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HISTOPATHOLOGICAL CHANGES IN THE GILL, LIVER, AND KIDNEY OF THE FISH *LABEO ROHITA* (HAMILTON) EXPOSED TO THIODICARB 75% WP

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Abstract:

Freshwater fish, *Label Rosita* was exposed to lethal & sub lethal concentration of a thiodicarb 75% wp insect side larvin technical grade for a period of 10 days. The histopathological changes in the gill include: necrosis, vacuolar degeneration, fusion and atrophy of primary and secondary gill lamellae. The tissue damages like degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, rupture in blood vessels and disposition of hepatic cords are the histopathological changes observed in the liver. The changes in the kidney include: necrosis, swelling of renal tubules, cellular hypertrophy and granular cytoplasm.

Key words: 75% wp, Alachlor, Lasso 50% EC, *Labeo rohita*, Gill, Liver, Kidney

INTRODUCTION

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 pests 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 (1999), 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, 1999). 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.

Histopathological studies have been conducted to help for establishment of casual 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 (Sakar and Jamal Al lail, 2005). 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.

Aquatic vertebrates are susceptible to non-target effects, because of their relatively restricted mobility, due to reduced pesticide dispersion leading to lengthy periods of exposure (Smith and Stratton, 1986). Fishes are particularly sensitive to a wide variety of pesticide chemicals, and toxic concentrations may raise not only from spillage of agricultural practices if their use is excessive but also from several other sources. Apart from causing death either directly or due to starvation by destruction of food organisms, many pesticides have been shown to effect growth rate, reproduction and behavior with the evidence of tissue damage. Fenvalerate induced histopathological and histochemical changes in the liver of the cat fish *Clarias gariepinus* (Sastry *et al*., 1979; Sakar *et al*., 2005). Korkmaz et al (2009) reported severe histopathological lesions and marked decline in protein level and glycogen level in different organs of Nile tilapia (Oreochromis niloticus) in response to the treatment of cypermethrin for 10 days and very good capability of recovery after their exposure to ascorbic acid. The sub-lethal concentrations of deltamethrin (0.25and 0.50 μg/l) for 10, 20 and 30 days were reported to cause determined histopathological changes in gills (desquamation, necrosis, epithelial hypertrophy, lifting of the lamellar epithelium, oedema, dilatation of the capillaries primary lamellae, aneurysm, epithelial hyperplasia and fusion of the secondary lamellae), liver (hypertrophy of hepatocytes, significant increase of Kupffer cells, circulatory disturbances, focal necrosis, fatty degeneration, nuclear pycnosis and narrowing of sinusoids) and gut (infiltration of mononuclear leucocyte and eosinophils towards lamina propria and necrotic tissues) of the mosquitofish, *Gambusia affinis* Cengiz, and Unlu (2006).

Changes similar to pesticide-induced histopathological effects were in the liver of Bluegills starved for 24 h. Similarly, while there were distinct pathological changes in the liver of fish exposed to 0.1 mg PCP/L for 24 h, those that were exposed for 96 h had normal liver (Venkatesam and Subramaian, 2007). Hence, it is difficult to appreciate the real significance of

pesticide-induced changes. (Sprague, 1971) stated that lack of basic information on fish histology makes the interpretation of any observed changes difficult.

Sudha Sing and Asja Mehrotra (1999) observed severe damage in the outermost layer serosa and muscle layers, necrosis in intestinal villi and increase in the number and size of mucus cells, under sub-lethal exposure of carbaryl for a period of one month. Ramachandra Mohan (2000) observed a significant reduction in the ovarian weight and diameter of developing oocytes and also degeneration of growing oocytes and resorption and yoiky oocytes exhibited atresia in *Glassogobius giuris* under sub-lethal exposure of Malathion. 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 (Hinton *et al*., 1992).

Tissue changes in test organisms exposed to sub-lethal and lethal concentrations 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 (Aruna *et al.,* 2000; Sambasiva Rao, 1999). Regarding histopathological effects of pesticides on various organs of fish are scanty (Dwivedi, 2000; Inbamani and Srinivasan, 1998; Adhikari, 1996).

In the present study, an attempt has been made to observe possible histopathological changes in certain vital tissues like gill, liver, and kidney of the freshwater fish *Labeo rohita* exposed to lethal and sublethal concentrations (1/10 of 96h LC_{50}) of thiodicarb (Larvin 75% WP) commercial grade for 96h.

