjit Singh, 1990]

The incidence of vacuolation in the cytoplasm of oocytes is explained in the light of Cameron’s (1964) statement that vacuolation indicated the onset of cytopathological changes and it is because of the prevention of the influx of electrolytes through the plasmamembrane. As a result, the control exerted by the plasma-membrane in ion-transport is lost and it leads to a change in the composition of the cytoplasm and even the nucleus of the cell. Such cells, show an increase in the volume which is called as hypertrophy
by Cameron (1964). It is because of hypertrophy extra space is to be vacuolated as the protoplasm cannot occupy the increased volume [Murugesan 1988].

As the yolk nuclei are involved in the synthesis of yolk (Viswanath, 1968), their reduction in size or their obliteration may be due to the deleterious impact of phosphamidon on the organellae which control the synthesis of yolk nucleus. The oocytes which lose their yolk nuclei cannot synthesis the nutrient.

Gall (1963) has established that the nucleolar extrusions are nothing but mRNA elaborated by the lampbrush chromosome loops which are ultimately dispersed into the cytoplasm. The diminished size of the nucleolar extrusions, the vacuolation of these bodies, the decrease in their number and their complete absence, indicate the extent to which mRNA production is affected by the pesticide pollutant. Comparatively the higher concentration of RNA positive material in the nucleolus and poor level of RNA positive material in the extranucleolar region and cytoplasm indicate that the nucleolar RNA (mRNA) synthesis is affected. Flickinger et al (1979) stated that the reduction of mRNA production was because of the inhibition of the enzyme RNA polymerase II.