



Chlorpyrifos Induced Histological Deformities In Hepato-Renal Organs Of Fish, *Channa Gachua* (F. Hamilton)

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<p>CC License CC-BY-NC-SA 4.0</p>	<p>Abstract</p> <p>Chlorpyrifos is used world wide to control different insect pests in agricultural, residential and commercial settings due to its low persistence and high toxicity. Such type of insecticides reaches to the fresh water bodies through agricultural, industrial and domestic runoff and exerts their detrimental effects on non target organisms. Therefore the present investigation was aimed to assess the histological deformities in liver and kidney of fresh water fish, <i>Channa gachua</i> after acute exposure to chlorpyrifos. In a short term (96 hrs.) study healthy fish were exposed to 35 ppm (LC0) concentration and 50 ppm (LC50) concentration of chlorpyrifos and simultaneously control group was run. In liver the significant alternation was also observed at both concentrations such as increased sinusoidal spaces, hepatic cord disarray, pyknosis, karyolysis, vacuolation, lipid infiltration, ruptured blood vessel, degeneration of hepatopancrease. In kidney at both concentration, sever degeneration and necrosis of hematopoietic tissue, vacuolization, degeneration of glomerulus and destruction of glomerular capillaries, hypertrophied renal tubular cells and nuclear hypertrophy, karyolysis in the renal tubular cells, epithelial lifting was also observed. Dose dependent profound effect was observed in liver and kidney of fish which were become more sever at higher concentration. The deformities in hepato-renal organs shows real picture of adverse consequences of toxic substances on commercially important and edible fishes. Thus results are significant from environmental pollution and human health point of view.</p> <p>Keywords: acute toxicity, karyolysis, pyknosis, hypertrophy, chlorpyrifos.</p>
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1. INTRODUCTION

Aquatic toxicology deals with the study of the effects of toxic substances on aquatic organisms at cellular and molecular level and subsequently on ecosystem (Rand and Petrocelli, 1985). It is a multidisciplinary field which includes toxicology, aquatic ecology and aquatic chemistry associated with environmental pollution. Freshwater, marine water and sediments in environment are included in this study. India is highly populated country. In last few decades the rate of population growth is tremendous. To meet the needs of increased population, development in modern technologies, industrialization and modernized agricultural practices is also increased. But this modernization brings undesirable and indiscriminate change in the environment. The usage of chemical pesticides in the field of modern agriculture practices has also been increased in order to increase the production to overcome the needs of increasing population. Farmers applied millions of tons of pesticides to the crops in order to protect them from pests. These toxic pesticides pose serious environmental problems particularly water pollution. After food and air, water is most essential for living organism. Besides

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it is required in agriculture, industries, recreation, drinking and domestic purposes. Water bodies get polluted by pesticide through surface runoff and spraying. The pesticides accumulate and showed their effects at each level of ecosystem. Presently several types of pesticides are used worldwide, amongst them three classes i.e. organophosphate, organochlorine, and carbamates are widely used by farmers.

Fishes are the good indicator of water pollution and most popular test organism. The fishes and other aquatic organisms are useful as nutritious food material to human being from all status and placed an important role in food chain. The fish accumulates toxic chemicals in their body and shows undesirable deleterious effects at cellular and molecular level. Due to that the quality and nutrition value of fish gets reduced and these toxic substances get entered in to food chain. Pesticidal toxicity mainly depends upon quality of water, concentration of pesticide and is species specific. The toxicant affects the general metabolism, biochemical composition and normal histological structure of vital organs in fishes. In toxicity studies the organisms which are ecologically relevant and sensitive to toxicants and have well-established available literature background are used. The pollutants disturb the regulatory mechanisms which leads into behavioural, biochemical, histopathological and physiological alterations and finally resulting into death (Chavan 2014).

Present study aimed that toxic effects of organophosphate pesticide chlorpyrifos on histological structure of liver and kidney in snakehead fresh water fish, *Channa gachua*.

2. MATERIAL AND METHODS

2.1 Selection of fish

In present research work the fresh water fish, *Channa gachua* locally known as 'Dokarya' was selected keeping in mind its availability throughout the year in local river Krishna around Karad city, Dist. Satara. The fish is sturdy for handling, commercially important, tasty, and edible and consumed by huge population so it was thought essential to check the effect of pesticide on this fish.