MATERIAL AND METHODS

Freshwater fish *Labeo rohita* was acclimatized to laboratory conditions for 10 days. They were exposed to sub-lethal and lethal concentrations of thiodicarb (Larvin 75% WP) for 96h. At the end of the exposure period, fish were randomly selected for histopathological examination.

Gill, liver, 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 h, 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 at 6 μ (microns) thickness and stained with Ehrlich haematoxylin and Eosin (dissolved in 70% alcohol) (Humason, 1972) and were mounted in *Canada balsam*.

RESULTS AND DISCUSSION

Pathology of Gill tissue under thiodicarb (Larvin 75% WP) toxicity:

Thiodicarb has induced marked pathological changes in fish gills. The changes include the bulging of tips of primary gill filaments. The secondary gill filaments lost their original shape and curling of secondary gill filaments was also observed. The pillar cell nucleus showed necrosis and development of vacuoles in the secondary gill epithelium. There is a tendency of fusion of disorganized secondary gill filaments (Plate.1.).

The damage of gills of fish exposed to the higher concentrations (lethal doses) was severe. Shortened and clubbing ends of the secondary gill lamellae, a 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 (Plate. I).

The epithelial layer of secondary lamellae of a gill of fish forms a barrier between the fish blood and surrounding water. Gaseous exchange needed to sustain life takes place through this barrier and any thickening induced by physical, chemical or biological agents hinders the respiratory function of this organ (Eller, 1971).

In fish, gill is the first organ to which the pollutant comes into contact. Hence, it is more vulnerable to damage than any other tissue. The proliferative gill lesions are often observed after exposure of fish to water-soluble toxicants.

Eller (1971) described endrin-induced histopathological changes in cut-throat trout and reviewed the gill lesions in freshwater teleosts. Couch (1975) reviewed the histopathological effects of pesticides and related chemicals on the livers of fish and concluded that many of them were non-specific. No characteristic trends of pathological changes for any class of pesticides were seen. On the other hand, examining the spots that were exposed to toxaphene, the control, and treated fish may be identified on the basis of gill changes alone (Lowe., 1966). The lamellae were slender and delicate in the controls and clubbed and distally thickened in the exposed fish the changes observed was severed necrosis cloudy swelling and granular vacuoles (Tilak *et al*., 2007).

A number of pathological changes have been reported in fishes exposed to different organochlorine and organophosphate pesticides (Lowe, 1966; Konar, 1970; Natarajan, 1981; Jayantha Rao, 1982; Jayantha Rao *et al*., 1985 Girija, 1987; Rama Murthy, 1988; Anita Susan, 1994; Vijaya Lakshmi, 1996; Yacobu, 1999; Ramana Kumari, 1999; Veeraiah, 2001; Tilak *et al* ., 2001a; Tilak *et al.,* 2001b; Tilak *et al.,* 2002; Tilak *et al*., 2005; Tilak *et al*., 2007; Venkatesam and Subramaian, 2007).

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 (Velmurugan, 2007; Sakr and Jamal Al lail, 2005; Tilak *et al*., 2001b; Veeraiah, 2002; Tilak *et al*., 2001a; Yacobu, 1999; Ramana Kumari, 1999; Vijayalakshmi, 1996). The nutritional gill disease consists of lamellar epithelial hyperplasia with an eventual fusion of secondary lamellae near the tips of gill filaments (Cowey and Roberts, 1978). The biological function of the inflammatory response is to distroy "WALL OFF" irritating substances so that damaged tissue may be healed. A number of pathological changes have been reported in fish exposed to different organochlorine and organophosphorus and synthetic pyrethroid compounds. Exposure of 'Sockeye' salmon fry to the butoxy ethyl ester of 2, 4-D for 96 hrs (1 mg/l) resulted in hypertrophy and hyperplasia of the epithelial cells of the gills Eller (1971) described endrin induced histopathological changes in cutthroat trout and reviewed the gill lesions in freshwater teleosts.

