CHAPTER - 5

HISTOPATHOLOGY
CHAPTER 5A

LIGHT MICROSCOPY
Histology, as the study of micro anatomy of specific tissues, has been successfully employed as a diagnostic tool in medical and veterinary science, first cellular investigation were carried out in the mid nineteenth century (Virchow, 1858). Exposure of animals to contaminated water also causes severe pathological changes at the tissues level. Pesticide that enter the body via internal digestive system after oral administration. They are not subjected initially either to the detoxifying reactions of the liver or to excrete via the biliary system. Compounds transported by oral feeding in effect can be distributed to all parts of the body in their unmetabolised form (Turner and Shanks, 1980).

The examination and study of normal cells and tissues by microscopy is called histology or microscopic anatomy. The study of abnormal cells and tissues is histopathology (Aughey and Frye, 2001). Toxicological histopathology gives useful data concerning the changes induced by chemicals at the tissue and cellular level. All the tissues and organs in the body of an animal may be potential targets for the toxic effects of any chemical or metal. A histopathological assessment throws light on the nature of tissue alteration and the extent of damage. This in turn indicates the toxic nature of the compound. Therefore, histology gives useful insight in to the tissue lesions prove to the external manifestations of the deleterious effects of heavy metals (Jayantha Rao, 1982).

Histology is the study of tissue sectioned as a thin slice, using a microtome. Histopathology is the microscopic study of diseased tissue and is an important tool of anatomical pathology since accurate diagnosis of cancer and other diseases usually requires histopathological examination of samples. The trained scientists who perform the preparation of histological sections are known as histotechnicians, histology technicians (HT), histology technologists (HTL), and medical scientists, medical laboratory technicians or biomedical
scientists. Their field of study is called histotechnology (Merck Source, 2002 and Sted man’s medical dictionaries, 2005).

To eradicate or control the pests nowadays farmers are using a number of pesticides in the operation of agricultural and other commercial crops protection. The adverse effect of a chemical agent on any animal depends of three variables 1) The vulnerability of individual tissues, 2) The mode of action of the agent and 3) The concentration of the agent. Susceptibility of chemical injury varies greatly in the tissues and cells of the same animal. It is even greater in different animals groups. However, the location of the major damage may be determined by the mode of action of the chemical. Some of the chemicals if present in the environmental media exert their effect locally at the portal of entry, leading to the damage to the proximal portion if the gastrointestinal track will be affected. Some other toxic compounds do not cause damage at the portal entry but affect the organs systematically in which they are accumulated.

Pesticides possess high toxicity not only to targets but also non-target organisms. These substances find their way in to places far from application (Reports of Secretary’s Commission on Pesticides and their Relationship to Environmental Health, 1969) and lead to alterations in metabolic activities of living organisms by bio-accumulation. Commonly pesticides accumulate to a greater extent in the liver (Edwards, 1973) which is the centre for pesticide metabolism. Pesticide residues in the tissues cause serious physiological alterations even at low levels (Dikshith et al., 1974, 1978; Mathur et al., 1981). Johnson (1973) and Verma et al., (1974) pointed out that a prolonged period of exposure to chemical compounds with very low concentration, results in the accumulation of more pesticide in the organs.

Pesticides which are ubiquitous in nature have become integral part in the tissues of animals. Pesticides find their way into places far from application and accumulate in significant concentrations in the tissues of
animals. The extent of severity of tissue damage is a function of the concentration and potentiality of toxic compound accumulated in the tissues as it is time dependent (Jayantha Rao, 1982). It is obvious that any chemical indult could cause pathological damage or injury to cells in an animal if it’s taken beyond the safe permissible limits. Susceptibility to chemical injury varies greatly among the tissues and cells of the same animal and more so among the tissues and cells of the same animal and in different animal groups.

The architectural dynamics of a tissue is very essential for maintaining the tissue integrity and for effective physiological, biochemical and metabolic functions. The cellular and sub-cellular constituents of tissue in terms of size, shape, number and position play an important role in the physiological and metabolic functions. Therefore, the histological structure of tissue in an animal has a profound influence on its function. Histology, the study of microanatomy of specific tissues, has been successfully employed as a diagnostic tool in medical and veterinary sciences since the first cellular investigations carried out in the nineteenth century (Virchow, 1858). The knowledge of the histology is useful to distinguish normal cells from abnormal or diseased ones, which helps in diagnosis of many diseases (Majumdar, 1980).

The physiological investigations may not help in the complete understanding of the chemical impact on a tissue. When coupled with cytoarchitectural studies, the toxicological studies seem to be complete so as to give a picture of the extent of pesticidal effect. The investigations of histopathological effects have not been persuaded with the same vigor compared to biochemical aspects.

However an alteration of cell morphology, even when localized and confined does not unequivocally indicate where or how a molecular derangement occurs, because they are several steps away from the initial disturbance. It can be suggested that both morphological and biochemical
assay should be applied for more accurate evaluation of pathological concepts. Moreover histopathological studies would help in assessing the extent of pollution in the ecosystem by pesticides and offer an exceptional opportunity to detect the effect of pollutants in various organs and organ system of an organism. Although much can be learned from the careful pathological examination of gross specimens; the finer cytoarchitectural changes produced during chemical intoxication can be traced by microscopic examinations of the tissues; such studies may explain to certain extent the tissue specificity of the drug action.

Histology and cytology are concerned primarily with morphologic characters of microscopic structures. This helps in understanding the chemistry of microscopic structure is termed histochemistry or cytochemistry, depending on whether the object of interest is the tissue or cell. Even though biochemical studies may give an idea of the pathological state of the animal, a clear picture of cytoarchitectural changes produced during the chemical intoxication can be produced during the chemical intoxication can be traced by histopathological studies. Several workers reported on the pesticides and pointed out the architectural damage to brain, gill, liver, kidney, heart, lung, muscle, testis, intestine in various animals (Jayantha Rao, 1982; Radhaiah, 1988; Vijay Joseph, 1989; Vani, 1991; Badri Sriman Narayanan et al., 1993; Manoranjitham et al., 1993; Pondy et al., 1997a, 1997b; Shukla et al., 2001. Glynn, 2003; Garg et al., 2004; Madhavelatha, 2006; Sivaiah, 2006; Rajendra Prasad, 2007; Sukanya, 2007; Kishandar, 2007; Madhava Rao, 2007; Nagarjuna, 2007; Rajeswari, 2008). But investigations at cell and ultra structural level are very low. Hence an attempt has been made to study histopathological effects of chlorpyrifos on liver, heart kidney and intestine in albino rats.
RESULTS

Normal histology of rat liver

Liver is partially divided into three hepatic lobes and incompletely invested by tunica serosa and a delicate lobe has two main constituents, an epithelial parenchyma and system of blood sinusoids. Liver contains a large number of hexagonal functional units called lobules. The classic lobule is traditionally described as roughly cylindrical with a venous channel, the central that course through its long axes. Irregular interconnecting sheets a plate-like arrangement of hepatic cells or hepatocytes radiate outward from the central vein and constitute the parenchyma of the lobule. Sinusoids separate the sheets of hepatic cells and empty in the central veins. At the angles of the hexagons are the portal canals which are loose stromal connective tissue characterized by the presence of the portal triads. It also has the lymphatic vessel. Connective tissue of portal area is ultimately continuous with the fibrous capsule of liver (Plate 5A.1; Fig. A). The portal canal is bordered by the outermost hepatocytes of the lobule. Sinusoids are lined by endothelial lining. Endothelium of sinusoids is discontinuous due to presence of large fenestrae and large gaps lining of sinusoids also contain second type of cells, called stellate sinusoidal macrophages of kupffer cells (Agarwal, 2001).

Histopathological changes of rat liver under chlorpyrifos intoxication

The microscopic examinations revealed that chlorpyrifos induced histopathological lesions in liver, after single, double and multiple doses administration the degree of severity differed from single dose to multiple dose of chlorpyrifos and it was more in the later period than former.

In single dose chlorpyrifos administration, central vein congestion, cellular swelling, cytoplasm porous in sub capsular hepatocytes, sinusoidal
hemorrhages and degenerative changes in hepatocytes was observed (Plate 5A.1; Fig. B).

In double dose administration, central vein congestion and dilated sinusoids, sinusoidal hemorrhages and focal necrotic areas were observed (Plate 5A.2; Figs. C&D).

Under multiple dose administration severe degenerative changes in central vein and diffused necrotic areas were observed (Plate 5A.3; Figs. E& F).