2.2 Selection of pesticide

In present study organophosphate pesticide i.e. chlorpyrifos was used for toxicity testing against the selected fish, which were purchased from local agro chemist shop.

2.3. Experimental set up

i) Acute toxicity

Healthy fishes from stock were divided in to three groups, group first and second as experimental group and group third as control. In experimental groups fishes were transferred to aquaria which contain 35 ppm (LC0) and 50 ppm (LC50) concentrations of particular pesticide solution for 96 hrs. The control group of fishes was maintained simultaneously in separate aquarium containing tap water.

ii) Histological Methods

For histology the fishes from control as well as treated group were taken out of aquaria at the end of exposure period. The live fishes were killed by decapitation. The desired organs like liver and kidney were dissected out. These organs were cut in to pieces about 2-3 mm in size and immediately fixed in fixative (CAF) at 40C. After fixation (24 hrs.) the tissues were washed under running tap water. The tissues were dehydrated through alcohol grades such as 30%, 50%, 70%, 90% and absolute alcohol for 30 minutes in each grade, cleared in xylene and embedded in paraffin wax at M.P. 58- 600C. The blocks were prepared by using cigarette packets. The sections were cut at 4-5 μ thickness. The sections were stained with H-E staining for histological or histopathological observations.

3. RESULTS

3.1 Liver

A) Histological observations

The histological structure of liver of control fish is shown in Figs. 1 & 2. These photomicrographs of H-E stained sections of liver showed hepatic cells within the network of bile canaliculi. Hepatic cells were roundish to polygonal in shape that contained centrally placed, spherical, blue colored nucleus (Figs. 1 & 2). Hepatic cells located around the sinusoids forming cord like structure called hepatic cell cords were not distinct. Bile canaliculus was seen located centrally in each of these cords (Fig. 2). There was no clear division of hepatic cells within the lobules and distribution of bile duct and blood vessels was irregular. Kupffer cells were occasionally seen lining the sinusoids with large amount of cytoplasm (Fig. 2). Between the cords of hepatocytes there was a network of irregularly dilated vessels called the sinusoids. The sinusoids were lined with endothelial cells and Kupffer cells. Between the two hepatic lobules there was a thin septum of connective tissue separating the hepatocytes from the exocrine pancreatic acini was noticed an interlobular septum (Fig.

2). In interlobular septum, bile duct, hepatic artery and portal vein, referred as portal triad were observed. Portal vein was seen easily distinguishable as it was covered with pancreatic acini together known as hepatopancrease (Fig. 2). Hepatic arteries were with narrow lumen covered with thick layer of endothelial cells. Intralobular central vein was observed in each of hepatic lobules and blood cells were observed in lumen of central vein.

B) Histopathological observation

Histopathological observations in the liver of fish, *C. gachua* exposed to different concentrations of chlorpyrifos showed discrete alterations as compared to the control fish, which are shown in Figs. 3-6.

a.35 ppm (LC0) concentration

Chlorpyrifos at this concentration has induced some histopathological changes which are shown in. 10, Figs. 3 and 4. Enlarged nucleus of hepatocytes, increased sinusoidal spaces, hepatic cords disarray, cytoplasmic vacuolization, pyknosis and karyolysis was seen (Fig. 3). Loss of normal architecture of the hepatic cells due to the rupture of their cell membranes was noticed Fig.

4. Spaces between the hepatic cells became very prominent. Disorganization of some of the blood vessels and rupture of wall of the central vein of hepatic lobule was also seen. Infiltration and deposition of lipids was seen in hepatocytes. Increased number of Kupffer cells and increased in the space of Disse was also noticed (Fig. 4).

b.50 ppm (LC50) concentration

When the fishes were exposed to LC50 concentration of this pesticide the liver showed significant degenerative changes as compared to previous one (Figs. 5 & 6). These histological lesions were characterized by significant disarray of hepatic cords, dilated sinusoids throughout the parenchyma, and increased sinusoidal spaces around the central vein and portal vein, complete degeneration of hepatocytes with severe necrotic patches etc (Fig. 5). There was degeneration of cytoplasm of the hepatocytes and nuclei became prominent and hypertrophied (Fig. 6). Plate No 1: Showing alterations in liver and kidney of *Channa gachua* exposed to 35 ppm and 50 ppm conc. of chlorpyrifos.