Baticodos *et al*., (1991) reported slight hyperplasia of gill epithelium in pinaeus monodon exposed to gusathion, a commonly used organophosphate. Inflammatory alterations of lamellar epithelium and hyperplasia were reported in the gills of freshwater major carp *Cirrhinus mrigala* (Hamilton) during 48 h exposure to a sub-lethal dose of Malathion (Roy and Datta, 1991). Edema with the lifting of lamellar epithelium and hyperplasia of lamellar epithelium were observed in the gills of all cat fish containing residues of endosulfan (Nowak and Barbara, 1992). Similar findings were noted in the gills of rainbow trout on exposure to zinc sulphate by Karuppasamy. (2000). Sakar. *et al.,*(2005)., Tilak. *et al*.,(2005). Sunitha and Sahai (1983) reported swellings of inflammation in almost all the respiratory lamellae of gills of *Rasbora daniconius* on exposure to HCH.

PLATE - I (GILL)

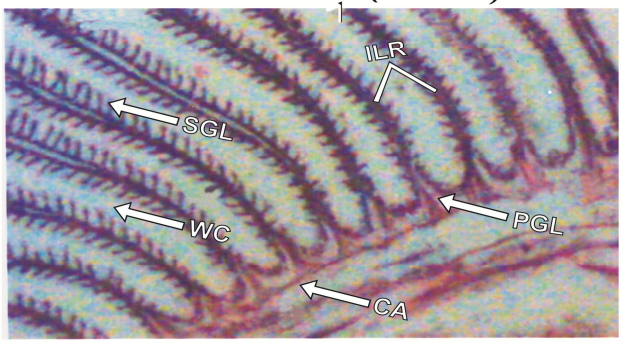

FIG-A
(CONTROL)

PGL - Primary Gill Lamella
SGL - Secondary Gill Lamella
CA - Central Axis
ILR - Inter Lamellar Region
WC - Water Channel

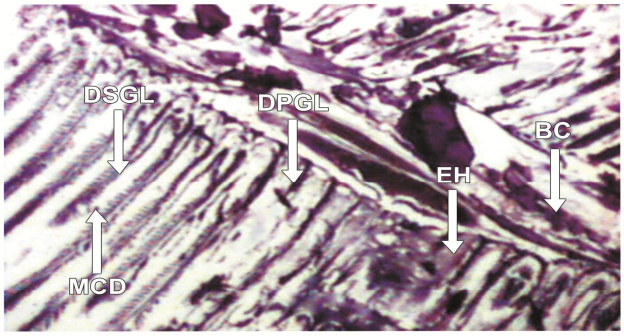

FIG-B
(SUBLETHAL)

DPGL - Degenerated Primary Gill Lamella
DSGL - Degenerated Secondary Gill Lamella
MCD - Marginal Channel Dilation
EH - Epithelial Hyperplasia
BC - Blood Congestion

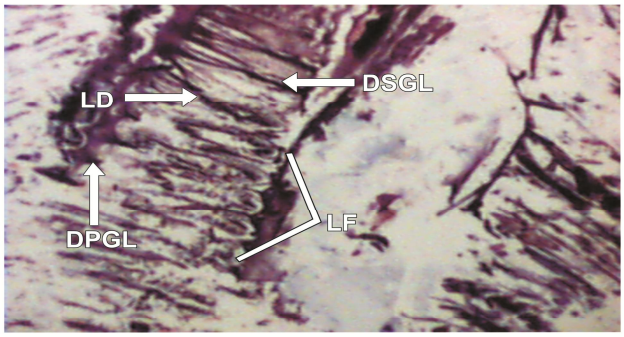

FIG-C
(LETHAL)

DPGL - Degenerated Primary Gill Lamella
DSGL - Degenerated Secondary Gill Lamella
LD - Lamellar Disorganization
LF - Lamellar Fusion

Plate.1
Fig. A. Control: Normal Gill lamella of *Labeo rohita* after 96h.
PGL: Primary gill lamella
ILR: Inter Lamellar Region
SGL: Secondary gill lamella
WC: Water Channel
CA: Central axis
Fig. B. Sub-lethal: Normal Gill lamella of *Labeo rohita* exposed to a Sub-lethal concentration of thiodicarb for 96h.
DPGL : Degenerated Primary gill lamella

EH: Epithelial Hyperplasia
MCD: Marginal Cell Dilation
BC: Blood Congestion
DGSL: Degenerated Secondary lamella
Fig. C. Lethal: Normal Gill lamella of *Labeo rohita* exposed to a lethal concentration of thiodicarb for 96h.
DPGL: Degenerated Primary Gill Lamella
DGSL: Degenerated Secondary Gill lamella
LD: Lamellar Disorganization
LF: Lamellar Fusion