**Normal histology of rat kidney**

Each kidney is enclosed in a tough connective tissue capsule extending into the parenchyma and has two regions – the cortex and the medulla. The nephron is the functional unit of the kidney. The major subdivisions are the renal corpuscle and the uriniferous tubule.

The blind end of the proximal tubule is identified with a network of capillaries and supporting cells to form a filtering system: the renal corpuscle. Each renal corpuscle consists of a glomerulus and a glomerular (Bowman's) capsule. The outer layer of the glomerular capsule is the capsular (parietal) wall, which is separated from the glomerular (visceral) layer by the capsular (urinary) space. The capillaries of the glomerulus are served by an afferent and an efferent arteriole, entering and leaving the renal corpuscle at the vascular pole. At the opposite pole is the capsular space, where the filtrate passes into the proximal tubule at the urinary pole of the renal corpuscle.

This nomenclature is based on the functional areas of the renal tubule. The proximal convoluted tubule (PCT) is long and lined with low columnar cells with a basal nucleus. The cytoplasm is deeply stained with eosin and the apical surface is a continuous brush border. The PCT is continued with the proximal straight tubule. It is similar in appearance and extends towards the
medulla where the epithelium changes abruptly to simple squamous. This part of the tubule descends into the medulla as the thin descending limb and bends sharply to return to the cortex as the thick ascending limb, which was previously known as the “loop of Henle.” In the cortex the epithelium becomes cuboidal or columnar and forms the distal straight tubule and coils near the glomerulus to become the distal convoluted tubule (DCT). The DCT is shorter than the PCT, the epithelium is cuboidal, the cytoplasm is paler and there is no brush border.

The DCT approaches the glomerulus at the vascular pole, where it thickens, and the cell nuclei of the tubule wall become crowded together to form the macula densa, part of the juxtaglomerular apparatus. Juxtaglomerular cells are modified smooth muscle cells in the walls of afferent arterioles close to the glomerulus. The collecting tubule or duct (lined with poorly staining cuboidal epithelium) is the terminal segment of the nephron, a continuation of the OCT within the medulla (Aughey and Frye, 2001) (Plate 5A.4; Fig. G).

Histopathological changes of rat kidney under chlorpyrifos intoxication

The microscopic examinations revealed that chlorpyrifos induced histopathological lesions in kidney, after single, double and multiple doses administration the degree of severity differed from single dose to multiple dose.

In single dose chlorpyrifos administration hemorrhage and hyperemic glomerulus was observed (Plate 5A.5; Fig. H & I).

In double dose administration hemorrhage and hyaline deposits in lumen was observed (Plate 5A.5; Fig. J).

Under multiple dose administration necrotic changes in tubular epithelial cells and hyaline deposits in lumen was observed (Plate 5A.6; Fig.K).
Normal histology of rat intestine

Histologically intestine has following four basic layers namely mucosa, sub mucosa (muscularis mucosa), muscularis externa and serosa. The function of the mucosa is absorption. Finger-like projections of the intestinal villi are long and thin. The core of each villus is formed by the lamina propria, which is vascular, cellular and reticular, with local aggregations of lymphoid cells.

The tall columnar cells that line the intestine have a striated border containing mucus-secreting goblet cells; these increase in number with distance from the stomach. At the bases of the villi, the epithelium dips into the lamina propria to form mucosal intestinal glands (the crypts of Lieberkuhn). The cells lining the crypts are columnar, secreting mucus, enzymes and local hormones, and are the stem cells that are active in the repair and replacement of the epithelium. The muscularis mucosa consists of two layers of smooth muscle, inner circular and outer longitudinal, and separates the crypts from the underlying mucosa.

A strip of muscle extends into each villus from the muscularis mucosae; a lacteal (lymphatic that transports chyle) is also present. The muscularis externa consists of two layers of smooth muscle dispersed in a gentle spiral, appearing as an inner circular and outer longitudinal layer. The serosa consists of loose connective tissue and the mesothelium with the visceral peritoneum (Aughey and Frye, 2001) (Plate 5A.6; Fig. L)

Histopathological changes of rat intestine under chlorpyrifos intoxication

The microscopic examinations revealed that chlorpyrifos induced histopathological lesions in rat intestine, after single, double and multiple doses administration the degree of severity differed from single dose to multiple dose.
In double dose chlorpyrifos administration degenerative changes in muscle layer, hypertrophy of goblet cells and slight infiltration were observed (Plate 5A.7; Fig. M & N).

Under multiple dose administration degenerative changes, infiltration and hyperemic changes in blood vessel were observed (Plates 5A.8; Figs. O & P).

**Normal histology of rat heart**

The cardiac wall consists of three layers: endocardium (inner), myocardium (middle) and epicardium (outer). The endocardium contains continuous squamous endothelial cells, vascular areolar connective tissue and conducting fibres. The myocardium is composed of cardiac muscle and also contains vascular areolar connective tissue. The epicardium is thicker than the endocardium, and fat deposits in the rather dense connective tissue and coronary blood vessels are often found. Fibrous rings support the heart valves. They provide a means of insertion for the cardiac muscle and may be referred to as the fibrous or cardiac skeleton (Aughey and Frye, 2001) (Plate 5A.9; Fig. Q).

**Histopathological changes of rat heart under chlorpyrifos intoxication**

The microscopic examinations revealed that chlorpyrifos induced histopathological changes in rat heart, after single, double and multiple doses administration the degree of severity differed from single dose to multiple dose.

In single dose chlorpyrifos administered rats, the heart did not show any marked pathological changes.

In double dose administration hemorrhage and diffuse areas with hemorrhage was observed (Plate 5A.9; Fig. R).
Under multiple dose administration diffused area with hemorrhage and rounded nucleus were observed (Plate 5A.10; Figs. S & T).

**Normal histology of rat testis**

The testis is covered by mesothelium continuous with the visceral layer of the tunica vaginalis. A thick dense connective tissue capsule, the tunica albuginea, encloses the testis. A variable amount of smooth muscle may be present. The tunica vasculosa is the inner vascular layer of the tunica albuginea. The capsule is reflected into the median plane of the testis to form a partition, the mediastinum, and gives off loose vascular connective tissue, the septa testis, to divide the testis into lobules to support the seminiferous lobules. The coiled seminiferous tubules are lined with a multilayered seminiferous epithelium of spermatogenic cells and sustentacular (Sertoli) cells. They rest on a basement membrane and are surrounded by a lamellated connective tissue with myoid elements. The specific interstitial (Leydig) cells are found in the loose vascular connective tissue separating the tubule.

In the prepubertal male there are two cell types: the sustentacular cell and the spermatogonium, the immature male germ cell. The sustentacular cells are tall columnar, extending from the basement membrane to the lumen of the tubule, with a pale vesicular basal nucleus and a prominent nucleolus. As the name suggests, the sustentacular cells support the later stages in the development of spermatozoa. Spermatogonia lie next to the basement membrane and are small round cells with a dark staining nucleus.

The primary spermatocyte divides meiotically to form two secondary spermatocytes, which each divide immediately to form two haploid spermatids. These are small cells with a spherical nucleus and lie close to the lumen of the tubule. The spermatids move into recesses in the sustentacular cells and metamorphose into spermatozoa, shedding the excess cytoplasm into the lumen of the tubule (**Aughey and Frye, 2001**) (Plate 5A.11; Fig. U).
Histopathological changes of rat testis under cypermethrin intoxication

The microscopic examinations revealed that chlorpyrifos induced histopathological changes in rat testes, after single, double and multiple doses administration the degree of severity differed from single dose to multiple dose.

In single dose chlorpyrifos administered rat testis showing congestion, degenerative changes in seminiferous tubules. (Plate 5A.11; Fig. V).

In double dose chlorpyrifos administration increased amount of connective tissue, congestion, degenerative changes in seminiferous tubules were observed (Plate 5A.12; Fig. W).

Under multiple dose administration increase amount of inter tubular connective tissues, degenerative changes, clumped spermatozoa, increased size of lumen in seminiferous tubules were observed (Plate 5A.12; Figs. X).

DISCUSSION

It is clearly indicated that the chlorpyrifos induced pronounced pathological changes in liver, kidney, heart, intestine and testis of rats exposed to single, double and multiple doses (Plates 5A.1 to 5A.12; Figs. A to X).