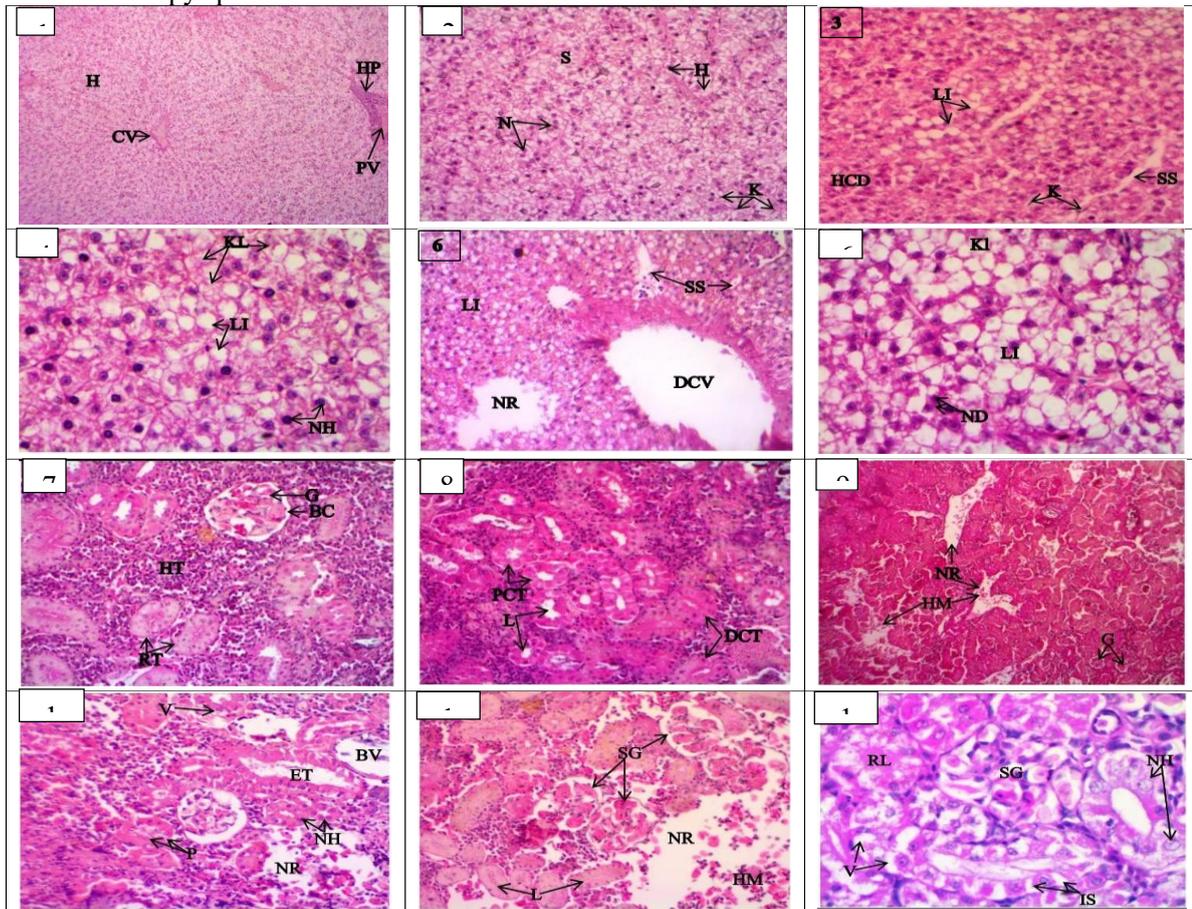


Fig. 1 & 2 T. S. of liver of control fish H&E 100x & 400x. **Fig. 3 & 4** T. S. of liver of fish exposed to 35 ppm conc. of chlorpyrifos for 96 hrs. H&E staining 400x & 1000x. **Fig. 5 & 6** T. S. of liver of fish exposed to 50 ppm conc. of chlorpyrifos for 96 hrs. H&E staining 400x & 1000x. **Fig. 7 & 8** T. S. of kidney of control fish.

H&E staining 400x & 400x. Fig. 9 & 10 T. S. of kidney of fish exposed to 35 ppm conc. of chlorpyrifos for 96 hrs. H&E staining 100x & 400x. Fig. 11 & 12 T. S. of kidney of fish exposed to 50 ppm conc. of chlorpyrifos for 96 hrs. H&E staining 400x & 1000x.