Anitha Kumari and Shree Ram Kumar (1997) observed decreased carbohydrate activity in the secondary lamellae and also in the respiratory epithelium of the freshwater teleost *Channa punctatus* under exposure to the polluted water of Hussain Sagar and states that the degeneration of respiratory epithelium and damages of gill tissue causes a decrease in energy metabolism. Similar changes were observed in rainbow trout exposed to sub-lethal concentrations of monocrotophos (Vijayalakshmi, 1994) to fenvalerate in *Labeo rohita* by Tilak *et al*., (2001a, 2001b) to cypermethrin in *Labeo rohita* (Veeraiah, 2001), Copper Sulphate in *Oreochromis mossambicus* Vekatesam and Subramaian.,(2007). Butachlor and Machete in *Channa punctatus* (Tilak *et. al.,* 2007).

Pathology of Liver tissue under thiodicarb 75% wp toxicity:

Thiodicarb has induced discrete pathological changes in the liver tissue of the fish *Labeo rohita*. These changes include degeneration of cytoplasm in hepatocytes, atrophy, the formation of vacuoles, a rupture in blood vessels, necrosis and disappearance of hepatocytic cell wall and disposition of hepatic cords (Plate.II). Liver, the first organ to face any foreign molecule through portal circulation is subjected to more damage (Jayantha Rao, 1982). The liver is an important organ of detoxification which breaks down toxic substances and metabolites of administered substances. This breakdown is carried out by an endoplasmic reticulum of hepatocytes. Due to these reasons, the hepatic cells are damaged severely.

Jayantha Rao, (1982) reported that in fish *Ophiocephalus punctatus* exposed to the toxicant resulted in vacuolation and necrosis in the liver.

Dubale and Shah (1979) reported that *Channa punctatus* under Malathion toxicity showed the degenerative changes in the liver. Rashatwar and Ilysas (1984) reported that in teleost fish *Nemachelius denesoni* (Day) exposure to phosphamidon caused highly vacuolated and claudy swelling and even the connective tissue was damaged in the liver. Ansari *et al*., (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 concentration of Malathion.

A few other reports are available which deal with the other than carbomate pesticides' effect on histology. Cruz and pilago (1989) reported that formalene treatment caused cloudy swelling, haemorrhage, deposition of pigments and necrosis in the liver of milk fish Changes fingerlings. Radhaiah and Jayantha Rao (1992) reported moderate cytoplasmic degeneration in hepatocytes, the formation of vacuoles, a rupture in blood vessels and appearance of blood cells among hepatocytes, the formation of vacuoles, picnotic nuclei in the liver of *T. 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.

