

## Histopathological Alterations Caused by Insecticides (Chlorpyrifos) in Carp Fishes in Khost, Afghanistan

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### ABSTRACT

Histology is the study of tissues is an important sensitive term to find out the influence of chemical compounds on the organ that are targeted and be used to find the health and growth of fish in polluted ecosystem (Van Dye and Pieterse, 2008). Histopathology is the study in which when water is polluted, the effect of this pollution is studied on the body and various tissues of the fish when these tissues come in contact with these chemicals. It not only give information about the function of the body but also inform us about the pollution of the water. It give us an idea about the side effects of human activities by disturbing the environment (Banaee, 2013). When these fishes are studied for parameters that changes observed indicate the toxic effect of these chemicals (Sudova et al, 2009).

**Keywords-** Carp fishes, Chlorpyrifos, Insecticides, Tissues, Toxicity.

## I. INTRODUCTION

Human made hazardous chemicals produce in the environment are due to the, transportation as well as utilization, are threat for the aquatic fauna. Therefore the judgment of ecological dangers due to hazard or toxic substances is a big challenge or dispute to Eco toxicologists and toxicologists (Braunbeck, 1994). Pollution is defined as any harmful change in the environment that affects living organisms badly. It is mostly caused because of human actions. Pollution of water is a type of environmental pollution that is most dangerous for all life on earth. It may be due high industrialization and activities of humans. When the natural environment of an area is disturbed it has a bad effect on living organisms and on the surroundings. Those things that cause pollution are known as Pollutants. Pollutants may be present in the environment naturally or it may be due to activities of human being like herbicides and pesticides. (Kurian, 1997).

### 1.1 Pesticides

In the previous fifty years, a gradual increase is observed in synthetic industrial chemicals such as herbicides and pesticides. Use of pesticides is more in the modern world to increase the demand of modern overpopulated world. Pesticides are specific for those pests for which it is formed and control the pest from increasing their progeny. It include substances that is made to increase the yield of a crop but it increase pollution. Effect of pesticides on environment has been studied much because of and frequent use in parallel with the new and modern technique of agricultural work and disperse pervasion of the environment with these chemicals. Pesticides having phosphate, nitrate have side effects on living things other pyrethroid and carbamate groups of pesticides and various types of inorganic chemicals and chemicals such as ammonium chloride, di-ammonium phosphate and urea are used. The advantage of these chemicals is that it increases the product and is very chief (Connell, 1984). Pesticides are found all over the world due to its excess use. In waters, air and all for areas where these pesticides are used. It

affect is not only restricted to that area but it spread to other areas and causes pollution (Loos et al., 2010; Yadav et al., 2010). Pesticides are chemicals when sprayed on the crops it stop the side effect of pests and thus the crop is protected. But these chemicals are added to air and also to water and soil and causes sediment, water and air pollution (Devault et al., 2009). When these chemicals are sprayed the reach to the soil and accumulate there. Also it accumulates in the water resources above the soil or near to the areas where these pest killing chemicals are applied (Byer et al., 2011).

### **1.2 Organ phosphorus pesticides**

These are basically anti-choline esterases and repress the neuron-muscular activities of both insects and humans (Nazarian and Amini, 2008). In agriculture use it has replaced other pesticides due to its high use after use it has small amount of residues, but it produce very intense effect afterward an increase side effects on the non-targeted organisms (Fulton and Key, 2001). No other study by Solé et al. (2000) the use of these chemicals decrease the living organisms of the water. But after that it has a bad effect on the coastal areas organisms is also been observed which indicate the high toxicity of these chemicals. It also has inhibitory effects on the cholinesterase (ChE) activity (Valbonesi et al., 2011) and has the ability to further increase the defects and change the physiology of the organisms (Almeida et al., 2010). The disturbance of food chain is also observed due to the use of this chemicals (Sun et al., 2011).