The rupture of blood vessel in large amount, and the complete rupture of wall of central and portal veins was also noticed. Elongation of blood vessel was also seen at certain regions (Fig. 5). The portal triad was disturbed and hepatopancrease was degenerated. Lipid infiltration in hepatocytes was observed in large amount (Fig. 6).

3.2 Kidney

A) Histological observations

Normal histological structure of kidney observed in the fish from control group is shown in Fig. 7 and 8. It consisted of number of nephrons or renal tubules. Each of nephron consisted of two parts the renal corpuscles or the glomerulus (G) arranged compactly in the cortex and tubules mostly situated in the medulla (T). The glomeruli and the renal tubules were surrounded by hematopoietic tissue. Glomerulus consisted of tuft of blood capillaries surrounded by double layer of flattened epithelial cells the Bowman's capsule (BC) (Fig. 7). The renal tubule consisted of single layer of epithelial cells surrounding the central lumen. The tubule was thin and short in the neck which continued into proximal convoluted tubule (PCT). PCT exhibited a narrow uneven lumen surrounded by a layer of cuboidal to low columnar epithelial cells with granular cytoplasm and basally situated nuclei (Fig. 8). They possessed densely arranged microvilli (brush boarder) towards the lumen. The cell boundaries of PCT lining cells were not well marked. Another convoluted tubule was distal convoluted tubule (DCT) exhibited large lumen with low cuboidal cells with faintly stained granular cytoplasm. Unlike the PCT the microvilli were not seen towards the apical side of the epithelial cells. Between the PCT and DCT there was thick and thin descending segment, and thin and thick ascending segment of loop of Henle. The loop of Henle was lined with a simple squamous epithelium. The end part of the renal tubule was collecting tubule (CT) it was composed of tall columnar cells with basally located nuclei. No brush boarder was seen in the lining cells of collecting duct.

B) Histopathological observations

Histopathological alterations observed in the kidney of fishes exposed to different concentrations of the chlorpyrifos are shown in Figs. 9-12.

a.35 ppm (LC0) concentration

On exposure of fishes to LC0 concentration of chlorpyrifos for a period of 96 hrs. changes observed in the structure of kidney are shown in Fig. 9 & 10. These structural changes were characterized by shrinkage of glomerulus, and expansion of periglomerular space within the Bowman's capsule and degeneration of hematopoietic tissue with hemorrhage (Fig. 9). Formation of intracytoplasmic vacuolation, pyknotic nuclei, necrosis and subsequent karyolysis in the renal tubular cells was also evident (Fig. 10). Blood capillaries in the tuft of glomerulus were dilated, some blood capillaries were disturbed. Epithelial cells in PCT get degenerated due to which its normal structure was collapsed. The squamous epithelial cells in the loop of Henle were disturbed. Few blood cells were appeared in the lumen of loop of Henle (Fig. 10).

b.50 ppm (LC50) concentration

At this concentration severe degeneration and necrosis of the hematopoietic tissue with formation of large vacuoles was seen (Fig. 11). The degeneration of glomeruli due to swelling and destruction of capillaries in them was evident (Fig. 11 and 12). In PCT the hypertrophied epithelial cells and pyknotic nuclei was observed (Fig. 11). In some tubular cells nuclear hypertrophy was seen (Plate no.2, Fig. 5). The cytoplasm of tubular cells was faintly stained with number of intra cytoplasmic vacuoles in them were also noticed (Fig. 12). Narrowing of lumen of PCT along with disturbed microvilli was also noticed (Fig. 12). There was increased diameter of Henle's loop and widening of lumen with karyolysis and development of intercellular spaces. Narrowing of lumen of DCT due to the swelling of its epithelial cells was also observed. Epithelial lifting from basement membrane was also seen (Fig. 12).