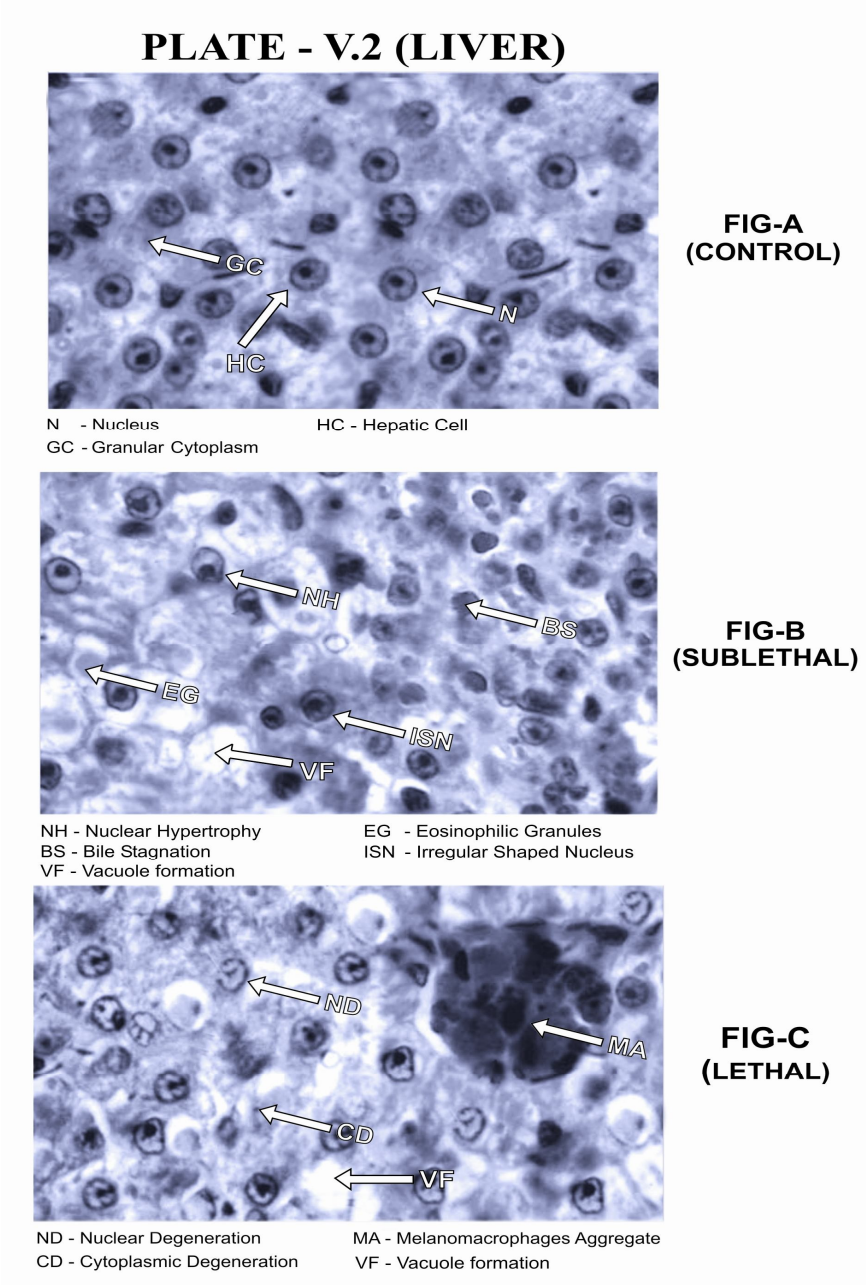

Plate.II.

Fig. A. Control: Normal structure of Liver in *Labeo rohita* after 96h.

N: Nucleus

HC: Hepatic Cell

GC: Granular Cytoplasm

Fig. B. Sub-lethal: Normal structure of Liver in *Labeo rohita,* exposed to a Sub-lethal concentration of thiodicarb for 96h.

NH: Nuclear Hypertrophy

BS: Bile Stagnation

VF: Vacuole Formation

EG: Eosinophilic Granules

ISN: Irregular Shaped Nucleus

Fig. C. Lethal: Normal Structural of Liver in *Labeo rohita* exposed to a lethal concentration of thiodicarb for 96 h.
ND: Nuclear Degeneration
CD: Cytoplasmic Degeneration
MA: Melanomacrophases Aggregate
VF: Vacuole Formation

Vijayalakshmi (1994) 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 drastically decrease in the hepatic cells of the freshwater teleost upon exposure to polluted waters of Hussain Sagar lake. Tilak *et al*., 2001a, 2001b reported the same degenerative changes in *Labeo rohita* and *Ctenopharyngodon idellus* under chlorpyrifos and fenvalerate toxicities. Tilak et. al. (2005) *Catla catla* under Chlorpyrifos toxicities. Tilak. et. al.,(2007) *Channa punctatus* exposed to a sub-lethal concentration of Butachlor. In the present study, thiodicarb has induced discrete pathological changes in the liver tissues of all the fish *Labeo rohita* the pathological changes noticed in the liver might effect the physiological activity of the fish such as reduction in enzyme synthesis (Sastry and Sharma, 1979; Rashatwar and Ilyas, 1984). This reduces the functional ability of liver which indirectly effects all metabolic activities of the organism.

The degenerative changes are intensified in lethal exposures. They include degeneration of cytoplasm in hepatocytes, atrophy, the formation of vacuoles, a rupture in blood vessels, necrosis and disappearance of hepatocyte wall disposition of hepatic cords decrease in size of nucleus pyknotic and vacuolar degeneration within the nucleus was evident (Plate.III).

Pathology of Kidney tissue under thiodicarb 75% wp toxicity:

Renal tissues of the fish *Labeo rohita* under thiodicarb toxicity evidenced marked pathological changes. Highly degenerative changes were observed in haemopoietic tissue which includes severe necrosis, cloudy swelling in renal tubules, cellular hypertrophy and granular cytoplasm. The epithelial cells of the distal convoluted tubule decreased in size. The interstitial renal tissue was less effected. Renal interstitial tissue showed the formation of vacuoles and cellular contours were not clearly distinguished (Plate.III).