### **1.3 Insecticides**

Insecticide is a type of chemical used to kill insects, like those that have infested a house or a farmer's crops. Words that end in -cide usually have to do with killing, such as genocide, suicide, and patricide. Another is insecticide, which is a word for a substance that kills insects and bugs. Insecticide is a type of chemical, and it often consists of a powder or gas. Insecticides change the physiology of insects and decrease their activities and thus kill the pests. Some kill them by stopping their nervous system, while some completely disrupt their body. The USDA at 2001 survey reported that insect killing chemicals play 12% of total pesticides give or sprayed on crops. In Unite states chemical like insecticides is mostly used and sprayed on the corns. It has various methods to give these chemicals to various plants most important method using spray of these chemicals. When the spray is done on the crops these pests are controlled by sucking the plants so these chemicals also enter and become part of the food of pests and degenerate their body. The spray of these chemicals also affects the growth and life of fish when they become part of the water sources (Betoulle et al. 2000).

### **1.4 Organ phosphorus insecticides**

These insecticides have good effect and increase the producing yield. Mostly the causes high rate of mortality and morbidity in land organisms specially

vertebrates (Stanley and Bunyan, 1979). Carbamates are used in lesser quantities than OPs, the majority of monitoring is concerned with OPs. Chemicals like diazinon have the ability to dissolve in water but not remain in environment and cannot absorb to soil, while chlorpyrifos have a vise versa relation and solubility in water and soil (Sharom et al, 1980). Survey of WHO indicate that these chemicals decrease the pest's activities and increase the yield of crop (Carod, 2002). The LD50 values of 3 of the 6 most commonly applied insect killing chemicals, carbofuran, dimethoate, and chlorpyrifos, indicate a high degree of toxicity to birds, whereas the other three (carbaryl, malathion, and deltamethrin) are relatively low in toxicity (Grue et al., 1986, Sheehan et al., 1987).

### **1.5 Chlorpyrifos**

Chlorpyrifos O-diethyl-O- (3,5,6-trichloro-2-pyridil phosphoro-thioate, is also an organophosphate class for killing insects that displays broad spectrum insect killing activity against many harmful arthropod pests (RACKE, 1993). Scientific study have found that as the chemical breaks down naturally in the environment, it releases chlorpyrifos Oxon, which has been found to be even more toxic than the original form of the chemical. (US EPA 2011). Hence, a study was undertaken to evaluate the aquatic toxicity of chlorpyrifos with special emphasis on behavioral, morphological, target enzyme interaction and bioaccumulation effect in the toxicant in different parts of body. Chlorpyrifos is normally supplied as a 23.5% or 50% liquid concentrate. The recommended concentration for direct spray pin point application is 0.5% and for wide area application is 0.03-0.12% mix is recommended (US). Chlorpyrifos kill the larvae and decrease their progeny and its population (Sharom et al., 1980).

### **1.6 Effects of Chlorpyrifos**

It brings high change in human. Paraoxonase is the body enzyme plays an important role in this changing body physiology. Paraoxonase is an enzyme that is activated by calcium whenever disturbance occur in tissues. Mostly this disturbance occurs in blood, liver and kidney level of calcium (US EPA 2011). Several (variations) polymorphisms of the paraoxonase gene effect both the level of expression of this enzyme and its enzymatic function, thus showing the works at which a person will detoxify a proper pesticides (Cal EPA 2008). paraoxonase levels change highly between individuals, as much as fourteen time between mothers and about twenty six times among children's that are newborn (Furlong et al., 2006). Fifty seven time great changes was studied in liver microsomes for metabolism (Croom et al. 2010). Additionally, chlorpyrifos causes damage to the dopaminergic neurons through stress effects on the mitochondria, this procedure lead to Parkinson's disease that is neurodegenerative disease (Lee et al., 2012). Chlorpyrifos has the same action on insects and human and it react with other organic substances having

phosphate and stop Ache. Acetylcholine is a neurotransmitter that work sympathetically and parasymphatically and is a cholinergic neuron is degenerate this neuron and effect the behavior of the organisms. Secretion of the neuron increasing when it is over stimulated this over or high stimulation is caused if acetylcholine is not blocked after immediate activation, because it is very quick in its work. This degeneration leaded to stop the function of motor neurons and lead to sensory and behavioral disturbances and then to death (Colborn 2006). Most of the studies shows that human is most susceptible to these chemicals as compare to other animals (US EPA 2011). Metabolite is the main thing of the cause of this disturbance. Main reason of this disturbance is the pesticides like chlorpyrifos (Slotkin 2004).