4. DISCUSSION

In toxicity studies and pollution monitoring the histology and histopathology is a valuable tool for assessing the toxic effects of any toxicant (pesticide, heavy metals, industrial effluents and toxic chemicals) on the different tissues of fishes. Intensity of lesions and damages were easily accessible with the help of histopathology which are dependent on exposure period and concentration of pesticide. In the present studies the histological and histopathological observations clearly indicated that the pesticides used in the present investigation produce significant pathological alterations in the structure of liver and kidney of *C. gachua*

exposed to pesticides. The alterations in above organs were dependent on concentration and the period of exposure of pesticide. The liver plays an important role in the metabolism and biochemical transformation of pollutants from the polluted water which inevitably shows the lesions and histopathological alterations in the structure of liver. It is also one of the organ which is most affected by contaminants in the water (Rodrigue and Fanta, 1998). The liver has ability to degrade toxic compound, but its regulating mechanism can be overwhelmed by increased concentration of toxic compounds and could be resulted in to structural damage (Brusley and Anadon, 1996).

After exposure to acute (96 hrs.) toxicity of chlorpyrifos in the present study the liver showed marked histopathological changes such as increased sinusoidal spaces, hepatic cord disarray, vacuolation, pyknosis and karyolysis in hepatocytes, nuclear hypertrophy, rupture of wall of central vein, and portal vein, lipid infiltration and breakdown of boundaries of hepatocytes. Similar pathological alterations have been reported by several investigators in the liver of different fishes in the influence of various pesticides such as Rodrigues and Fanta (1998) in fish, Brachio rario, Kunjamma et al. (2008) in *O. mossambicus*, Velmurugan et al. (2009) in *C. mrigala*, Thripathi et al. (2011) in *C. catla*, Olufayo and Alade, (2012) in *Heterobranchus bidorsalis*, Deka and Mahanta (2012) in *H. fossilis*, Vijayakumar (2013) in *C. punctatus* and Latif et al. (2014) in *L. rohita* exposed to different pesticides.

In present study the histopathological changes such as considerable degradation in cellular structure, cytoplasmic vacuolation, degeneration of hepatocytes, sever necrotic patches with hemorrhage, hepatic cord disarray, pyknosis, karyolysis, congestion, significant sinusoidal spaces, ruptured blood vessels and severe lipid infiltration were observed in liver of fish exposed to lethal and sublethal concentrations of both the pesticides. More or less similar alterations were observed by Day and Saha (2016) in liver of *L. rohita* exposed to lambda-cyhalothrin 5% EC and marshal (carbosulphan 25% EC). According to them necrosis is a passive mode of cell death which shows the capacity to maintain homeostasis which was affected by pesticide. Thus, occurrence of necrosis may be one of the important reason for decreased lysosomal membrane stability leading to the leakage of lysosomal marker enzyme, acid phosphatase to the soluble fraction. Pyknotic nuclei indicate that the cell become hypofunctional, pyknosis results in irreversible condensation of chromatin in the nucleus of hepatocyte. Chavan and Muley (2014) also reported that the degenerative effects and necrosis of hepatocytes may be due to the cumulative effect of the toxic pollutant and increase in their concentration in liver Hartly et al. (1996) reported hepatopancrease atrophy and apoptotic nuclei, loss of contact between hepatocytes and pancreocytes. From these observations they suggested that the inhibition of protein synthesis, energy depletion and aggregation of microtubules resulting from vacuolation of hepatocytes. They also stated that pathologic condition may be because of the loss of energy in the detoxification process increase in metabolic by products caused aggregation of melanomacrophages. Melanomacrophages are the elements of the immune system in fish, which stimulates immune response against diseases or foreign materials. The abnormality may be attributed to direct toxic effect of pesticide on hepatocytes in liver which is the site of detoxification of all types of toxins and chemicals (Latif et al., 2014).

From the review of earlier workers it assumed that, due to intoxication and toxicants excreted in the bile the sinusoids and blood vessels get ruptured and because of subsequent stasis of blood may be responsible for the hepatocytes degeneration and necrosis. Hypofunction of cells and condensation of chromatin material in the influence of pesticides might be leads in to pyknosis and karyolysis. The fatty change in liver indicates imbalance between the rate of entry and utilization of fatty acids in metabolic processes in the hepatocytes and the rate of their release in to the blood circulation. The cellular degeneration in liver may be due to the O₂ deficiency because of vascular dilation and intravascular hemolysis in the blood vessels with subsequent stasis of blood as well as significant increase in Kupffer cells in liver due to pollutant accumulation.

Kidney performs an important function of electrolyte and water balance and maintain internal environment in the body of fish. It excretes the nitrogenous metabolic waste products such as ammonia, urea and creatinine. histopathological alterations in the kidney under pesticide stress serve as good indicator of environmental pollution.

