HISTOPATHOLOGICAL CHANGES IN THE GILL, LIVER, BRAIN AND KIDNEY OF THE FISH CHANNA PUNCTATUS EXPOSED TO QUINALPHOS TECHNICAL GRADE AND 25% EC

The aquatic environment is continuously being contaminated with toxic chemicals from industrial, agricultural and domestic activities. Pesticides are one of the major classes of toxic substances used in India for management of pest in agricultural lands and control of insect vectors of human disease. The runoff from treated areas enters the river and aquaculture ponds that are supplied by rivers. Such rivers and the adjacent aquaculture ponds are likely to be contaminated by pesticides (Begum, 2004).

According to Robinson (1996), first world countries investigated methods to detect the initial signs of decreasing water quality, to prevent increasing pollution problems. Organisms inhabiting in aquatic environments are considered biologically sensitive, due to their ability to respond to changes that occur in the water. The biotic integrity of an ecological system is therefore reflected in the quality and quantity of its fauna (Robinson, 1996). Changes, occurring specifically of biochemical, histological and physical alterations, in fish populations due to chemical stress, are manifestations resulting and can give a relatively rapid indication of how environmental conditions affect fish populations. Fish populations will either adapt to environmental changes, or may result in mortality in low concentrations. To manage healthy fish populations, it is necessary to identify and are early detectable warning signs of damage on cellular level, before physiological and behavioral processes are affected and can be achieved as bioindicator through histological analysis.

Histopathological biomarkers are closely related to others of stress since many pollutants either toxic or non toxic have to undergo metabolic activation in order to be able to culminate cellular change in the affected organism. The mechanism of action of several xenobiotics could initiate the formation of a specific enzyme activity that causes changes in metabolism, further leading to cellular intoxication and finally death. These manifests as necrosis, as well as from chemical insult, lesions may arise and other degenerative alterations to which the organism responds with an inflammatory, defensive reaction (Velkova-Jordanoska, 2002; Roganovic-Zafirova et al., 2003).

Histopathological studies have been conducted to help for establishment of causal relationships between contaminant and exposure and other various biological responses. These
investigations have also been proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments (Sakr and Jamal Al lail, 2005; Machado and Fanta, 2003; Schwaiger et al., 1996) Such analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and gonads (Dutta, 1996). Investigation of such nature may therefore prove to be a cost effective tool to determine the nature of fish populations, hence reflecting the well being of an entire aquatic ecosystem.

Mode of action of different chemicals varies leading to effects of different nature, on various body tissues. Some toxins exert their effect locally at the portal of entry; resulting in damage to external surface of the body and some when ingested, affect even the different regions of gastro intestinal tract. Contrary to this there are toxins that do not cause deleterious effects of the portal or entry but they systematically affect the tissue in which they get accumulated. Thus, various chemicals with their varied mode of action affect different tissues thereby bringing about certain architectural changes ultimately culminating either death of the organism or making the organism less viable for its survival.

Changes in organs/tissues have been widely used as biomarkers in the evaluation of the good commodity of fish exposed to contaminants, both in the laboratory (Wester & Canton, 1991; Thophon et al., 2003) and field studies (Hinton et al., 1992; Schwaiger et al., 1997; Teh et al., 1997). One of the great advantages of environmental monitoring is that this category of examining specific target organs, including gills, kidney and liver, which are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish (Gernhofer et al., 2001) and all can be revealed only by such study. Furthermore, the alterations found in these organs are normally easier to identify than the functional ones (Fanta et al., 2003), and serve as warning signs of damage to animal health (Hinton & Laurén, 1990).

The toxicity of any pollutant is either acute or chronic. The chronic studies include both histochemistry and pathology. Although, toxicant impairs the metabolic and physiological activities of the organisms, such studies alone do not satisfy the complete understanding of pathological conditions of tissue under toxic stress. The extent of severity of tissue damage is a consequence of the concentration of the toxicant and is time dependent. Hence, it is useful to have an insight into the histological analysis. Also, the severity of damage depends on the toxic
potentiality of a particular compound or pesticide accumulated in the tissue (Jayantha Rao, 1984).

Tissue changes in test organisms exposed to a sub-lethal concentration of toxicant are a functional response of organisms which provides information on the nature of the toxicant. Numerous reports are available to understand the biochemical physiological and metabolic alterations that are created by the chronic effects of pesticides on animals and fishes (Sornaraj et al., 2005; Aruna et al., 2000; Geetha et al., 1999; Ranjitsingh et al., 1996; Sambasiva Rao, 1999). Although major advances have been made in recent years in science, the histology and histopathology of fish and other aquatic invertebrates are still to be studied when compared with mammals (Rand and Petrocelli, 1985). Regarding histopathological effects of pesticides on various organs of fish are scanty (Dwivedi, 2000; Inbamani and Srinivasan, 1998; Banerjee and Shelley, 1997; Usha, 1997; Adhikari, 1996).

The susceptibility of animal tissue to different chemicals may vary from animal to animal and also within the same animal and even the different tissues of the same individual. Incorporation of the parent and/or their metabolites in lower organisms in the tissues of fishes, birds and mammals have been recorded to cause serious morphological alterations in vital tissues even at the very low concentrations (Mathur et al., 1981; Tilak et al., 2001b). A number of pathological changes have been reported in fishes exposed to different organochlorine and organophosphate pesticides (Altinok and Capkin, 2007; Velmurugan, 2007; Sakr and Jamal Alail, 2005; Machado and Fanta 2003; Tilak et al., 2001b; Veeraiah, 2002; Tilak et al., 2001a; Yacobu, 1999; Ramana Kumari, 1999; Vijayalakshmi, 1996).

In the present study, an attempt has been made to observe possible changes in certain vital tissues like gill, liver, brain and kidney of the freshwater fish *Channa punctatus* (Bloch) exposed to lethal (96 hr LC$_{50}$) and sublethal concentrations (1/5$^{th}$ of 96 hr LC$_{50}$) of quinalphos technical grade and 25% EC formulation for 8 days.