### 1.7 Carps fishes

This species has a great tolerance to the environment. It is a filter feeder and its rate of growth is very rapid and high. (Shen, 2009). Larger family of fishes is this known as Cyprinidae. Fishes of this family live in fresh waters (Nelson 1994). The silver carp is find in fresh water and is called fresh water specie. (Zhao, H. 2011). Some are now introduced to the aquarium and they adapted successfully for example carpio specularis and C. carpio nudus have gained entry into the riverine ecosystem through accidental or short introduction. Larvicidal fishes like, Lebistesreticulatus Nothobranchus species. And Gambusiaaffinis were added for consisting the insect larvae in limit waters. In Bengal, other species such as carpio had formed producing new populations and add more than seventy % of the stock. (Dudgeon, 1992).

### 1.8 Histopathology of Fish

Histology is the study of tissues is an important sensitive term to find out the influence of chemical compounds on the organ that are targeted and be used to find the health and growth of fish in polluted ecosystem (Van Dyk and Pieterse, 2008). Exposition to these chemicals produce toxic effect on the body of fish that increase with passage of time and lead to the mortality of the fish (Dyk et al., 2009). Histopathology is the study in which when water is polluted, the effect of this pollution is studied on the body and various tissues of the fish when these tissues come in contact with these chemicals. It not only give information about the function of the body but also inform us about the pollution of the water. It give us an idea about the side effects of human activities by disturbing the environment (Banaee, 2013).

## II. METHODS

### Experimental

The present research was performed to find out the toxicological effect of chlorpyrifos on tissues of fresh water carp fish. The carp fish was procured from mondozi district. The experiment was carried out in aquarium for the assessment of acute and chronic

toxicity of chlorpyrifos. This research is a basic field or operational research. The fishes were collected from farm at khost city and kept in water poles. The carp fishes were treated with different concentrations of insecticides. The data were collected and evaluated with Microsoft excel. The toxic properties of the insecticides substances that occur in the carp fishes were evaluated and the fishes were grouped and distributed with different groups. The groups as follows

**The first group:** used as a control, fishes were not treated with insecticides

**The second group:** In this group, insecticides were used in such a way that the concentration was 2.5 mgr. /L and Seventy hours' time was considered for this group.

**The third group:** In this group, insecticides were used in such a way that the concentration was 1, 7 mgr. /L and Seventy hours' time was considered for this group.

**The fourth group:** In this group, insecticides were used in such a way that the concentration was 1 mgr. /L and Seventy hours' time was considered for this group.

### Procedure for Histopathology

Gills, liver, intestine, kidneys tissues of 1 cm size were cut for tissue processing. Samples were threaded and were washed in bottle water. Tissues were washed in such a way that water did not fall directly on tissues as it might damage the tissues. Tissues were washed overnight. After washing tissues were placed in ascending grade of alcohol for dehydration with reducing time period in Automatic tissue processor. Tissues can be placed in 35% alcohol for unlimited time, and kept in various grades of alcohol for definite time as follows.

### Dehydration

30%	alcohol	
4-5 hrs.		
50%	alcohol	
3 hrs.		
70%	alcohol	
3 hrs.		
80%	alcohol	
2 hrs.		
90%	alcohol	
2 hrs.		
Absolute	alcohol	I
1 hrs.		
Absolute	alcohol	2
1 hrs.		

### Clearing

Alcohol	and	Xylene
40 min		
Xylene		I
32 min		
Xylene		II
16 min		

### Impregnation

For impregnation tissue subjects were placed in paraffin melted at 70°C.

Paraffin	I
1.5 hrs.	
Paraffin	II
1.5 hrs.	

**Embedding**

After processing then the tissues blocks were formed. Tissue blocks were made in automatic tissue embedding assembly. Blocks were, .made by pouring melted paraffin. Over the tissues placed in plastic cassettes. Blocks were then shifted to cold plate of Tissue Take and were allowed to dry.

**Staining**

Slides were placed for staining after final drying. For staining of slide section Hematoxylin and Eosin (H & E) staining was used. Automatic slide stainer (Tissue-Tek® DRST™2000 Sakura, Japan) was used for staining purpose. Staining was performed according to a standard protocol.