Since liver is the major metabolic centre to detoxify the pesticide, it was also adversely affected with central vascular congestion, cellular swelling and cytoplasm porous in sub capsular hepatocytes, sinusoidal hemorrhages and degenerative changes in hepatocytes (Plates 5A.1 to 5A.3; Figs. A to F). These changes in turn may result altered hematological profiles and also some metabolites and enzymes of protein metabolism as evidenced in the present investigation (Chapter II & III).
Several authors have reported histopathological changes in liver in different animal models under pesticidal toxicity.

Hepatic lesions including hypertrophy, vacuolation, nuclear pycnosis, karyolysis and fatty degeneration of hepatocytes in a freshwater fish *Punctius conchonius*, which was chronically exposed to sublethal concentrations of endosulfan, phosphomidon and aldicarb (Gill et al., 1990). Garg et al. (1992) reported very mild degenerative changes as evidenced by cloudy swelling and fatty degeneration in liver tissues of rats exposed to fluvalinate.

Impact of endosulfan on the liver *Cyprinus carpio* and expressed that extensive vacuolation, indistinct cell boundaries, loss of polygonal shape of the cell and degenerative necrosis are the respective histopathological changes (Jonsson and Jayabal, 1993). Majumder et al. (1994) reported congestion, vacuolar degeneration and accumulation of fat in centrilobular area, focal to extensive necrosis, hyperplasia of kupffer cells, and dilation of sinusoids in fenvalerate treated birds.

Abou-Zaid and El-Balshy (1995) observed necrosis, blood vessel congestion and leucocytic infiltration in the liver of newly born mice that inhaled “Ezalo”, a commercial formulation of synthetic pyrethroid for 15 days. Luty et al. (1997) reported small lymphocytic infiltrations in the areas of blood vessels and accumulation of nuclei of hepatocytes in the sub capsular layer in liver tissues of rats exposed to paraquat.

Luty et al. (1998) observed increased porosity of the cytoplasm of hepatocytes in the liver of rat exposed to α-cypermethrin. Luty et al. (1998) observed more porous cytoplasm in sub capsular hepatocytes and focal sub capsular hyperemia in liver of rat exposed to dichlorvos.

Latuszynska et al. (1999) reported a few small infiltrations consisted of lymphocytes and histiocytes and in single hepatocytes signs of parenchyma degeneration in the liver of rats exposed to chlorpyrifos and cypermethrin.
Sakr (1999) reported rats inhaled the pyrethroid tetramethrin showed destruction of liver architecture, cytoplasmic vacuolation of the hepatocytes and leucocytic infiltrations. Tos-Luty et al. (2001) reported slight infiltrations of mononuclear cells between hepatocytes and also sporadically degenerative changes in individual hepatocytes in the liver of rats exposed to carbaryl.

Tos-Luty et al. (2001) observed degenerative changes of hepatocytes with infiltration of lymphocytes, kupffer cells proliferation and nuclear anisocytosis in hepatocytes of mice liver in the liver of rats exposed to fenvalerate.

Lynch et al., (2003) reported liver is the primary site of toxicity in rats and mice exposed to inhaled dimethylformamide (DMF) for 13 weeks. Centrilobular hepatocellular necrosis seen in exposed rat was accompanied by increased activities of hepatic intracellular enzymes in the serum, and also by increases in relative liver weights. Tos-Luty et al. (2003) observed parenchymatous degeneration of hepatocytes with slight infiltration in the liver of rats dermal exposed to malathion.

Tos-Luty et al. (2003) observed the presence of fine sub capsular infiltrations, diffused parenchymatous degeneration of single hepatocytes, and the presence of fine foci constructed of plasmatic cells and histiocytes located between hepatic plates in the liver of rats orally exposed to malathion. Degeneration of hepatocytes, necrosis, disappearance of hepatocytes wall, atrophy, formation of vacuoles, necrosis and pycnotic nuclei have been reported in the Ctenopharyngodon idellus exposed to fenvalerate (Tilak et al., 2001).

Sakr and Hanafy (2002) observed the normal structural organization of the hepatic acini impaired, cytoplasmic vacuolation of hepatocytes, blood vessels congested and abundance of leucocytic infiltrations in toads exposed to fenvalerate. Wade et al. (2002) observed hypertrophied hepatocytes with
many highly vacuolated cells in liver tissues of rats exposed to complex mixture of persistent contaminants.

Choudhary et al. (2003) reported congestion, vacuolar degeneration and accumulation of fat in centrilobular area, focal to extensive necrosis, hyperplasia of kupffer cells, dilation of sinusoids, nuclear aberrations, cytoplasmic degranulation and pycnotic nuclei in the liver tissues of rats exposed to endosulfan. Cloudy swelling, bile stagnation, focal necrosis, atrophy and vacuolation have been reported in the Corydoras paleatus exposed to methyl parathion (Fanta et al., 2003).

Manna et al. (2004) reported congestion and hemorrhages in the liver tissues of rats exposed to α-cypermethrin. Manna et al. (2005) reported congestion and fatty changes in livers of rats exposed to deltamethrin. Purohit (2005) observed mild hemorrhages and fatty changes would be due to decomposition and metabolism of the acephate into methamidophos in liver of white leghorn birds exposed to acephate.

Sarkar et al. (2005) found significant changes as hyperplasia, disintegration of hepatic mass, focal coagulative necrosis in Labeo rohita exposed to cypermethrin.

Velisek et al. (2006) observed degeneration of hepatocytes in the periportal zones and affected hepatocytes showed pycnotic nuclei and many small or one big vacuole in the cytoplasm in the liver of Oncorhynchus mykiss exposed to cypermethrin.

Cengiz and Unlu (2006) reported hepatic lesions in the liver tissues of fish exposed to deltamethrin were characterized by hypertrophy of hepatocytes, significant increase of kupffer cells, circulatory disturbances, focal necrosis, fatty degeneration, nuclear pycnosis and narrowing of sinusoids. Sinusoidal congestion and parenchymatous degeneration of hepatic
Light Microscopy

cells and fibrosis in hepatic portal areas were observed in White Leghorn Cockerels liver exposed to acephate (Tripathi et al., 2007).

Hepatic lesion in the liver tissues of Cirrhinus mrigala exposed to fenvalerate were characterized by congestion, cloudy swelling of hepatocytes and focal necrosis (Velmurugan et al., 2007). Nagarjuna (2007) observed perivascular infiltration, cytoplasm porous in sub capsular hepatocytes, cellular swelling, congestion, hemorrhage and hypertrophy of kupffer cells in cypermethrin induced albino rats.

Rajeswari (2008) reported congestion, cellular swelling, cytoplasmic degeneration, bi nucleated condition in nucleus and pushing of nucleus to the periphery of hepatocytes in acephate treated albino rats.

Some toxic substances do not cause damage at the portal of entry but affect the organs systematically in which they are accumulated. Since Kidney happens to be an organ for excretion of undetoxified chemical, it is also affected badly in the present investigation. The pathological changes include hemorrhage and hyperemic glomerulus hyaline deposits in lumen and necrotic changes in tubular epithelial cells (Plates 5A.4 to 5A.6; Figs. G to K). These changes in turn may result altered hematological profiles and also some metabolites and enzymes of protein metabolism as evidenced in the present investigation (Chapter II & III).

Several authors have reported histopathological changes in kidney in different animal models under pesticidal toxicity.

Hypertrophy of glomeruli with complete obliteration of bowman’s space along with mild generalized degenerative changes and progressive hyalinization of cortical tubule in kidney tissues of rats exposed to fluvalinate (Garg et al., 1992).
Abou El-Zahab et al. (1993) observed congestion of blood vessels, hemorrhages, necrosis and inflammatory leucocytes in kidneys of rats inhaled pyrethroids. Abdeen et al. (1994) reported treating mice with fenvalerate induced renal damage of the epithelial lining of the renal tubule, ruptured of the distal tubules and enlargement of the glomeruli with hydropic degeneration.

Elsan treatment in Channa punctatus resulted in a significant decrease in the dimension of Bowman’s capsule and glomerulus, and the tubules lost their regular shape due to precipitation of cytoplasm and karyolysis (Banerjee and Bhattacharyya, 1994). Hypertrophy of renal cells, changes in the nuclear structure, formation of vacuoles, necrosis and degeneration of renal components were noticed on the renal cells of Cyprinus carpio exposed to malathion and sevine (Dhanapakiam and Premlatha, 1994).