**MATERIALS AND METHODS**

Freshwater fish *Channa punctatus* (size 6-8 cm in length 6.5-7.5 g in weight) were acclimatized to laboratory conditions for one week. All the precautions laid down by APHA et al., (1998) are followed, for maintaining the fish. The fish were exposed to organophosphorus pesticide quinalphos technical and 25% EC to 96 hours LC$_{50}$ Technical lethal (2.9136 mg L$^{-1}$), Technical sublethal ($1/_{10}^{th}$ of 96 hr LC$_{50}$ i.e., 0.2913 mg L$^{-1}$), 25% EC Lethal (2.3228 mg L$^{-1}$) and
25% EC sublethal ($\frac{1}{10}$ of 96 hr LC$_{50}$ i.e., 0.2322 mg L$^{-1}$) concentrations for 8 days. At the end of the exposure period, fish were randomly selected for histopathological examination.

Gill, liver, brain and kidney tissues were isolated from normal (not exposed to the toxicant) and experimental fish. Physiological saline solution (0.75% NaCl) was used to rinse and clean the tissue. They were fixed in aqueous Bouins solution for 48 hr, processed through graded series of alcohols, cleared in xylene and embedded in paraffin wax. Gills alone were processed by double embedding technique. Sections were cut of 4-6 µ (microns) thickness; stained with Hematoxylin-Eosin (dissolved in 70% alcohol) (Humason, 1972) and were mounted in Canada balsam. Histopathological lesions were examined and photographed with the help of Intel Pentium QX3 computer attached microscope under 400X lens.
OBSERVATIONS AND DISCUSSION

General histology of fish gill

Teleosts have five pairs of gill arches. In the front four pairs, the slender gill filaments form two lines facing towards the back and these two lines are joined to each other at the base by a gill septum. The last pair of gill arches generally transforms into the pharyngeal bone and does not play a role in respiration.

Numerous semicircular secondary gill lamellae are lined up along both sides of the gill filament. The surface of the gill lamellae is covered with simple squamous epithelial cells and many capillaries separated by pillar cells (PC) run parallel along the surface. Numerous semicircular secondary gill lamellae are lined up along both sides of the primary gill lamellae. The primary gill lamellae (PGL) consist of centrally placed rod like central axis (CA) with chloride cells and with blood vessels on either side. The secondary lamellae (SGL), also termed as respiratory lamellae are highly vascularised and covered with a thin layer of epithelial cells (EC). Blood vessels (BV) are extended into each of the secondary gill filaments. The blood cells of the secondary gill lamellae have a single nucleus which is flattened in appearance. The region between the two adjacent secondary gill lamellae is known as inter lamellar region (ILR) (Plate VI.1, Fig. A).

Pathology of Gill tissue under quinalphos toxicity

Quinalphos technical and 25% EC exposures have induced marked pathological changes in fish gills architecture. The changes include epithelial lifting (EL), bulging of tips of primary gill filaments (BTPG), Degenerated secondary lamella (DGSL), Curling of secondary gill filaments (CSG), Atrophy secondary lamella (ASL), Fusion of secondary gill filaments (FSG) (Plate VI.1, Fig B, C, D and E).

The damage of gills of fish exposed to the higher concentrations of lethal nature was severe. Shortened and clubbing of ends of the secondary gill lamellae, fusion of adjacent secondary gill lamellae and necrosis in the primary lamellae were well marked. Hyperplasia and hypertrophy of nuclei were also seen. Besides these changes pyknotic nuclei, vacuolization and degeneration of epithelial cells and pillar cells and lifting of the epithelial layer from the secondary lamellae were also significant.
LEGEND FOR FIGURES
Plate VI.1

Fig. A. Normal Gill lamella of *Channa punctatus*; Bouin, HE x400
- (CA) Central axis
- (PGL) Primary gill lamella
- (SGL) Secondary gill lamella
- (ILR) Inter lamellar region
- (PC) Pillar cell
- (EC) Erythrocyte

Fig. B. Gill lamella of *Channa punctatus* exposed for 8 days to sublethal concentration of quinalphos technical grade; Bouin, HE x400
- (EL) Epithelial lifting
- (BTPG) Bulging of tips of primary gill filaments
- (CSG) Curling of secondary gill filaments
- (DGSL) Degenerated secondary lamella

Fig. C. Gill lamella of *Channa punctatus* exposed for 8 days to lethal concentration of quinalphos technical grade; Bouin, HE x400
- (EL) Epithelial lifting
- (CSG) Curling of secondary gill filaments
- (DGSL) Degenerated secondary lamella
- (DEC) Degeneration of epithelial cells
- (FSG) Fusion of secondary gill filaments

Fig. D. Gill lamella of *Channa punctatus* exposed for 8 days to sublethal concentration of quinalphos 25% EC; Bouin, HE x400
- (EL) Epithelial lifting
- (ASL) Atrophy of secondary lamella
- (DGSL) Degenerated secondary lamella
- (FSG) Fusion of secondary gill filaments

Fig. E. Gill lamella of *Channa punctatus* exposed for 8 days to lethal concentration of quinalphos 25% EC; Bouin, HE x400
- (BTPG) Bulging of tips of primary gill filaments
- (CSG) Curling of secondary gill filaments
- (FSG) Fusion of secondary gill filaments
- (DGSL) Degenerated secondary lamella
- (DEC) Degeneration of epithelial cells
The immediate morpho-pathological response of the gills of fish exposed to ambient xenobiotics is often manifested by a significant increase in the density of its mucous cells (Bradbury, 1987; Wise et al., 1987; Dutta, 1997; Hemalatha and Banerjee, 1997a; 1997b). The large quantity of mucous secretion acts as a defence mechanism against several toxic substances (Handy and Eddy, 1991; Mazon et al., 1999). The regular sloughing of mucus from the surface of gills into the media helps to remove the bound pathogens, toxicants and foreign matters (Powell et al., 1992) which adhere to the gills.

The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants (Poleksic & Mitrovic-Tutundzic, 1994; Mazon et al., 2002; Fernandes & Mazon, 2003). Alterations like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Mallatt, 1985; Hinton & Laurén, 1990; Poleksic & Mitrovic-Tutundzic, 1994; Fernandes & Mazon, 2003).