From the body of fish, the waste products are eliminated through the kidney. The non-detoxified pesticide molecules must be eliminated by the kidney of fish and hence, it is susceptible to chemical compounds when exposed to lethal or sub-lethal dose of thiodicarb while it was eliminated through it, kidney might have caused degenerative changes in renal tubule and glomeruli, i.e. necrosis in the proximal tubules with development of vacuoles (Plate.III).

Srivatsava and Srivatsava (1990) reported the bursting of glomeruli and tubules and degeneration of cellular boundaries and clumping of glomeruli at some places in the kidney of *Cirrhinus mrigala* exposed to urea. Cloudy swelling of renal tubule, marked loss of haemopoitic tissue, shrinkage of glomeruli were reported in *Namachelius denisoni* (Day) exposed to phosphamidon (Rashatwar and Ilyas, 1984). In monocrotophos treated mice thickening of glomerular basement membrane, tubular degeneration and compensatory diletion and the fecal collection of chronic inflammatory cells in the interstitial tissue were reported by Malaya Gupta *et al*., (1988).

Degenerative changes in epithelial cells of proximal tubules and haemopoitic 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). Cytological break down of glomeruli was reported in kidneys of the stickle back *Gasterosteus aculeatus* exposed to dissolved cadmium (Oronsaye, 1989). Anitha Kumari and Shriram Kumar (1997) observed the mild activity of carbohydrates in the

cytoplasm, nuclei and the luminar border of the proximal and distal tubules in the kidney of the fresh water 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; Malaya Guptha *et al*., 1988; Ramana Kumari, 1999; Yacob, 1999; Tilak *et al*., 2001a, Tilak *et al*., 2001b and Tilak *et al*., 2002) who observed renal damage, rupture in the glomeruli and reduced renal tubules and its lumen in *Channa punctatus* exposed to Dabb. EI-Zalabani and Soliman (1981) and Feng *et al*., (1982) also reported necrosis in the renal epithelium, swelling of mitochondria in the renal tubules in animals administered with methothrin and pyrethrin respectively. Such sort of pathological conditions causing dis-function of kidney tissue has been reported under pesticide toxicity by Radhaiah (1985and1988); Ramamurthy (1988); Tilak., *et al*.,*(*2005.,2007), Venkatesam. (2007).
Pesticides, in general, allowed an expansion of agricultural activities in order to supply the increasing world population with food, mainly after World War II. However, a superficial knowledge of the toxicity of these products leads to an exaggerated use, resulting in uncontrolled environmental contamination. The pollutants affect food chains in different environments and also humans, not only due to the ingestion of contaminated food but also by the inadequate handling of the toxic products.

Thiodicarb is a carbamate insecticide and pesticide that consists of two methomyl groups linked by amino nitrogen through sulfur molecules. The physical characteristics of this crystalline powder include a color ranging from white to tan and a faint sulfurous odor. While this carbamate is relatively stable in light and ambient conditions, it does degrade relatively easily at temperatures over 100 degrees Celsius (212 degrees Fahrenheit) into several byproducts, including dimethyl disulfide and carbon dioxide. The main byproduct of thiodicarb decomposition, however, is methomyl, which is achieved through hydrolysis catalyzed by acidic or alkaline conditions.

The chemical name for thiodicarb is 3,7,9,13-tetramethyl-5,11-dioxa-2,8,14-trithia-4,7,9,12-tetraazapentadeca-3,12-diene-6,10-dione. However, thiodicarb is also known by several alternate common names, including bismethomyl thioether, carbamic acid and UC 51762, among others. It is also sold under the trade name of Larvin.

Thiodicarb is commonly used to protect agricultural crops from Lepidopterous pests, such as Beet armyworm, Corn earworm, and Black cutworm. As an insecticide, thiodicarb is effective against eggs as well as larvae, although the latter must feed upon treated foliage in order to be controlled. Heavy infestations of larvae may require higher applications than the standard dose, but not to exceed 60 ounces (1.77 liters) per acre (4047 square meters) per season.