Luty et al. (1997) reported slight foci of lymphatic infiltrations in the cortex and medulla of kidney in rat exposed to paraquat. Luty et al. (1998) observed parenchymatous degeneration in single cells in the proximal tubuli in the kidney of rat exposed to α-cypermethrin.

Luty et al. (1998) observed lymphocytic infiltrations of the paraglomerular region and in the outlet part of the kidney rats exposed to dichlorvos. Latuszynska et al. (1999) observed a few infiltrations of mononuclear cells between the proximal tubules or around blood vessels in the kidney of rats exposed to chlorpyrifos and cypermethrin.
Santhamma et al. (1999) have reported separation of epithelial layer, degenerative changes in proximal tubules besides atrophy of proximal tubules and degenerative changes in haemopoietic tissue of kidney of the fish, *Tilapia mossambica* in response to monocrotophos exposure.

Tos-Luty et al. (2001) observed atrophy of the glomerule, hypertrophy of Bowman’s capsule and hyaline deposits in renal tubuli in the kidney of rats exposed to deltamethrin. Tos-Luty et al. (2001) observed inflammatory infiltrations between the tubuli and parenchymatous degeneration of single cells in the kidney of rats exposed to fenvalerate. Tos-Luty et al. (2003) observed parenchymatous degeneration of the cells of renal tubules and hyperemia of the cortical part of the kidney, especially of renal glomeruli, as well as infiltrations between the proximal tubules in the kidney of rats orally exposed to malathion.

Das and Mukherjee (2000) reported dilation of tubules, necrosis changes characterized by karyorrhexis and karyolysis at the nuclei of affected cells of *Labeo rohita* exposed to hexachlorocyclohexane. Haratym-Maj (2000) observed a few infiltrations of mononuclear cells between the proximal tubules in kidneys of mice exposed to α-cypermethrin. Tilak et al. (2001) observed severe necrosis, cloudy swelling in the renal tubules, cellular hypertrophy, granular cytoplasm, vacuolation in kidney tissues of *Ctenopharyngodon idellus* exposed to fenvalerate.

Sakr and Hanafy (2002) observed renal tubules degenerated and atrophied glomeruli in the kidneys of toads exposed to fenvalerate. Choudhary et al. (2003) reported chronic glomerulonephritis, glomerulosclerosis, adenoma and glomerulus deposits in kidney tissues of rats exposed to endosulfan. The kidney of fish receives the largest proportion of postbranchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution.
Manna et al. (2005) reported congestion of blood vessels in kidneys of rats exposed to deltamethrin. Purohit (2005) observed intertubular hemorrhages, cystic dilatation of tubules, tubular degeneration and fatty changes in kidney of white leghorn birds exposed to acephate. Cengiz (2006) described degeneration in the epithelial cells of renal tubule, pycnotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen in Cyprinus carpio exposed to deltamethrin.

Necrosis of tubular epithelium, pycnotic nuclei in the hematopoietic tissue, hypertrophied epithelial cells of renal tubules, narrowing of the tubular lumen, expansion of space inside the Bowman’s capsule and contraction of the glomerulus were observed in kidney tissues of Cirrhinus mrigala after exposure to fenvalerate (Velmurugan et al., 2007).

Nagarjuna (2007) observed hyperemia, hyaline deposits in lumen, fragmentation of glomerulus, hypertrophy of Bowman’s capsule changes and increased size of lumen in proximal and distal convoluted tubules in cypermethrin treated albino rats.

Rajeswari (2008) reported slight thickening of Bowmans capsule atrophy of glomelurus hyaline deposits in lumen in acephate treated albino rats.

Since gut is considered to be main route for absorption of pesticide, the duodenum also have degenerative changes in villi and goblet cells, infiltration, degenerative changes and hyperemic changes in blood vessel (Plates 5A.6 to 5A.8; Figs. L to P). Such changes would definitely result poor absorption of nutrients in experimental animals. This in turn may result altered hematological profiles and also some metabolites and enzymes of protein metabolism as evidenced in the present investigation (Chapter II & III).
Several authors reported histopathological changes in intestine in different animal models under pesticidal toxicity.

Mandal and Kulshrestha (1980) described the lesion formation in villi of *Clarias batrachus* after exposure to sumithion. Histological analysis of intestine tissue of *Channa striatus* and *Heteropneustes fossilis* inhabiting the polluted water showed degenerative changes in the serosa, mucosa and submucosal layers, focal necrosis, proliferation and desquamation of the superficial parts of villi (Kumari and Kumar, 1997). Braunbeck and Appelbaum (1999) have found that in the intestine, exposure to endosulfan is associated with changes in the epithelial lining, which indicates disturbances of intestinal absorption.

Cengiz, (2006) reported edema, degeneration, accumulation of lymphocytes in the lamina propria, pyknotic state of nuclei and necrosis in the intestine of *Gambusia affinis* exposed to endosulfan. Cengiz and Unlu (2006) reported infiltration of mononuclear leucocytes and eosinophils towards lamina propria, necrosis in the intestine tissues of *Gambusia affinis* exposed to deltamethrin. The intestine is a very important absorption place for the toxic compounds (Timbrell, 1991).

Atrophy of epithelial cells, necrosis of epithelial cells, desquamation of mucosal epithelium and infiltration of lymphocytes into the lamina propria were detected in intestine tissues of *Cirrhinus mrigala* after exposure to fenvalerate (Velmurugan et al., 2007). Nagarjuna (2007) observed hypertrophy of goblet cells, necrotic changes at tip of villi, infiltration, congestion in submucosa, fragmentation of villi, heavy infiltration, and necrotic changes in epithelial and glands of rat intestine exposed to cypermethrin.
Rajeswari (2008) reported infiltration, congestion in sub mucosa, fragmentation of villi and heavy infiltration under acephate toxicity in albino rats.

In the present investigation chlorpyrifos toxicity exhibited clear histopathological changes in cardiovascular tissue viz., heart. These pathological changes include hemorrhage and diffuse areas with hemorrhage and rounded nucleus (Plates 5A.9 to 5A.10; Figs. Q to T). These changes in turn may result altered hematological profiles and also some metabolites and enzymes of protein metabolism as evidenced in the present investigation (Chapter II & III).

Several authors reported histopathological changes in heart in different animal models under pesticidal toxicity.

Luty et al. (1997) reported focal hypertrophy of the interstitial tissue, homogenous of basophilous cytoplasm in muscle fibres and between the fibres of heart muscle occur small focuses of infiltrations containing mononuclear cells in exposed rat to paraquat. Luty et al. (1998) observed infiltration of lymphocytes between cardiomyocytes in heart of rats exposed to dichlorvos.

Tos-Luty et al. (2001) observed inflammatory infiltration between the cardiac fibres in the heart of rats exposed to carbaryl. Tos-Luty et al. (2003) observed focal parenchymatous degeneration of cardiomyocytes and the presence of single basophils in the heart of rats orally exposed to malathion. Purohit (2005) observed mild congestion in cardiac muscle fibres due to acephate in synthetic white leghorn birds.

Nagarjunna (2007) observed congestion and slight infiltration in cardiac muscle exposed to cypermethrin in albino rats. Rajeswari (2008) congestion and infiltration was observed in acephate treated albino rats.
In the present investigation chlorpyrifos exhibited clear histopathological changes in reproductive tissue viz., testis. These pathological changes include congestion, increase amount of intertubular connective tissues, clumped spermatozoa, increased size of lumen in seminiferous tubules, reduced number of spermatids and degenerative changes (Plates 5A.11 to 5A.12; Figs. U to X).

Several authors have reported histopathological changes in testis in different animal models under pesticidal toxicity. Farag et al. (2000) observed exfoliation of the germinal cells into the lumen with ill-defined spermatogenesis in the testis of mice exposed to acephate. Cypermethrin is known to reduce fertility in male rats through affecting of testosterone, follicle-stimulating hormone and luteinizing hormone and the number of cell layers of the seminiferous tubules as well as to cause congestion and hemorrhages in testis (Elbetieha et al., 2001).

Khan et al. (2001) observed the loss of spermatozoa, and complete derangement of cellular organization of testis in rats treated with novel phosphorothionate. Manna et al. (2004) reported edema between seminiferous tubules, vacuolation and hyalinization in the tubules of rats testes exposed to α-cypermethrin.

Manna et al. (2005) reported edematous fluid accumulation between the tubules and vacuole formation within the tubules in testes of rats exposed to deltamethrin. Purohit (2005) observed increase amount of intertubular connective tissues in testis due to acephate in synthetic white leghorn birds.