Several other xenobiotics are also known to induce fusion of the secondary lamellae of gills (Leino et al., 1987; Dutta, 1996; Wendelaar Bonga, 1997). During the present study also, the slimy coatings over the gills showed compositional alterations and sloughed off several times, which might have led to the fusion of the secondary lamellae. According to Mallatt (1985) induced alterations in gill histology are mostly non-specific in nature, which partially represent the damage, and partially the compensatory response of the fish. Examples of the first are necrosis of the epithelial cells of the secondary lamellae, epithelial lifting, dilatation of the blood sinuses of the secondary lamellae, and lamellar aneurysm. The main compensatory responses are hypertrophy and hyperplasia of the respiratory epithelial and chloride cells, hyperplasia of the mucous cells (including decrease due to exhaustion, followed by an increase in their density) and infiltration of the dilated intercellular spaces by leukocytes. Dutta (1996), categorised the structural alteration in the gill morphology into two groups: (1) direct deleterious effect of the xenobiotics causing necrosis and rupture of the branchial epithelium. Such type of effect is mostly dose dependent and very often reported under lethal conditions (Mallatt, 1985). They also suggested that death of branchial cells and their rupture usually develops either by autolysis or
by rapid lyses caused by the direct action of toxicants on the cells’ constituents (Abel, 1976), and (2) branchial defence response achieved by mucus hyper secretion, chloride cell proliferation, epithelial lifting, swelling, hyperplasia and lamellar fusion. According to Peuranen et al., (1994) any discontinuity of epithelial lining of the gill due to massive wear and tear may lead to a negative ionic balance and to changes in the haematocrit and mean cellular haemoglobin values of the blood. Similar observation has been made which is vivid from chapter-IV.

Most part of the gill lesions caused by sublethal exposures affects lamellar epithelium (Hinton & Laurén, 1990); however, some alterations in blood vessels may also occur, when fishes suffer a more severe type of stress. The damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel, blood congestion or even an aneurysm (Takashima & Hibiya, 1995; Rosety Rodríguez et al., 2002). The formation of an aneurysm is related to the rupture of the pillar cells (Heath, 1987; Martinez et al., 2004) due to a bigger flow of blood or even because of the direct effects of contaminants on these cells.

Several other studies have shown similar effects of pesticides on fish gills (Cengiz and Unlu, 2002 & 2003). Many investigators have reported the histopathological changes in gills of different fish species exposed to pesticides. Hemorrhage in the primary and secondary gill lamellae, degeneration and necrosis of epithelial cells, distortion of the secondary lamellae, disruption of epithelial cells from pillar cells were observed in gill tissues of Anabas testudineus exposed to monocrotophos (Santhakumar et al., 2001). Degenerative changes in gills, such as detachment and lifting of the epithelial linings from the surface of the gills, uncontrolled regeneration of the primary lamellae and secondary lamellae, hypertrophy, hyperplasia, necrosis of the epithelial cells, dilation of the blood sinuses of the secondary lamellae, lamellar aneurysm, hemmorhages were noticed after exposure of sublethal concentration of profenofos (Rao et al., 2006). Coutinho & Gokhale (2000) found epithelial lifting in the gills of carp (Cyprinus carpio) and tilapia (Oreochromis mossambicus) exposed to the effluents of a wastewater treatment plant.

According to Mallat (1985) such alterations are non-specific and may be induced by different types of contaminant. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired, which is evident as observations vide chapter III. Toxic substances can injure gills, thus reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms (Saravana Bhavan and Geraldine et
However, fishes have the capacity to increase their ventilation rate, to compensate low oxygen uptake (Fernandes & Mazon, 2003).

According to Altinok and Capkin (2007) gills of rainbow trout (*Oncorhynchus mykiss*) after exposure to different concentrations of methiocarb (2.5 and 3.75 mgL$^{-1}$) or endosulfan (0.6 and 1.3 µgL$^{-1}$) for 21 days showed lesions in gills exposed to either concentrations of endosulfan and found that there was no concentration-related effect observed on the histopathological lesions. Guimarães et al., (2007) found that an organophosphate (trichlorfon) which is used in the treatment of parasitic organisms in aquaculture induced several histopathological alterations on gill of *Oreochromis niloticus*. But only edema and blood congestion were observed up to 72 hr. No inflammatory processes were observed after 96 hr of exposure.

A study by Yildirim et al., (2006) in Nile tilapia (*Oreochromis niloticus* L.) fingerlings exposed to 5 µg L$^{-1}$ deltamethrin revealed severe morphological alterations in the gills, like hyperemia, fusion of secondary lamellae and telangiectasis. According to Rao et al.,(2006; 2003) effect of profenofos on mosquito fish, *Gambusia affinis* for 20 days on structural integrity of gill in sublethal concentration of 0.13 mg L$^{-1}$ (1/5th of LC$_{50}$) found deformities in the primary and secondary lamella of gill and electron microscopy studies on gill tissue of *Oreochromis mossambicus*, exposed to profenofos by the same author revealed an abnormal gill morphology, with distinct breakages in gill arches and rakers, along with deep lesions and erosions in the epithelium.

Studies by Rao et al., (2005) on subacute studies of monocrotophos on mosquito fish, *Gambusia affinis*, carried out in vivo for 24 days in sublethal concentration of LC$_{10}$ (7.74 mg L$^{-1}$) revealed that the toxicant induced deformities in the primary and secondary lamellae of gill which was dependent on the duration of exposure and has resulted in failure of exchange of gases. According to Tilak et al., (2005a) *Cirrhinus mrigala* when exposed to the sublethal and lethal concentrations of technical grade as well as 20% EC of Chlorpyrifos for 8 days caused marked histopathological changes in the tissues of fish gill.

Histopathological changes in the gill tissue of the fish *Channa punctata* exposed to sublethal concentration of Butachlor and Machete, an Herbicide was studied by Tilak et al., (2005b) and found marked pathological changes such as bulging of tips of primary gill filaments and fusion of disorganized secondary gill filaments. Similarly the toxic sublethal concentration of fenvalerate technical grade in the gill of *Cirrhinus mrigala* was evaluated and found marked
pathological changes like necrosis, progressive degeneration in the gill tissue (Anita susan and Tilak, 2003).