In the present study the kidney of fish *C. gachua* exhibited several histopathological alterations after pesticides exposure. When fish is exposed to any toxicant it ultimately reaches to kidney for its excretion, while the toxicant or its intermediate metabolite passing along with the nephron it is causing several deleterious effects on it. In the acute toxicity test the kidney shows shrinkage of glomerulus that results in to expansion of periglomerular space within Bowman's capsule, severe necrosis with hemorrhage in hematopoietic tissue, intracytoplasmic vacuolation, pyknotic nuclei, karyolysis and necrosis in renal tubular cells, degeneration of

capillaries in glomerulus, resulting into its shrinkage, reduced tubular lumen, which might be because of hypertrophied tubular epithelial cells. While at certain places widening of tubular lumen was noticed due to degeneration of tubular epithelial cells with karyolysis and intercellular spaces and epithelial lifting in tubules. Many of earlier workers have reported similar histopathological alterations in glomerulus and tubular epithelium in the kidney of different fishes exposed to various pesticides such as Bhuiyan et al. (2001) in *C. punctatus* exposed to sumithion, Cengiz, (2006) in common carp exposed to deltamethrin, Velmurugan et al. (2007) in *C. mrigala* exposed to monochrotophos, Prashanth (2011) in, *C. mrigala* exposed to cypermethrin, Sing (2012), in *C. carpio* exposed to dimethoate (EC 30%), and Kumar and Gautam, (2014) in *C. punctatus* exposed to nuvan. Sharma and Sharma (2016) suggested that atrophy of glomerulus and hemorrhage due to entry of pesticide in the kidney disrupt their normal functioning by histopathological alterations. The necrosis of renal tubules affects the metabolic activities that promotes metabolic abnormalities. Abubakar et al. (2014) revealed neutrophil and lymphocytic infiltration in tubular cells. Inflammatory cells were seen in hematopoietic tissues in kidney of *C. gariepinus* exposed to sniper 1000EC. They claimed that hydropic degeneration observed in the kidney of exposed fish might be due to increase in the permeability of tissues to water and increased urine output and subsequent impairment in the infiltration function of the kidney. Khatan et al. (2016) also showed extensive damage to the renal tubular epithelium with vacuolation, necrosis and complete loss of hematopoietic tissues with occurrence of golden brown hemosiderin pigments, degeneration of glomerular tufts and loss of glomerulus in the kidney of cat fish, *H. fossilis* after chlorpyrifos treatment. According to Pala and Day (2017) the alterations might be related to the depletion of ATP which finally leads to the death of the cells. The degenerations and necrosis might greatly impairs filtration function. They further suggested that vacuolation in cytoplasm may be the indicator of hydropic degeneration.

In the present investigation formation of vacuoles in the cytoplasm of the tubular cells were noticed in the kidney of fishes exposed to both concentrations of pesticides which might be also because of hydropic degeneration in the cells. The results obtained in the present study were more or less identical in pattern of cellular damage to that of in kidney of fishes exposed to different pesticides as studied earlier by various researchers. The pesticides chlorpyrifos might have altered the chemistry of the internal environment. To cope up with these drastic changes, the organ systems might not be equipped as in the normal environment under heavy load of work they must be getting crumpled as proposed by (Kamble, 1999). Thus, when fish is exposed to pesticide they might suffer irreversible cellular architectural changes in kidney which led to fish in danger or it cannot be survived. These histopathological changes alter the internal balance of metabolites and ions in the body. Findings of earlier workers strengthen the results in present investigation. The pesticides once entered into the gastrointestinal tract and their metabolites came in to circulatory system and subsequently they affect the vital organs, such as liver and kidney. Histopathological changes in different tissues results into the failure of normal physiological functions and metabolism might be the main cause of fish mortality.

5. CONCLUSION

The pesticides used regularly in agricultural and domestic field to kill the harmful pests may produce definite and permanent effects on non-targeted organism and subsequently enter into food chain and reach to human population via food. Consumption of fishes from water contaminated with pesticides pose harmful threats and several physiological disorders and the health of people at high risk. Thus, organophosphate pesticides and their metabolites produced several drastic degenerative changes which leads in to malfunctioning of organ system and collapsed physiology of fish. Cellular damage and collapsed metabolism affect the ability of fish to store energy and increased toxicant stress enhance the use of nutrients and energy in long term stability to survive.

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