Sivaiah (2006) reported necrosis in the connective tissue, degeneration of spermatids, atrophied seminiferous tubules and reduced lumen, atrophied spermatozoa, clumping of spermatozoa and necrosis in interstitial cells in mice testis exposed to monocrotophos and azadirachtin.
Chlorpyrifos exposure has resulted clear architectural changes in liver, heart, kidney, intestine, testis and muscle tissues of exposed rats.

In general, it is obvious that in the tissues studies, the structural integrity was found to be disrupted to a greater extent, suggesting that cypermethrin even in sublethal dose causes deleterious effects and leads to the death of the animal, when exposed to the intervals even beyond the period investigated in the present study.

Severity of histopathological changes has been observed in multiple dose chlorpyrifos administered rats than those of single and double dose. This clearly indicated that repeated exposure to low doses/concentrations causes deleterious effects and making them less fit for better survival. It is reported that the damage in tissues of non-target organisms was time and concentration/dose dependent (Amminikutty and Rege, 1978; Dubale and Shah, 1979; Nagaratnamma, 1982).
LEGEND FOR FIGURES

Plate 5A.1

Fig. A: Control rat liver showing hepatocytes (H) with centrally placed prominent nucleus (N) with sinusoids (S) and central vein (CV). H & E. 10x.

Fig. B: Single dose chlorpyrifos administered rat liver showing central vein congestion (CVC), cellular swelling (CS) cytoplasm porus in sub capsular hepatocytes, sinusoidal hemorrhage (SH) and degenerative changes in hepatocytes. H & E. 10x.
LEGEND FOR FIGURES

Plate 5A.2

Fig. C: Double dose chlorpyrifos administrated rat liver showing central vascular congestion (CVC) and dilated sinusoids (DS). H & E. 10x.

Fig. D: Double dose chlorpyrifos administrated rat liver showing sinusoidal hemorrhage and focal necrotic areas (FNA). H & E. 40x.
LEGEND FOR FIGURES

Plate 5A.3

Fig. E: Multiple dose chlorpyrifos administrated rat liver showing severe degenerative changes in central vein (SDCV) and diffused necrotic areas (DNA). H & E. 10x

Fig. F: Multiple dose chlorpyrifos administrated rat liver showing diffused necrotic areas (DNA). H & E. 40x
LEGEND FOR FIGURES

Plate 5A.4

Fig. G: Control rat kidney showing glomerulus (G) glomeruli with stalk (GS), Bowman’s capsule (BC), proximal convoluted tubule (PCT) and distal convoluted tubule (DCT). 10x.

Fig. H: Single dose chlorpyrifos administrated rat kidney showing hemorrhage (H). H & E. 10x.
LEGEND FOR FIGURES

Plate 5A.5

**Fig. I:** Single dose chlorpyrifos administered rat kidney showing hypertrophy of goblet cells (HG). H & E. 10x

**Fig. J:** Double dose chlorpyrifos administered rat kidney showing hyaline deposits in the lumen (HY) and hemorrhage (H). H & E. 10x
PLATE - 5A.5
LEGEND FOR FIGURES

Plate 5A.6

Fig. K: Multiple dose rat kidney showing hyaline deposits in the lumen (HY) and necrotic changes in tubular epithelial cell (NCTEC). H & E. 10x

Fig. L: Control rat intestine showing serosal layer (SL), longitudinal muscle layer (LML), sub mucosa (SM), lamina propria (LP), villus (V), epithelial layer (EL). H & E. 10x
PLATE - 5A.6
Plate 5A.7

**Fig. M:** Double dose chlorpyrifos rat intestine showing degenerative changes in the mucosal layer (DCML). H & E. 10x.

**Fig. N:** Multiple dose administered rat intestine showing degenerative changes in villi (DCV) and hemorrhage (H). H & E. 10x
LEGEND FOR FIGURES

Plate 5A.8

**Fig. O:** Multiple dose chlorpyrifos rat intestine showing Hypertrophy of goblet cells (HGC) and Infiltration (I). H & E. 10x

**Fig. P:** Multiple dose administered rat intestine showing hyperemic changes in blood vessel (HCBV) and infiltration (I). H & E. 10x
LEGEND FOR FIGURES

Plate 5A.9

Fig. Q: Control rat heart showing cardiac muscle fibres with nucleus (N). H & E. 10x

Fig. R: Double dose chlorpyrifos administered rat heart showing hemorrhage (H) diffused area with hemorrhage (DAH). H & E. 10x
LEGEND FOR FIGURES

Plate 5A.10

Fig. S: Multiple dose heart showing diffused areas with hemorrhage (DAH). H & E. 10x

Fig. T: Multiple dose chlorpyrifos administered rat heart showing hemorrhage (H) diffused area with hemorrhage (DAH) and rounded nucleus (RN). H & E. 10x
LEGEND FOR FIGURES

Plate 5A.11

Fig. U: Control rat testes showing seminiferous tubules (ST), Germinal epithelial layer (GEL), Spermatids (SP), matured spermatozoa (S) and lumen of seminiferous tubules (LST). H & E. 10x

Fig. V: Single dose chlorpyrifos administered rat testes showing congestion (C) degenerative changes in seminiferous tubules (DCS). H & E. 10x
LEGEND FOR FIGURES

Plate 5A.12

**Fig. W:** Double dose chlorpyrifos administered rat testes showing congestion (C) and degenerative changes in seminiferous tubules (DCS) clumped spermatozoa (CS). H & E. 10x

**Fig. X:** Multiple dose chlorpyrifos administered rat testes showing increased connective tissue (ICT) degenerative changes in seminiferous tubules (DCS), clumped spermatozoa (CS), increased size of lumen (ISL). H & E. 10x
PLATE - 5A.12
CHAPTER -5B

TRANSMISSION ELECTRON MICROSCOPY
Electron microscopy is widely used as a basic research tool, and necessary for other components of a toxicity study such as biochemical, metabolism and kinetic studies. Transmission electron microscopy also has a well established role in the characterization of sub cellular structural alterations in tissues which have been modified by the effects of xenobiotics.

Electron microscopy provides a static morphological assessment of cells, its ability to characterize changes in sub cellular organelles can provide valuable information about any functional deficits. Electron microscopy, in contrast to light microscopic examination, allows the characterization of changes such as proliferation of the exclusion of sub cellular degeneration in vital organs such as the heart when unexplained macroscopic or weight changes are seen without a light microscopic correlate.

Electron microscope is a scientific instrument that use a beam of highly energetic electrons to examine objects on a very fine scale. This examination can yield Topography, Morphology, Composition and Crystallographic information. The transmission electron microscope (TEM) was the first type of Electron microscope to be developed and is patterned exactly on the light transmission microscope except that a focused beam of electrons is used instead of light to “See through” the specimen. It was developed by Max Knoll and Ernst Ruska in Germany in 1931. Despite the technological advances in transmission electron microscopy, it is highly selective and only small samples of tissues can be examined. So that appropriate and defined objectives are selected and examined with in the context of a toxic study.

The use of larger resin embedded sections is a cost effective compromise between electron microscopy and conventional light microscopy. Sometimes termed ‘high resolution light microscopy’, light microscopic evaluation of semi thin sections can provide a means of avoiding extensive use of the electron microscopy, because it can locate cytoplasmic organelles in a way sometimes not possible in paraffin embedded material.
In spite of the apparent similarities there are great differences between the Light and the Electron Microscopy. In case of EM, molecules or supramolecular structures is now possible to obtain more detailed information. The electron microscope (EM) permits a direct study of biological ultra structure. Its resolving power is much greater than that of the light microscope. (Bozzola and Russell, 1992; Aughey and Frye, 2001).

Physiological studies do not satisfy in complete understanding the impact of any deleterious chemical. The toxicity of any chemical necessarily impairs the metabolic strategy of animal physiology. To have a clear understanding, as to how these chemicals cause injury to the tissues, it is essential to have an insight into histopathological analysis of the tissues, wherein, one can envisage a better understanding of the pathological conditions of tissues under toxic stress of pestilent. Thus, histopathology helps in diagnosing the damages of the tissues of an animal subjected to toxic stress of pestilence (Jayantha Rao, 1982). Several workers reported that the pesticides cause damage at cell organelles (Latuszynska et al., 1999; Haratym-Maj, 2000; Tos-Luty et al., 2001, 20003; Madhaveelatha, 2006; Madhava Rao, 2007; Nagarjuna, 2007; Rajeswari, 2008). In view of the histopathological changes observed in the present investigation (Chapter 5B; Plates 5B.1 to 5B.4), an attempt has been made to study the transmission electron microscopy (TEM) study to observe the possible changes at cell organelles under chlorpyrifos intoxication in albino rats.