Cengiz and Unlu (2003) studied the histopathological effects of malathion, an organophosphate pesticide, on the gill tissues in mosquitofish, *Gambusia affinis*, under light microscope after exposing the fish to sublethal concentrations (0.01 and 0.02 mgL$^{-1}$) of malathion for 10, 20 and 30 days and found a variety of histopathological effects which include gill lesions, necrosis and desquamation of secondary lamellar epithelium, lifting up of epithelium, intraepithelial oedema, fusion of adjacent secondary lamellae, haemorrhage at primary lamellae, disorganization and rupture in secondary lamellae, hypertrophy and hyperplasia of epithelial cells and concluded that the alterations were time- and dose-dependent.

Santhakumar *et al.*, (2001) studied histopathological effects of sublethal doses of monocrotophos on the gills by exposing the fish for a period ranging from ten to twenty days and observed disruption of epithelial cells from pillar cells, haemorrhage in the primary and secondary gill lamellae, degeneration and necrosis of epithelial cells and distortion of the secondary lamellae very prominently and concluded that the extent of damage to gills was dependent on the dose and duration of exposure. Tilak *et al.*, (2001a) observed hydropsy, vascular degeneration and bulging and severe necrotic changes in the secondary in the gill tissues of fish *Labeo rohita* exposed to the sublethal concentrations of technical as well as 20% EC of chlorpyrifos after 8 days.

According to Dutta *et al.*, (1998) Scanning electron microscopic studies of the gills of catfish (*Heteropneustes fossilis*), exposed to sublethal concentrations of malathion (4 mg L$^{-1}$ and 6 mg L$^{-1}$) revealed that 24 hr exposure to 4 mg L$^{-1}$ had a mild effect. However, severe damage was found after 48 and 72 hr exposures. After a 24 hr exposure to a 6 mg L$^{-1}$ concentration, more severe damage ensued. The microridged epithelial cells of the gill arch became perforated and the central portion of the filament appeared elevated. Numerous mucous gland openings also became visible. After 48 and 72 hr exposures, the damage and structural changes were more pronounced when compared with the 4 mg L$^{-1}$ exposure. Enlarged mucous gland openings were found on the gill arch. The lamellar surface had many crevices, elevations and depressions. Broken microridges in the gill arch surface were visible at a 72 hr exposure. At 96 hr of exposure, structural recovery occurred to some extent in both the 4 and 6 mg L$^{-1}$ exposures. Corrugation and dissociated epithelium along with some interlamellar bridges were evident after
72 hr of exposure to both concentrations. Such deleterious effects caused reduction in available water supply as well as available respiratory area that lead to result in decreased oxygen uptake. Consequently, fish failed to get in sufficient oxygen leading to asphyxia.

Inflammatory alterations of lamellar epithelium and hyperplasia were reported in the gills of freshwater major carp *Cirrhinus mrigala* (Hamilton) during 48 hr exposure to sublethal dose of malathion (Roy and Munshi, 1991). Edema with lifting of lamellar epithelium and hyperplasia of lamellar epithelium were observed in the gills of all catfish containing residues of endosulfan (Nowak and Julli, 1991).

A number of pathological changes have been reported in fish exposed to different organochlorine and organophosphorus and synthetic pyrethroid compounds. The other reports on these lines to different pesticides are Vijayalakshmi and Tilak, 1996; Das and Mukherjee, 2000; Rodrigues *et al*., (2001); Tilak *et al*., 2001a; Tilak *et al*., 2001b and Anita susan and Tilak, 2003; Ortiz *et al*., (2003); Cengiz and Unlu, 2003; Machado and Fanta, 2003; Altinok and Capkin, 2007; Velmurughan *et al*., (2007), which are in agreement with the observed histopathological changes under quinalphos exposure.

**General histology of Liver**

The surface of liver is covered with serous membrane and some connective tissue extends inward into parenchyma. It is composed of parenchymal cells (hepatic cells) (HC) and lattice fibres, which support the former. Hepatic cells are roundish polygonal, containing clear spherical nucleus (N). They are located among sinusoids forming cord like structures known as hepatic cell cords. In fish, these structures are generally obscure. Bile canaliculus (BC), is centrally located in each cord. Fairly large quantities of lipid glycogen granules (LGG) are also observed in the cytoplasm of fish hepatic cells (Plate VI.2, Fig A).

Hepatic cells have many vital functions. Other than the secretion of bile, they play an important role in protein, lipid and carbohydrate metabolism. They serve as storage sites for some nutrients and detoxification is another function attributed to them.

**Pathology of Liver tissue under quinalphos toxicity**

Quinalphos technical and 25% EC exposures have induced discrete pathological changes in the liver tissue of the fish *Channa punctatus*. These changes include Degenerated hepato pancreatic tissue (DGHP), Blood cells among hepatocytes (BC), Appearance of Blood streaks among hepatocytes (ABS), Formation of vacuoles (FV), along with atrophy, necrosis and
disappearance of hepatocytic cell wall and disposition of hepatic cords (Plate VI.2, Fig B, C, D and E). The degenerative changes are intensified in lethal exposures.

The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply (Van der Oost et al., 2003) it is also one of the organs most affected by contaminants in the water (Rodrigues and Fanta, 1998). The liver has the ability to degrade toxic compounds, but its regulating mechanisms can be overwhelmed by elevated concentrations of these compounds, and could subsequently result in structural damage (Brusle & Anadon, 1996).