Thiodicarb is formulated to include several liquid products and one powdered product that must be mixed with water before using. Application to agricultural crops may take place on the ground or from the air. Which crops are treated with thiodicarb depends on how this agent is registered with each state. For instance, in Florida, thiodicarb is registered as one of the insecticides that may be applied to sweet corn. In California, thiodicarb may be used on head lettuce, leaf lettuce, spinach, corn, and celery, as well as on cotton.

Thiodicarb is considered moderately toxic. Animal-based studies indicate that the metabolic byproduct of thiodicarb, a potential carcinogen known as acetamide, is eventually formed by the breakdown of methomyl in the stomach. However, this substance is further metabolized and excreted as carbon dioxide through respiration and urination. Since toxicity tests have only been conducted on animals, the long-term impact of carbamate pesticides on human health is still largely unknown.

PLATE - III (KIDNEY)

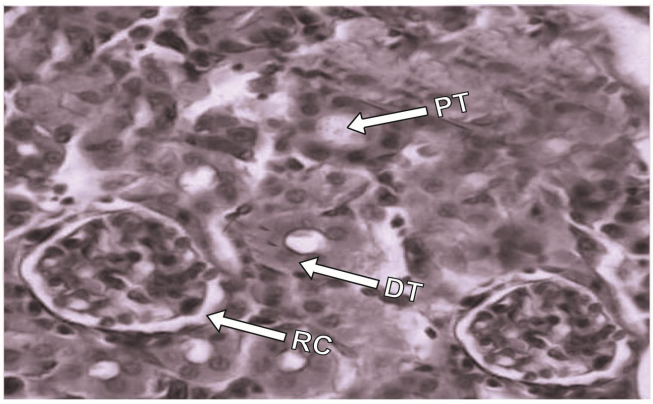

FIG-A
(CONTROL)

RC - Renal Corpuscle(showing glomerulus & bowman's space)
PT - Proximal Tubule
DT - Distal Tubule

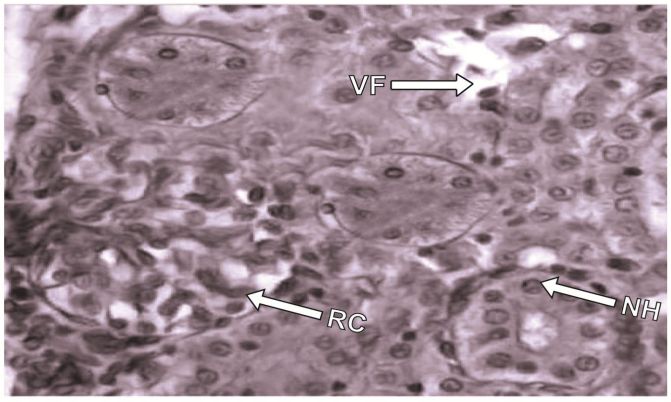

FIG-B
(SUBLETHAL)

RC - Renal Corpuscle (showing glomerular expansion & absence of bowman's space)
NH - Nuclear Hypertrophy VF - Vacuole formation

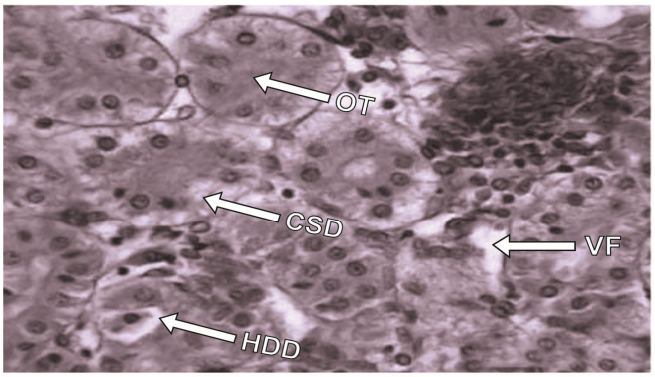

FIG-C
(LETHAL)

OT - Occlusion of Tubular lumen HDD - Hyaline Droplets Degeneration
CSD - Cloudy Swelling Degeneration VF - Vacuole formation

Plate. III.
Fig. A. Control: Normal structure of Kidney in *Labeo rohita* after 96h.
RC: Renal Corpuscle (showing glomerulus & bowmen's space)
PT: Proximal Tubule
DT; Distal Tubule
Fig. B. Sub-lethal: Structure of Kidney in *Labeo rohita,* exposed to a Sub-lethal concentration of thiodicarb for 96 h.