RESULTS

Normal electron microscopic structure of rat liver cells (hepatocytes)

The cells of the liver are called hepatocytes. They are large sized and polygonal cells. Hepatocytes have prominent nuclei uniformly distributed chromatin and centrally placed, uniform distribution of cell organelles, rough endoplasmic reticulum, smooth endoplasmic reticulum, golgi apparatus, many
mitochondria, lysosomes, rich in peroxisomes, rich in secretory vesicles and secondary lysosomes.

Hepatocytes are rich in glycogen granules and fat droplets. Each cell has central nucleus with distinct nuclear membrane and one or more prominent nucleoli. The mitochondria of the hepatic cells are spherical, rod shaped or filamentous, depending on the location of the cell within the lobule and on the functional state. The Golgi apparatus lies either near the edge of the cell or close to the nucleus. The rough endoplasmic reticulum shows continuity with smooth endoplasmic reticulum. The cell shows glycogen in the form of rosettes of dense granules. Clear granular mitochondria were seen (Agarwal, 2001; Aughey and Frye, 2001; Lodish et al., 2004) (Plate 5B.1; Figs. A & B).

**Ultra structural changes of rat liver hepatocytes under chlorpyrifos intoxication**

Ultra structural changes in hepatocytes following oral multiple dose administration of the chlorpyrifos showed paler nucleus with dense condensation of chromatin centrally very little condensed chromatin is attached to periphery of nucleus, nucleus at the periphery site of the cell, condensation of cell organelles towards the nucleus, loss of cytoplasmic organelles, cell organelles absent at the periphery site of cell, lucent areas of cytoplasm containing the residues of cell organelles and lipid vacuoles, total cell size decrease, ruptured mitochondria, disappearance of secretory vesicles, decrease in the number of mitochondria, increase in the number of lipid vacuoles, increase in the number and rupture of peroxisomes, proliferation of endoplasmic reticulum and nick of plasma membrane. Mitochondria were usually swollen, showed a clearance of the matrix and destruction of cristae (Plates 5B.2; Figs. C & D).
Normal electron microscopic structure of rat kidney

Each kidney is enclosed in a tough connective tissue capsule extending into the parenchyma and has two regions — the cortex and the medulla.

Nephron

The nephron is the functional unit of the kidney. The major subdivisions are the renal corpuscle and the uriniferous tubule. The blind end of the proximal tubule is indented with a network of capillaries and supporting cells to form a filtering system: the renal corpuscle. Each renal corpuscle consists of a glomerulus and a glomerular (Bowman’s) capsule. The outer layer of the glomerular capsule is the capsular (parietal) wall, which is separated from the glomerular (visceral) layer by the capsular (urinary) space. The capillaries are lined with a fenestrated endothelium resting on a basal lamina.

The visceral epithelial cells, or podocytes, closely invest the capillary endothelium of the glomerulus and develop primary processes wrapped around each capillary. These processes develop secondary foot processes called pedicles. The foot processes of adjacent podocytes interdigitate, resulting in the formation of small gaps called slit pores. The podocyte basal lamina is fused with the endothelial basal lamina and blood passing through the capillary is filtered through this common basal lamina into the capsular space. Mesangial perivascular cells are present between the endothelium and the basal lamina. The capillaries of the glomerulus are served by an afferent and an efferent arteriole, entering and leaving the renal corpuscle at the vascular pole. At the opposite pole is the capsular space, where the filtrate passes into the proximal tubule at the urinary pole of the renal corpuscle.

This nomenclature is based on the functional areas of the renal tubule. The proximal convoluted tubule (PCT) is long and lined with low columnar
cells with a basal nucleus. The cytoplasm is deeply stained with eosin and the apical surface is a continuous brush border. The basal plasma membrane is folded, with mitochondria in the cytoplasm giving a striated effect, and functions to increase the surface for transport. Columnar cells have prominent nucleus, rough endoplasmic reticulum, smooth endoplasmic reticulum, Golgi complex secretory vesicles and uniformly distributed cell organelles. The PCT is continued with the proximal straight tubule. It is similar in appearance and extends towards the medulla where the epithelium changes abruptly to simple squamous.

This part of the tubule descends into the medulla as the thin descending limb and bends sharply to return to the cortex as the thick ascending limb, which was previously known as the loop of Henle. In the cortex the epithelium becomes cuboidal or columnar and forms the distal straight tubule and coils near the glomerulus to become the distal convoluted tubule (DCT). The DCT is shorter than the PCT, the epithelium is cuboidal, the cytoplasm is paler and there is no brush border. The DCT approaches the glomerulus at the vascular pole, where it thickens, and the cell nuclei of the tubule wall become crowded together to form the macula densa, part of the juxtaglomerular apparatus. Juxtaglomerular cells are modified smooth muscle cells in the walls of afferent arterioles close to the glomerulus.

The collecting tubule or duct (lined with poorly staining cuboidal epithelium) is the terminal segment of the nephron, a continuation of the DCT within the medulla, joining with others to form straight ducts: the papillary ducts. Here the epithelium becomes columnar, and then becomes urethelium towards the opening into the renal pelvis (Aughey and Frye, 2001) (Plate 5B.3; Fig. E).
Ultra structural changes of rat kidney cells of renal proximal tubules under chlorpyrifos intoxication

Ultra structural studies of the cells of renal proximal tubules of chlorpyrifos treated albino rat showed reduced nucleus size and dense chromatin condensation along the nuclear periphery, ruptured brush border, increase in the number of autophagous vacuoles and secondary lysosomes, reduced the number of secretory vesicles and lucent areas of cytoplasm containing the residues of cell organelles, secondary lysosomes and autophagous vacuoles, showed clearance of matrix and destruction of cristae (Plate 5B.3; Fig. F).

Normal histology of rat testes

The testis is covered by mesothelium continuous with the visceral layer of the tunica vaginalis. A thick dense connective tissue capsule, the tunica albuginea, encloses the testis. A variable amount of smooth muscle may be present. The tunica vasculosa is the inner vascular layer of the tunica albuginea.

The capsule is reflected into the median plane of the testis to form a partition, the mediastinum, and gives off loose vascular connective tissue, the septula testis, to divide the testis into lobules to support the seminiferous lobules. The coiled seminiferous tubules are lined with a multilayered seminiferous epithelium of spermatogenic cells and sustentacular (Sertoli) cells. They rest on a basement membrane and are surrounded by a lamellated connective tissue with myoid elements. The specific interstitial (Leydig) cells are found in the loose vascular connective tissue separating the tubule.

The testis of rat consists several seminiferous tubules. The seminiferous tubules are covered with connective epithelial layer. The seminiferous tubule under microscope showed spermatids, matured.
spermatozoa and sertoli cells. Interstitial cells are present in between the seminiferous tubules.

The electron microscopic picture of seminiferous tubule of rat testis showed with matured spermatozoa with tail and head, sertoli cells and junction layer which separate the seminiferous tubules and uniform distribution of leydig cells were observed (Plate 5B.4. Fig. G).

**Ultra structural changes of rat testis under chlorpyrifos intoxication**

Ultra structural changes in rat testis following oral multiple dose administration of the chlorpyrifos showed acrosomal degeneration, atrophy of interstitial cells and degenerative changes in the epithelial layer of spermatozoa and degeneration of interstitial cells were observed (Plate 5B.4; Fig. H).

**DISCUSSION**

The electron microscopic observation of liver and kidney under multiple dose of chlorpyrifos administration showed pronounced pathological changes in cell organelles.

Ultra structural changes in hepatocytes were dense condensation (clumping) of chromatin, fragments of rough endoplasmic reticulum, proliferation of endoplasmic reticulum, ruptured mitochondria showing clearance of matrix and destruction of cristae, increase and rupture of peroxisomes, increase in the number of lipid vacuoles, disappearance of secretory vesicles and decrease in the number of mitochondria.

Ultra structural studies of the cells of proximal tubules showed reduced nucleus size and dense chromatin condensation (clumping) along the nuclear periphery (nuclear Pycnosis), ruptured brush border and increase in the number of autophagous vacuoles and secondary lysosomes.
The pathological changes observed in the present investigation clearly indicate that chlorpyrifos not only caused damaged at cellular level of these organs but also caused damage at sub cellular level.