Fish liver histology could therefore serve as a model for studying the interactions between environmental factors and hepatic structures and functions. Some of these environmental factors include biotoxins, parasites, infectious germs, physiochemical parameters and pollutants, for example pesticides, hydrocarbons, PCB’s (polychlorinated biphenyls) and heavy metals (Brusle and Anadon, 1996).
PLATE V1.2
LIVER

Figure - A : Control

Figure - B : Technical Sublethal

Figure - C : Technical Lethal

Figure - D : 25% EC Sublethal

Figure - E : 25% EC Lethal

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**LEGEND FOR FIGURES**

**Plate VI.2**

Fig. A. Normal structure of Liver in *Channa punctatus*; Bouin, HE x400

- (HC) Hepatic cell
- (N) Nucleus
- (BC) Bile canaliculus
- (LGG) Lipid and glycogen granules

Fig. B. Liver of *Channa punctatus* exposed for 8 days to sublethal concentration of quinalphos technical grade; Bouin, HE x400

- (DGHP) Degenerated hepato pancreatic tissue
- (BC) Blood cells among hepatocytes
- (ABS) Appearance of Blood streaks among hepatocytes
- (FV) Formation of vacuoles

Fig. C. Liver of *Channa punctatus* exposed for 8 days to lethal concentration of quinalphos technical grade; Bouin, HE x400

- (DGHP) Degenerated hepato pancreatic tissue
- (BC) Blood cells among hepatocytes
- (ABS) Appearance of Blood streaks among hepatocytes
- (FV) Formation of vacuoles

Fig. D. Liver of *Channa punctatus* exposed for 8 days to sublethal concentration of quinalphos 25% EC; Bouin, HE x400

- (DGHP) Degenerated hepato pancreatic tissue
- (BC) Blood cells among hepatocytes
- (ABS) Appearance of Blood streaks among hepatocytes
- (FV) Formation of vacuoles

Fig. E. Liver of *Channa punctatus* exposed for 8 days to lethal concentration of quinalphos 25% EC; Bouin, HE x400

- (DGHP) Degenerated hepato pancreatic tissue
- (BC) Blood cells among hepatocytes
- (ABS) Appearance of Blood streaks among hepatocytes
- (FV) Formation of vacuoles
Tissue changes in liver are linked with histological abnormalities of kidney and gill. Once absorbed, toxicant is transported by blood circulation to liver for transformation and/or storage, and if transformed in the liver it may be excreted through the bile or pass back into blood for possible excretion by kidney or gill (Lindstoma-Seppa et al., 1981).

Vacuoles in the cytoplasm of the hepatocytes can contain lipids and glycogen, which are related to the normal metabolic function of the liver. Depletion of the glycogen in the hepatocytes is usually found in stressed animals (Hinton & Laurén, 1990; Wilhelm Filho et al., 2001), because the glycogen acts as a reserve of glucose to supply the higher energetic demand occurring in such situations (Panepucci et al., 2001).

Significant changes were observed in the liver tissue in lethal and sublethal concentrations of quinalphos technical grade and 25% EC where marked swelling of the hepatocytes in places with areas of diffuse necrosis (Plate VI.2, Fig B, C, D and E). The normal architecture of liver tissue as markedly disrupted. Sinusoids in most cases were distended and central veins appeared severely damaged due to marked swelling and degeneration of the endothelial lining cells.

A study by Yildirim et al., (2006) in Nile tilapia (Oreochromis niloticus L.) fingerlings exposed to 5 µg L⁻¹ deltamethrin revealed severe morphological alterations in liver, where hydropic degenerations in liver was observed. Sakr and Jamal Alail (2005) observed histopathological changes induced in the liver after exposing the fish Clarias gariepinus to fenvalerate which are mainly represented by cytoplasmic vacuolization of the hepatocytes, blood vessel congestion, inflammatory leucocytic infiltration necrosis and fatty infiltrations. Marked reduction in glycogen contents and total protein contents of the liver cells as compared with the control fish was also noticed which was time dependent.

Ortiz et al., (2003) observed reduction in the diameter of the hepatocytes and cellular vacuolization with hypertrophy of the hepatocytes in liver, when fish was exposed to lindane. Rodrigues et al., (2001) observed hepatocytes which were tumefied, with vacuolation, cytoplasmic granulation, nuclear lateralization. Along with nuclei varying in diameter, density and condensed chromatin in the central region with pyknosis and areas of necrosis in the liver of Prochilodus lineatus exposed to a sublethal concentration of the organophosphate insecticide Dipterex 500® (Trichlorfon). Swelling of the hepatocytes with diffuse necrosis and marked
swelling of blood vessels were observed in the liver tissue by Das and Mukherjee (2000) when *Labeo rohita* was exposed to hexachlorocyclohexane.

Anomalies such as irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position, close to the cell membrane, were also described in the siluriform *Corydoras paleatus* contaminated by organophosphate pesticides (Fanta *et al*., 2003). Pacheco & Santos (2002) described increased vacuolisation of the hepatocytes as a signal of degenerative process that suggests metabolic damage, possibly related to exposure to contaminated water.

The study of *Brachydanio rerio* hepatic tissue showed morphological alterations in individuals exposed to organophosphates, even when they were exposed to sublethal doses considered safe (Rodrigues and Fanta, 1998). Ansari and Kumar, (1987) reported significant alterations in the hepatic cell count and the nucleocyton plasmic index in the liver of zebra fish *Brachydanio rerio* (cyprinid) exposed to 0.9 mg L\(^{-1}\) concentration of malathion. According to Ansari and Kumar (1987) and Gill *et al*., (1988), the liver is an organ that frequently undergoes changes when exposed to pesticides at sublethal doses. Rashatwar and Ilysas (1984) reported that in teleost fish *Nemachelius denesoni* (Day) exposure to phosphamidon caused highly vacuolated and cloudy swelling and even the connective tissue was damaged in liver. Narayan and Singh (1991) observed extensive degeneration of cytoplasm with pyknosis of nuclei and loss of glycogen in liver tissue of *Heteropneustes fossilis* while subjecting them to acute thiodan toxicity.

A few other reports are available which deal with the other than organophosphate pesticides effect on histology, Radhaiah and Jayantha Rao (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels and appearance of blood cells among hepatocytes, formation of vacuoles, picnotic nuclei in the liver of *Tilapia mossambica* exposed to fenvalerate. Similar changes were observed in three Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* exposed to fenvalerate by Anita Susan (1994) and Vijayalakshmi (1994) observed the same changes in *Labeo rohita* under fenvalerate and monocrotophos synergistic exposure. Yacobu (1999) reported the same observations in *Ctenopharyngodon idellus* exposed to fenvalerate. Anitha Kumari and Shree Ram Kumar (1997) observed an uneven distribution of carbohydrate content and a drastical decrease in the hepatic cells of the freshwater teleost upon exposure to polluted waters of Hussain Sagar lake. Tilak *et al*., (2001c), reported the same degenerative changes in *Ctenopharyngodon idellus* under fenvalerate toxicity. Anita Susan and Tilak (2003) observed the toxic sublethal concentration of
fenvalerate technical grade induced atrophy, appearance of blood streaks among hepatocytes in liver of *Cirrhinus mrigala*. Similar reports were observed in liver of *Cirrhinus mrigala* was exposed to the sublethal and lethal concentrations of technical grade and 20% EC of Chlorpyrifos, Tilak *et al.*, (2005a).