RC: Renal corpuscle (showing glomerular expansion & absence of bowman's space)
NH: Nuclear Hypertrophy
VF: Vacuole Formation
Fig. C. Lethal: Structure of Kidney in *Labeo rohita* exposed to a lethal concentration of thiodicarb for 96 h.
OT: Occlusion of Tubular lumen
CSD: Cloudy Swelling Degeneration
HDD: Hyaline Droplets Degeneration
VF: Vacuole Formation

Carbamates such as thiodicarb are cholinesterase-inhibiting, meaning that they disrupt the action of certain enzymes involved in central nervous system functioning. Symptoms of thiodicarb toxicity include increased salivation, headache, muscle weakness, dizziness, nausea, vomiting, abdominal cramping, and profuse sweating. In the environment, decomposition of thiodicarb is dependent on sufficient aeration, microbial activity, temperature, and soil density and pH. With a half-life of less than seven days, thiodicarb does is not expected to accumulate in the environment or contaminate groundwater.

Methomyl is a water-soluble crystalline solid that gives off a sulfurous odor. Highly toxic, it is classified as a carbamate insecticide that is designated as being a *Restricted-use Pesticide* (RUP) by the U.S. Environmental Protection Agency (EPA). Since the late 1960s, the substance has been used as a pesticide on commercial fruit and vegetable crops as well as stored products. Its application as an insecticide is highly effective against a wide variety of pests, particularly those that are resistant to organophosphorus pesticides.

Initially, Dupont registered methomyl with the EPA for use as an insecticide for commercially grown chrysanthemums in 1968. However, its use soon gained favor to help protect livestock and even commercial real estate from pests. Since that time, it has been used to treat almost all commercial production of lettuce, rhubarb, asparagus, artichokes and pomegranates in the U.S.

While there have been very few clinical studies conducted to determine the potential adverse effects of methomyl on human health, it is reasonable to assume that information collected from animal-based models can be extrapolated to people. For this reason, modifications on the regulated usage of methomyl have taken place since the mid-1990s. For example, it is no longer to be used in greenhouses or as an additive to fly bait. In addition, it is now required that a bitter agent is added to preparations to discourage children from accidentally ingesting them.

Methomyl is readily absorbed through the skin and by inhalation of fine particles. Its mechanism of action is to inhibit cholinesterase, an enzyme produced in the liver that regulates nervous system functioning. The telltale symptoms of toxicity by this route are uncontrolled muscle movements, spasms, convulsions, etc. The insecticide is also absorbed through the intestinal tract. In fact, ingestion of this substance equates to a fast-acting poison in both humans and animals. However, if the ingested dose is not too high and action is taken quickly, poisoning may be counterchecked by one or more injections of atropine. Metabolism of methomyl occurs by hydrolysis before it degrades into the byproducts carbon dioxide and acetonitrile. Thus, when fish is exposed to Sub-lethal and lethal concentrations of thiodicarb pesticide for 96h, 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.

CONCLUSION

The histopathological changes in certain vital tissues like gill, liver, and kidney in the fish *Labeo rohita* exposed to sub-lethal and lethal concentrations of Thiodicarb were studied. Thiodicarb 75% WP caused marked pathological changes in the gill than liver and kidney. They include progressive degeneration, bulging of tips of primary gill lamellae, club-shaped secondary gill lamellae and other severe necrotic changes in the epithelial cells of secondary gill lamellae. Thiodicarb 75% WP also caused profound pathological changes under chronic exposures in liver tissues of the test fish such as degeneration of cytoplasm in hepatocytes, atrophy, formation of vacuoles, necrosis, disappearance of hepatocytic wall, disintegration of lattice fibres and appearance of blood streaks among hepatocytes. All these changes indicate the hepato-toxic nature of the Thiodicarb 75% WP. Since carbamate pesticides are neuropoisons, the Thiodicarb 75% WP intoxication caused atrophy, chromatolysis, i.e., dissolution of the nissel bodies and loss of stainable substances within the cytoplasm was noticed. Renal excretion is one of the ways of eliminating the non-detoxified toxicant molecule resulting in severe pathological changes in haemopoietic tissue, severe necrosis; cloudy swelling of renal tubules, disintegration of interstitial tissue, pyknotic nucleic, etc were also noticed.

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