Dense chromatin condensation and fragments of rough endoplasmic reticulum observed in the present investigation might have resulted in drastic altered in some metabolites and enzymes of protein metabolism (Chapter III).

Accordingly, proliferations of endoplasmic reticulum suggest that this cytoplasmatic structure may participate in the metabolism of chlorpyrifos. On the ultra structural level, the changed cytoplasmatic structures represent microsomal component of the liver, which according to Mac Pherson et al. (1991) and Tos-Luty et al. (2001) are responsible for carbaryl metabolism.

Increase in the number of autophagous vacuoles observed in the present investigation. This may suggest an intensification of the processes of intracellular digestion. Changes in mitochondria were connected with disturbances in oxido-reduction processes taking place in the organelle. Increase in the number of peroxisomes may be associated with the cellular response to the toxic effect of free radicals induced by chlorpyrifos, as peroxisomes contain enzymes which inactive these radicals.

The areas of cytoplasm were observed in the present investigation to be deprived of normal cellular organelles, with an increased number of lipid vacuoles. These changes confirm the focal degeneration of the cytoplasm.

Focal degeneration of the cytoplasm was observed in the present investigation in tubular cells, manifested by the presence of autophagous vacuoles. Changes of this type are irreversible and are undoubtedly associated with the destruction of the protein-lipid structure of intracellular membranes and lysis of cytoplasm.
According to the authors, this is a manifestation of the toxic effect on cells, because the integrity of protein-lipid membranes ensures the normal functioning of the cells (Videira et al., 2001). In studies in vitro concerning the effect of organophosphorus pesticides, on protein-lipid membranes in mammals it was shown that these compounds change physical and chemical properties of the membranes.

Due to their hydrophobic nature and small molecular size, chlorpyrifos passes through the cell membrane and reaches the nucleus. It is suggested that within the nucleus chlorpyrifos binds to DNA through the reactive groups of its acid moiety, leading to destabilization as well as unwinding of the DNA, which could be possible for its genotoxicity.

Cypermethrin induced DNA damage in other vital organs like liver and kidney. This could be attributed to the fact that cypermethrin exposure induces free radical mediate tissue damage in rat liver and kidney (Kale et al., 1999). This provides evidence that DNA damage to form condensed chromatin at the centre of nucleus observed after cypermethrin exposure could be a consequence of free radical attack to DNA.

The appearance of DNA ladder confirmed the apoptotic death induced by chlorpyrifos in avian hepatocytes and splenocytes. DNA fragmentation is one of the well known features of apoptosis. The DNA Ladder assay is based upon the principle that during apoptosis, cellular nuclear DNA is non-randomly cleaved into 180-200 base pair units. When run on an agarose gel, this DNA ladder can be detected, and is an indicator of apoptotic cells. DNA fragmentation has been suggested to be one of the first irreversible events to trigger mature immune cell apoptosis (Tripathi et al., 2007).

The increase in total protein and albumin may be the result of hypertrophic changes in the rats' liver in response to the mixture of persistent contaminants and the proliferation of endoplasmic reticulum implied by the
obvious cellular hypertrophy and increased cytochrome P450 activity (Wade et al., 2002).

Bone matrix is synthesized in the rough endoplasmic reticulum of osteoblasts (Cooper et al., 1966) and disorganization of endoplasmic reticulum in DDT intoxicated chicken has been noticed (Reyes and Moore, 1979). Proliferation of agranular endoplasmic reticulum with concomitant vesiculation of rough endoplasmic reticulum of liver cells due to DDT in chicks has been reported (Svendson, 1973). Garg et al. (2004) reported pesticide treatment caused significant reduction in total serum protein in the broiler chicks. Thus it is possible that the rough endoplasmic reticulum of osteoblasts is adversely altered due to low dose feeding of different pesticides in broiler chicks causing lower synthesis of bone matrix and diminished appositional bone growth.

Carbosulfan and cypermethrin have definite interactions with DNA metabolism in mice, resulting in sister chromatid exchanges, indicating potential mutagenic effects (Sarbani Giri et al. 2003).

Several authors reported ultra structural changes in hepatocytes in different animal models under pesticidal toxicity.

Luty et al. (1997) reported the appearance of pleomorphic mitochondria, widening of the channels of the smooth endoplasmic reticulum and slight increase in the number of peroxisomes in liver tissues of rats exposed to paraquat.

Luty et al. (1998) observed increase in the quantity of electron dense bodies which occurred primarily in the form of secondary lysosomes of varying size and internal structure, widening of the tubules of rough endoplasmic reticulum, overgrowth of smooth endoplasmic reticulum and
enlarged and irregular shape of mitochondria in the liver of rat exposed to α-cypermethrin.

Luty et al. (1998) observed vacuoles of low electron density of various sizes and shapes and high concentrations of lipid-like bodies in liver of rat exposed to dichlorvos. Latuszynska et al. (1999) observed slight empty cytoplasm spaces within hepatocytes with a small amount of membranous and granular material and also increased number of peroxisomes in the liver of rats exposed to chlorpyrifos and cypermethrin.

Haratym-Maj (2000) observed considerable increase in the lipid-like bodies of various sizes in hepatocytes. These changes were usually accompanied by an increased number of fine peroxisomes in livers of mice exposed to α-cypermethrin.

Tos-Luty et al. (2001) reported decrease in amounts of the rough endoplasmic reticulum, as well as an increase in amounts of the smooth endoplasmic reticulum in the cytoplasm of hepatocytes in the liver of rats exposed to carbaryl. Tos-Luty et al. (2003) observed widening of ergastopasma tubules and slightly swollen mitochondria in hepatocytes of rat liver dermal exposed to malathion. Tos-Luty et al. (2003) observed lucent areas of cytoplasm containing the residues of cell organelles and lipid vacuoles. Mitochondria were usually swollen, showed a clearance of the matrix and destruction of crists in the liver of rats orally exposed to malathion.

Madhaveelatha (2006) reported reduction in nucleus size, scattered chromatin, necrotic changes in the mitochondrial membrane and degeneration in endoplasmic reticulum in mice liver hepatocytes exposed to monocrotophos.
Nagarjuna (2007) reported paler nucleus with dense condensation of chromatin centrally very little condensed chromatin is attached to periphery of nucleus, nucleus at the periphery site of the cell, condensation of cell organelles towards the nucleus in rat liver cell exposed to cypermethrin.

Rajeswari (2008) reported ruptured mitochondria showing clearance of matrix and destruction of cristae, increase and rupture of peroxisomes, increase in the number of lipid vacuoles, disappearance of secretory vesicles in rat liver cell exposed to acephate.

Several authors reported ultra structural changes in cells of renal proximal tubules of kidney in different animal models under pesticidal toxicity.

The amount of digestive vacuole in cytoplasm increased, and their size and internal structure were different in the renal proximal tubule. Electron bright vacuoles, giant mitochondria and some of the slightly swollen mitochondria loss their cristae and brightness of the matrix in the renal proximal tubule. The intertubular space is wide and contains the interstitial elements was observed in the kidney of rats exposed to paraquat (Luty et al. 1997).

Sporadically, the lack of invagination of the cell membrane, as well as thickening of the basal lamina of proximal tubules was observed in the kidney of rats exposed to α-cypermethrin. Luty et al. (1998) observed the widening of rough endoplasmic reticulum, Golgi apparatus, swollen mitochondria with brightened matrix and electron light structures in the epithelial cells of proximal tubuli.

Luty et al. (1998) observed the widened spaces between the proximal tubules infiltrated with lymphocytes and large electron light vacuoles and slight changes in peroxisomes in the cells of proximal tubules in the kidney of
rat exposed to dichlorvos. Latuszynska et al. (1999) observed empty spaces and partial vacuolization of the cytoplasm and also increase in the number of electron dense bodies in some cells of proximal tubules. The disorders also affected a few renal glomeruli and were manifested as vacuolization and lighting of basic cytoplasm of podocytes in the kidney of rats exposed to chlorpyrifos and cypermethrin.

Haratym-Maj (2000) observed in the cells of proximal tubules in the kidney an increase in the number and size of autophagous vacuoles as well as accumulation of electron dense bodies and a clear widening of the Golgi structures in kidneys of mice exposed to α-cypermethrin. Tos-Luty et al. (2001) observed increase in number of autophagous vacuoles in the cells of proximal tubuli in the kidney of rats exposed to carbaryl.