Quinalphos induced pathological changes in the liver observed in the present study might effect the physiological activity of the fish such as reduction in enzyme synthesis and reduces the functional ability of liver which indirectly affects all metabolic activities of the organism.

**General Histology of fish Brain**

Five major regions are distinguished in the brain of fishes. They are telencephalon, diencephalon, mesencephalon, metencephalon and mylencephalon. In fishes, the roof of the telencephalon is covered with membranous tissue and lateral ventricles do not exist. The diencephalon is the region that contains the third ventricle and is composed of the epithalamus, thalamus and hypothalamus. The epithalamus contains the ends of the nerve fibres from the telencephalon and also the habenula, which connects with the thalamus, hypothalamus and the olfactory areas of the telencephalon. The mesencephalon contains the centre of the visual sense, as well as the integration center between this sense and the other senses of locomotion.

Metencephalon occupies the interior portion of the dorsal wall of the fourth ventricle and is composed of a cortex and medulla. The metencephalon is the integration center between the auditory sense and the sense of the lateral line. The main part of the mylencephalon, the medulla oblongata, is shaped like the spinal cord opened on its dorsal side. (Plate VI.3, Fig A).

**Pathology of Brain tissue under quinalphos toxicity**

Quinalphos technical and 25% EC exposures induced marked pathological changes in the brain of exposed fish.
LEGEND FOR FIGURES
Plate VI.3

Fig. A. Normal structure of Brain in *Channa punctatus*;
Formalin, Golgi. Cox x75
(DOA) Dorsal olfactory area
(VOA) Ventral olfactory area
(SA) Septal area
(TOM) Tractus olfactorius medialis
(TOL) Tractus olfactorius lateralis

Fig. B. Brain of *Channa punctatus* exposed for 8 days to sublethal concentration of quinalphos technical grade; Formalin, Golgi. Cox x75
(DDOA) Degenerated dorsal olfactory area
(DVOA) Degenerated ventral olfactory area
(BS) Blood streeks
(DSA) Degenerated septal area

Fig. C. Brain of *Channa punctatus* exposed for 8 days to lethal concentration of quinalphos technical grade; Formalin, Golgi. Cox x75
(DDOA) Degenerated dorsal olfactory area
(DVOA) Degenerated ventral olfactory area
(BS) Blood streeks
(DSA) Degenerated septal area

Fig. D. Brain of *Channa punctatus* exposed for 8 days to sublethal concentration of quinalphos 25% EC; Formalin, Golgi. Cox x75
(DDOA) Degenerated dorsal olfactory area
(DVOA) Degenerated ventral olfactory area
(BS) Blood streeks
(DSA) Degenerated septal area

Fig. E. Brain of *Channa punctatus* exposed for 8 days to lethal concentration of quinalphos 25% EC; Formalin, Golgi. Cox x75
(DDOA) Degenerated dorsal olfactory area
(DVOA) Degenerated ventral olfactory area
(BS) Blood streeks
(DSA) Degenerated septal area
These changes include Degenerated dorsal olfactory area (DDOA); Degenerated ventral olfactory area (DVOA); Blood streeks (DS), Degenerated septal area (DSA), atrophy, necrosis, pycnosis, dissolution of the nissel bodies, swelling of the axon and demylenation or vacuolisation of the mylin sheath of nerve fibres (Plate VI.3, Fig B, C, D and E). Since quinalphos is an organophosphate compound and organophosphates are neuropoi sons, the quinalphos intoxication caused atrophy, chromatolysis i.e. dissolution of the nissel bodies and loss of stainable substances within the cytoplasm. Congestion in the medulla oblongata, which inturn causes abnormalities in the blood circulation.

Similar changes were observed by Yacobu, (1999) reporting swelling of the axon, atrophy, necrosis and pycnosis in the fish *Ctenopharyngodon idellus* under fenvalerate toxicity, and Tilak et al., (2005a) on *Cirrhinus mrigala* exposed to the sublethal and lethal concentrations of technical grade as well as 20% EC of Chlorpyrifos for 8 days and the severity of damage is more in lethal exposures than in sublethal exposures. Quinalphos technical grade caused more degenerative changes in brain than in 25% EC exposures (Plate VI.3, Fig. B, C, D and E).

Das and Mukherjee (2000) reported that hexachlorocyclohexane was neurotoxic and induced vacuolation of brain parenchyma and moderate swelling of pyramidal cells of the cerebrum and opined that vacuolation may have been due to glycolysis leading to microsomal and mitochondrial dysfunctions. Loss of Nissl substances and glial cell reaction, with evidence of glial nodule formation in places, were proof of the the neurotoxic nature of the chemical.

Santhakumar et al., (2000) studied the pathological effects of monocrotophos on the brain by exposing the fish *Anabas testudineus* to sublethal concentrations 1.9, 4.75 and 9.5 mg L$^{-1}$ for 21 days and reported that the pesticides produced rupture of cortex, atrophy of molecular and granular layer, necrosis of neurofibrillar region, vascular dilation, nuclear pyknosis, fibrosis, vacuolation, cerebral oedema and interzonal detachment. The histopathological changes were found to be dose dependent. The effect of monocrotophos on brain showed vascular dilation that is corresponding to earlier observations by Cope et al., (1970), the rupture of the wall of brain found in the present work coincide with the work done by Kennedy et al., (1970), hemorrhage of meninx pimitiva and swelling of myelin sheaths around nerve fibres observed in the present study was also reported with exposure of pesticides like malathion (Walsh and Ribelin , 1975); 2,4-D (Cope et al., 1970) and methooxychlor (Kennedy et al., 1970)
The vacuolation, dilation of blood capillary, fibrosis, agglutination of neurons and loss of definite demarcation between layers were observed on optic tectum of *Channa punctatus* by Karuppasamy, (2000). The above findings were also in support to the observation of Bhattacharya and Mukherjee (1978) in the optic tectum of *Clarias batrachus* and *Channa punctatus*; Joshi and Dubey (1984) *Oxygaster bucarila* to industrial effluents.