**Mounting of the coverslip**

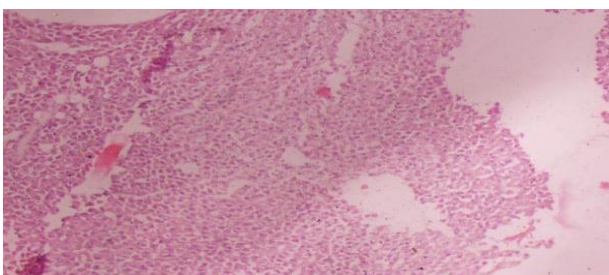
After completion of staining process slides were cleaned properly, DPX (Scharlau) was poured on slide sand cover slips were placed in such a manner on slides to avoid formation of bubbles otherwise. There will be hindrance in reading of the slides.

**III. RESULT AND DISCUSSION**

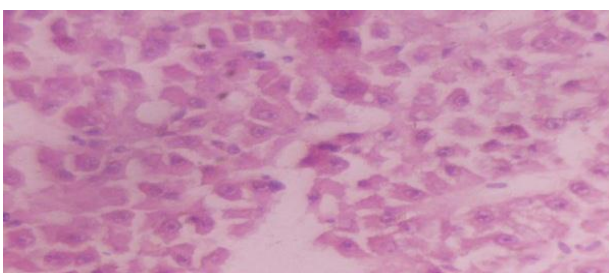
At the end of the research, it was revealed that in comparison with the control group, the samples of Chlorophyrips from the group of insecticides with different concentrations were used or pre-applied. The results of this research are shown in graphs, pictures and tables.

**Histology of carp fishes**

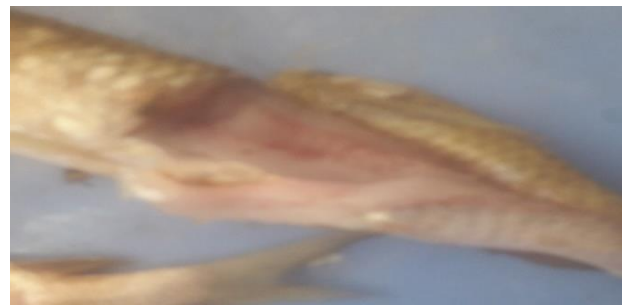
Acute concentration 2.5mgr/L, 1.7mgr/L, 1mgr/L and Normal group



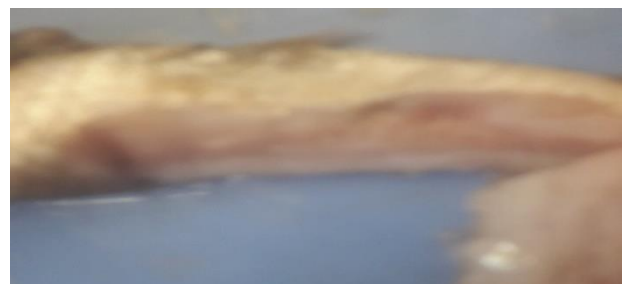
**Figure 1. Normal group**



**Figure 2.**



**Figure 3.**



**Figure 4.**

The fishes were dissected and studied for histopathology. Degeneration of the liver was higher at high concentration of chlorpyrifos. Intestine and kidney were also affected more as the concentration and time of exposure was increased. The less affected organ was gills. But when the time of exposure increased the gills were also affected and lamellar fusion of the gills occurs. Same the result was recorded in the previous study.

**IV. CONCLUSION**

From the present study that was done on histopathological alteration caused by insecticide (Chlorpyrifos) in carp fish following conclusions can be drawn.

Various Concentration of chlorpyrifos was added to water quality. The concentration of chlorpyrifos was from 1.3µl/L to 1.8µl/L. The sacrifice of the fishes was studied at different time periods from day 1 to 3. The fishes were then dissected and studied for histopathology. Degeneration of the liver was higher at high concentration of chlorpyrifos. Intestine and kidney were also affected more as the concentration and time of exposure was increased. The less affected organ was gills. But when the time of exposure increased the gills were also affected and lamellar fusion of the gills occurs.

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