Tos-Luty et al. (2003) observed vacuoles with damaged external membrane as well as swollen and pleomorphic mitochondria in the cells of renal proximal tubules of the rat kidney orally exposed to malathion. Madhaveelatha (2006) reported reduced nucleus size, loss of brush border and vacuoles formation in the cells of renal proximal tubules in the kidney of mice exposed to monocrotophos.

Several authors reported ultra structural changes in rat testis in different animal models under pesticidal toxicity.

Chlorpyrifos in the present investigation exhibited clear lesions in reproductive tissue viz., testis. These include degeneration of spermatids, atrophied seminiferous tubules and reduced lumen, atrophied spermatozoa, necrosis in interstitial cells and degenerative changes in the epithelial cells (Plate 5B.4 Fig.H).

Epididymis consists of three parts. They are 1) the caput epididymis: which lies at the end of the testis t which the spermatic cord is attached. 2) the
Electron Microscopy

carpus epididymis or central body and 3) the cauda epididymis which is present at the apposite pole of the testis. Holocrine and principal cells are present in the rat epididymis. The lining epithelium of the epididymis is composed of two types of cells, narrow and tall columnar cells and round basal cells. The columnar cells bear numerous very long microvilli, which are largely nonmotile and named as stereocilia. The basal cells, which are numerous in number, rest on the basement membrane. They are spherical in shape and with almost central round nuclei. These cells are thought to be the progenitors of the columnar cells.

Robaire and Hermo (1988) have revealed the histoarchitecture of the various cell types in the ductus epididymal epithelium of control rats. In treated rats, the cytoplasm of epididymis vacuolrized and nucleus was placed towards apical end, with the decrease in cell height and width. The cytoplasmic granules were increased in size and also depletion of circulating levels of androgens in the principal cells of the caput epithelium of rats (Akbarsha and Siva swamy, 1998).

The degenerated interstitial cells may also result in the failure of testosterone in male. More changes were found in multiple dose of chlorpyrifos exposed testis. Germinal epithelium is a multi layered structure formed by two kinds of cells, sertoli cells and germ or spermatogenic cells. Sertoli cells are bigger, some what triangular cells with broad bases and distinct out lines. The fully formed sperms remain attached to the edges of the sertoli cells. Spermatogenic cells are several kinds with 4 to 8 layered in the epithelium between the basement membrane and the lumen (Majumdar, 1980).

Decrease in sperm reserve appears to be a reason in the reduction of epididymis weight in the rats exposed to drugs (Sarkar et al., 1997). The seminiferous tubules of testis also revealed perceptive changes like
concentration of oedematous fluid in the interstitium, cessation of
spermatogenesis and atrophy of leydig cells while the seminiferous tubular
diameter decreased at day 15. On day 30 the degenerative changes were
clearer wherein the loss of the cellular identity of the germinal epithelium and
complete arrest of spermatogenesis were noticed.

Testis of treated rats showed dose dependent gradual histological
changes in the testicular tissue but presence of a few normal seminiferous
tubules together with damaged ones was a common feature in testicular
architecture. The exposure of animals to organophosphorus pesticide causes
so many testicular histopathological changes in mammals and other animals
as reported in rats by Dikshith, and Duuta (1972), Chouha et al., (1974),
Roy Choudary et al., (1981), Bhaumik et al., (1990), Borania and Sahai
and Modi (1993) reported many histopathological changes in the testicular
tissue of albino rats due to pesticide intoxication.

Newly developed techniques in high resolution Transmission Electron
Microscopy (TEM) and for tissue processing procedures have been applied to
an investigation of structure of various cells in rat testis. A series of high
resolution TEM micrographs are presented which survey the testis cells which
also illustrate ultra structural features of some of their intracellular organelles.

Electron microscopy enables more detailed evaluation of sperm
alterations (Baccetti, et al 1984; Zamboni et al., 1987; chemes et al., 1998),
and permits a distinction to be made between phenotypic and genotypic
defects (Baccetti et al.,2001) hereditary male sterility due to genotypic sperm
defects is a possibility. The original idea of generic human male infertility
correlated to chromosome abnormalities could give way to the concept that
male infertility is due to particular defects, caused by chromosome anomalies,
heralding research in to the genes responsible for hereditary sperm
characteristics and their mutations. Recently, fibrous sheath dysplasia of 'stump' spermatozoa was associated with pericentric inversion of chromosome 9 (Baccetti et al., 1997). However the clearest connection between a sterilizing human sperm defect and a specific chromosome alteration was the discovery of mutations in the DNA11 gene, mapping to 9 p13-p21, in three Kartagener's syndrome patients (Guichard et al., 2001). In studying the association between chromosome mutations and sperm defects in sterile men, immature sperm was found in a human carrier of Robertsonian translocation 14;22 (Baccetti et al., 2002). This anomaly was most likely in a chromosome region involved in spermatogenesis.

Jayantha Rao et al., (1984, 1986) reported various histological changes and histochemical change in a fresh water fish, Tilapia mossambica under sub lethal concentration of phosphomidon and heptachlor.

Sivaiah (2007) reported degenerative changes in plasma membrane of spermatozoa, spermatids and sertoli cells, reduced length in spermatozoa, atrophy of leydig cells, degenerative changes in sertoli cells of mice exposed to monocrotophos and azadirachtin.

Chlorpyrifos caused hematological and some metabolites and enzymes of protein metabolism changes besides irreparable architectural changes in different tissues of experimental rats. All these changes were more pronounced in multiple dose chlorpyrifos administered rats clearly indicates that the frequent exposure of non-target organisms including human beings to pesticides may result vulnerability and eventual death. In nature's conservationist point of view, pesticides should not affect the non-target life adversely but should degrade in the ecosystem at a faster rate, otherwise problems like biomagnifications and cumulative effects will arise.
LEGEND FOR FIGURES

Plate 5B.1

**Fig A:** Electron micrograph of control rat liver showing prominent nucleus (N), clear chromatin (C), nuclear membrane (NM), rough endoplasmic reticulum (RER), secondary lysosomes (SL), peroxysomes (P) and secretory vesicles (SV) (Stain: Uranyl acetate and lead citrate). 2Kx

**Fig. B:** Electron micrograph of control rat liver cell showing rough endoplasmic reticulum (RER), more number of mitochondria (M) and secretory vesicle (SV) (Stain: Uranyl acetate and lead citrate). 10Kx
LEGEND FOR FIGURES

Plate 5B.2

Fig. C: Electron micrograph of multiple dose chlorpyrifos exposed rat liver cell showing dense condensation of chromatin (CC), ruptured peroxisomes (RP), lucent area of cytoplasm (LAC) consist of residual organelles and lipid vacuoles (LV) (Stain: Uranyl acetate and lead citrate). 4Kx

Fig. D: Electron micrograph of multiple dose chlorpyrifos exposed rat liver cell showing lucent area of cytoplasm (LAC) consist of residual organelles and vacuoles (V), degenerative changes in nuclear membrane (Stain: Uranyl acetate and lead citrate). 6Kx
LEGEND FOR FIGURES

Plate 5B.3

**Fig. E:** Electron micrograph showing nucleus (N), chromatin (C), nuclear membrane (NM), golgi apparatus (GA), mitochondria (M), secretory vesicles (SV), brush border (BB) and basal membrane (BM) in a control rat renal proximal tubule cell (Stain: Uranyl acetate and lead citrate). 3Kx

**Fig. F:** Electron micrograph of multiple dose rat renal proximal tubule cell showing degenerative changes in nucleus (N), lucent areas of cytoplasm (LAC), Pushing of cell organelles to the periphery (Stain: Uranyl acetate and lead citrate). 3.5Kx
LEGEND FOR FIGURES

Plate 5B.4

Fig. G: Electron micrograph of control rat testis showing spermatozoa with prominent structure, leydig cells and sertoli cells are uniformly distributed and the epithelial cells of spermatozoa is completely covered (Stain: Uranyl acetate and lead citrate). 6Kx

Fig. H: Electron micrograph of multiple dose chlorpyrifos exposed rat testis showing severe degenerative changes in acrosome, irregular arrangement of epithelial cells, atrophy of interstitial cell (Stain: Uranyl acetate and lead citrate). 6.5Kx
PLATE - 5B.4

Fig. G

Fig. H

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