Altinok and Capkin (2007) reported increasing methiocarb concentrations on fish rainbow trout (*Oncorhynchus mykiss*) caused telangiectasis and necrosis between the molecular and granular layers of the cerebellum where Purkinje cells are located. Similar findings were also observed by Dalela *et al.*, (1979). Tricklebank (2001) found the same result when damselfish, *Parma microlepis*, was exposed to aldrin and dieldrin.

Histological changes in brain due to zinc toxicity to *Labeo rohita* exposed to 5 mg L\(^{-1}\) showed swelling of pyramidal cells with binucleated nuclei and at 10 mg L\(^{-1}\) exposure severe necrosis of neuronal cells of cerebrum was observed by Loganathan *et al.*, (2006), indicating loss of nissl substances mild vacuolar changes with empty spaces appeared due to increased concentration and duration.

The extent of damage to the brain in the present study was more in lethal than in sublethal concentrations exposed to quinalphos, which may alters the physiological and behavioural functions of the fish. Various regions in fish brain are concerned with many functions such as feeding, vision, proper body motion can be directly related to the impaired neuronal dysfunction of central nervous system due to inhibition of brain AChE activity and all of them altered the complete behaviour and reduce the fitness of fish in its environments.

**General Histology of fish Kidney**

Teleostean kidney consists of head and body kidneys. Head kidney is the anterior portion of the kidney and consists of lymphoid tissue. Body kidney is composed of many nephrons and interstitial lymphoid tissue. The interstitial tissue is the major haematopoitic tissue in the body. Each nephron consists of two parts, the glomerulus (G) and the urinary tubule. The glomerulus capsule consists of an inner and outer layer of single flattened epithelia. Renal tubules (RT) consist of single layer of epithelial cells. Mesangium fills the space between the loops of glomerular capillaries (Plate VI.4, Fig. A). Renal tubules are thin and short in the neck segment. The proximal convoluted segment is divided into two parts i.e. segment I and segment II. The renal tubules are composed of cuboidal epithelial cells with densely arranged microvilli in the
tubular lumen. In segment II, renal tubules are composed of cuboidal epithelial cells. Cilia and microvilli are found in the tubular lumen. In the distal convoluted segment, epithelial cells have no microvilli. The cells of this segment are stained with eosin more faintly than those of proximal convoluted segment. Thus, it is easy to distinguish between proximal and distal convoluted segments under light microscopy (Oguri, 1982).

**Pathology of Kidney tissue under quinalphos toxicity**

Renal tissues of the fish exposed to quinalphos technical and 25% EC showed some common pathological changes. Highly degenerative changes were observed in haemopoietic tissue which include Shrinkage of glomerulus (SG), Expansion of space inside Bowman’s capsule (ESBC), Hypertrophied cells (HTC) and Lumen tubules diminished (LTD). In 25 % EC Intra cytoplasmic vacuoles in epithelial cells of renal tubules (ICV), Degenerating haemopoietic tissue with erythrocytes (DGHTE) more prominently observed. (Plate VI.4, Fig B, C, D and E). Besides the above changes severe necrosis, cloudy swelling in renal tubules and granular cytoplasm was also observed. From the body of fish, the waste products are eliminated through kidney. The non-detoxified pesticide molecules must be eliminated through the kidney of fish and hence, it is susceptible to chemical compounds when exposed to lethal or sublethal doses. Quinalphos, while it was eliminated through kidney might have caused degenerative changes in renal tubule and glomerulus.

The posterior kidney of freshwater fishes is largely dedicated to the production of copious dilute urine and it has little responsibility for ion or acid-base balance. In marine fish, where they are opposite osmotic gradients, urine flow is severely reduced by elimination of all but the proximal tubules. In some marine species, water loss is further reduced by elimination of glomerular filtration altogether and renal function depends solely on tubular secretion.
PLATE VI.4
KIDNEY

Figure - A : Control

Figure - B : Technical Sublethal

Figure - C : Technical Lethal

Figure - D : 25% EC Sublethal

Figure - E : 25% EC Lethal
LEGEND FOR FIGURES
Plate VI.4

Fig. A. Normal structure of Kidney in *Channa punctatus*; Bouin, HE x400
   (G) Glomerulus
   (RT) Renal tubule

Fig. B. Kidney of *Channa punctatus* exposed for 8 days to sublethal concentration of quinalphos technical grade; Bouin, HE x400
   (SG) Shrinkage of glomerulus
   (ESBC) Expansion of space inside Bowman’s capsule
   (HTC) Hypertrophied cells
   (LTD) Lumen tubules diminished

Fig. C. Kidney of *Channa punctatus* exposed for 8 days to lethal concentration of quinalphos technical grade; Bouin, HE x400
   (SG) Shrinkage of glomerulus
   (ESBC) Expansion of space inside Bowman’s capsule
   (HTC) Hypertrophied cells
   (LTD) Lumen tubules diminished

Fig. D. Kidney of *Channa punctatus* exposed for 8 days to sublethal concentration of quinalphos 25% EC; Bouin, HE x400
   (SG) Shrinkage of glomerulus
   (ESBC) Expansion of space inside Bowman’s capsule
   (HTC) Hypertrophied cells
   (LTD) Lumen tubules diminished
   (ICV) Intra cytoplasmic vacuoles
   (DGHTE) Degenerating haemopoietic tissue with erythrocytes

Fig. E. Kidney of *Channa punctatus* exposed for 8 days to lethal concentration of quinalphos 25% EC; Bouin, HE x400
   (SG) Shrinkage of glomerulus
   (ESBC) Expansion of space inside Bowman’s capsule
   (HTC) Hypertrophied cells
   (LTD) Lumen tubules diminished
   (ICV) Intra cytoplasmic vacuoles
   (DGHTE) Degenerating haemopoietic tissue with erythrocytes
The kidney of the fish receives the vast majority of post branchial blood, and because of that, we can expect renal lesions in the fish when toxicant agents exist in the environment. Therefore, a study of these possible kidney changes may be expected to be a good indicator of environmental pollution.

In the present study, kidney of the fish showed cloudy swelling in tubule cells. This alteration can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm. Initial stage in the degeneration process can progress to hyaline degeneration, characterized by the presence of large eosinophilic granules inside the cells. These granules may be formed inside the cells or by the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Hinton & Laurén, 1990; Takashima & Hibiya, 1995). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima & Hibiya, 1995). The presence of tubule degeneration, coupled with the absence of necrosis in the kidney in the present study indicates that the kidney suffered damage after exposure to lethal or sublethal doses of quinalphos.

Most common alterations found in the kidney of fishes exposed to water contamination are tubule degeneration (cloudy swelling and hyaline droplets) and changes in the corpuscle, such as dilation of capillaries in the glomerulus and reduction of Bowman’s space (Takashima & Hibiya, 1995). Following exposure of fish to toxic agents such pesticides, histological alterations have been found at the level of the tubular epithelium and glomerulus (Teh et al., 1997; Thophon et al., 2003). Similar alterations were found in fishes exposed to organic contaminants (Veiga et al., 2002) and mixed environmental contaminants (Schwaiger et al., 1997; Pacheco & Santos, 2002).

When *Cirrhinus mrigala* was exposed to the sublethal and lethal concentrations of technical grade as well as 20% EC of Chlorpyrifos for 8 days, marked histopathological changes were observed in the fish kidney such as severe necrosis, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm Tilak et al.,(2005a). Histopathologic changes in the kidney of *Prochilodus lineatus* exposed to a high Dipterex 500® (Trichlorfon) concentration showed an enlargement of intercapsular space with glomerular atrophy, hypertrophy of the kidney tube cells with small granules on its cytoplasm and little nuclear alteration. Blood overflowing from capillaries which in many cases did not permit to see the vessel limit was also noticed. Some areas exhibited necrosis with pyknotic nuclei and vacuoles in the cytoplasm. After
forty-eight hours, the kidney tissue showed glomerular expansion rendering impossibility to visualize the intercapsular space as well as cytoplasm limit of many cells. The parietal capsular epithelium and the basal membrane presented loss of cell content, the tubular cells appeared swollen vacuolated and with thin and thick cytoplasmatic granulations. Some of the cell nucleus kept relatively regular form with a condensed chromatin on its central region, while others showed themselves relatively small and pyknotics advancing to a cariolisis forming necrosis focus. Veiga, et al., (2002)

Cloudy swelling of renal tubule, marked loss of haemopoictic tissue, shrinkage of glomeruli were reported in *Namachelius denisonii* (Day) exposed to phosphamidon (Rashatwar and Ilyas, 1984). Similar observations were made by Csepai (1978) in *Cyprinus carpio* chronically exposed to Anthio 40 EC, Satox an Basuden 10G, the organochlorine and organophosphate compounds. El-Zalbani and Soliman (1981) and Feng *et al*., (1982) also reported necrosis in renal epithelium, swelling of mitochondria in the renal tubules in animals administered with methothrin and pyrethrin respectively. Such sort of pathological conditions causing disfunction of kidney tissue have been reported under pesticide toxicity by Radhaiah (1985 & 1988); Rama Murthy (1988).

Degenerative changes in epithelial cells of proximal tubules and haemopoictic tissues, severe necrosis in the proximal tubules leading to the formation of vacuoles, degenerative changes in epithelial cells of collecting tubules of *Tilapia mossambica* exposed to fenvalerate has been reported by Radhaiah (1988). The toxic sublethal concentration of fenvalerate technical grade in the kidney of *Crirrhinus mrigala* showed changes in haemopoietic tissue which included severe necrosis, vacuoles around renal tubules and hemorrhage. (Anita Susan and Tilak, 2003) Similar report by Tilak *et al*., (2001c) in kidney tissue of the fish *Ctenopharyngodon idellus* when exposed to technical and sublethal concentration of 20% EC fenvalerate was observed with tissue damage like necrosis, vacuolar degeneration and atrophy.

Histopathological changes in the kidney tissue of the fish *Channa punctata* exposed to sublethal concentration of Butachlor and Machete, an Herbicide was studied and the changes observed were severe necrosis, cloudy swelling, cellular hypertrophy and granular cytoplasm. The distal convoluted tubules decreased in size and formations of vacuoles were reported by Tilak *et al*., (2007).
In lindane polluted fish, the kidney showed a disintegration of the convoluted tubules, and large intracytoplasmic vacuoles in the epithelial cells of the tubules. Shrinkage of the glomerulus and increased space within the Bowmans capsule were also observed by Ortiz et al., (2003). In *Oryzias latipes* (medaka) exposed to a lindane isomer, Wester and Canton (1986) found prominent glomerular hyalinosis as an indicator of renal toxicity.

Gupta and Dalela (1987) reported histological changes in kidney of *Notopterus notopterus*, exhibiting degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis following subtle exposure to phenolic compounds. Anitha Kumari and Shreeram Kumar (1997) observed mild activity of carbohydrates in the cytoplasm, nuclei and the luminar border of the proximal and distal tubules in the kidney of the freshwater teleost *Channa punctatus* under exposure to the polluted water of the Hussain Sagar Lake.

The present observations are in agreement with the reports of Goel and Veenagarg, 1980; Mandal and Kulshreshtha, 1980; Dubale and Awasthi, 1982; Malaya Guptha et al., 1988; Dhanapakiam and Premlatha, 1994; Ramana Kumari, 1999; Yacobu, 1999; Tilak et al., 2001a & 2001b; Tilak et al., 2005a & 2005b and Tilak et al., 2007) who observed renal damage, rupture in the glomeruli, reduced renal tubules and its lumen in different fish exposed to different toxicants.

Thus, when fishes are exposed to pesticides, they suffer irreparable architectural changes in various vital organs making the fish less fit for better survival. These histopathological changes can alter various physiological activities of the fish such as release of various enzymes and the metabolic processes as evidenced from Chapter-V. Thus, the histological changes observed in the gills, liver, brain and kidney of the freshwater fish *Channa punctatus* exposed to quinalphos technical grade and 25% EC indicate that the fish were responding to the direct effects of the contaminants as much as to the secondary effects caused by stress.