Histopathological Effects Of Black Molly Fish *Poecilia latipinna* Exposed By Trifluralin.

Dr. Mukhtar Khamis Haba
Department of biology/College of science for women/University of Baghdad.

Abstract

Sublethal concentrations of herbicide trifluralin were investigated with black molly fish *Poecilia latipinna*. Fish were exposed to (0.09,0.18,0.28 trifluralin m/L) for 30 days toxicity test. Trifluralin caused many histopathological effects in gill, liver and kidney. Gill disorders include hypertrophy of chloride cells, hyperplasia, lifting of epithelial cells and fusion of secondary lamellae. Liver suffered from herbicide as swelling of hepatocytes, infiltration of leukocytes, necrosis and fibrosis. Kidney also affected by trifluralin,Degeneration, hypertrophied cells and narrowing of the tubular lumen, shrinkage of the glomerulus, hyperplasia, hyalinization, Atrophied of tubular cells with Oedema.

Introduction

Herbicides are generally less toxic to fish and aquatic life than insecticides, many are short-lived and do not accumulate in the environment , however, some are highly toxic to aquatic animals and should be avoided or used with extreme caution near water ways and aquatic environment. Trifluralin is toxic to fish and other aquatic organisms(WSSA,1989).many studies were done on different species of fresh water fishes to determine the toxicity of trifluralin on fish by estimating the LC50 (lethal concentration for half number of fish),such as The ranges of 96-h LC50 values were 10–330 µg×L1 for rainbow trout *(O. mykiss)*, 8.4–400 µg×L1for bluegill sunfish *(Lepomis macrochirus)*, 105–160 µg×L1for fathead minnows *(Pimephales promelas)*, and 210–2200 µg×L1for channel catfish *(Ictalurus punctatus)* (Mayer and Ellersieck1986). (0.036–0.051)mg/L for carp (Poleksi and Karan,1999).

Tissue organisms exposed to a sub-lethal concentrations of toxicants are functional response of organisms which provides information on the nature of toxicant (Das and Mokherjee,2000) . Wide varieties of insecticides and other toxins tend to accumulate in high concentrations with it (Metelev *et al*.,1971).Du to residual effects of pesticides ,important organs like the kidney,liver,gill are damaged(Rahman *et al*.,2002). Poleksive and Karan, 1999 noted sever histological changes in the gills and kidney of carp fish affected by trifluralin . The study reported here includes observations on the pathology conditions associated with trifluralin exposure in black molly *Poecilia latipinna*, we determined the histological changes in gill, liver and kidney within microscoping examination. Many authors recorded and observed histological abnormalities in gills, liver and kidney for fishes contaminated by pesticides.(Visoottiseth *et al*.,1999; Das &Mukherjee ,2000; Macelo *et al*.,2002; Velmurugan *et al*.,2007).

Material and methods

Black molly fish *Poecilia latipinna* selected for this study because it is small, available and suitable for histopathological studies on the cellular level.Fishes bought from Alghazel market. fish acclimatized for two weeks in aquariums (60 *30*30 cm),average length (4+0.5 cm).48 fishes divided in to four groups ,each 12 1n aquarium (3L capacity).first group for control ,the others for toxicity test. fish exposed to (0.009,0.018,0.028 mg/L) FOR 30 day as a period of exposure. Fish were fed by commercial tropical food once a day. Water exchange every couple of days intervals with fresh test solutions in each experimental aquarium with well aerated. After 30 days test fish were killed for histopathological study. Histological

preparations have been done according to (Bancroft and Steven, 1982). Organ tissues fixed in 10% formalin, were prepared for paraffin blocks (58-60c) and sectioned at 6Mm.Stained sections examined with Olympus compound binocular microscope (LH)fitted with photomicrographic attachment.

Result and Discussion

Histopathological changes have been widely used as biomarkers in the evaluation of the health 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 advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs including gills, kidney and liver that are responsible for vital infections, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish(Gernhofer *et al*.,2001).Furthermore the alternations found in these organs are normally easier to identify than functional ones(Fanta *et al*.,2003),and sever as warning signs of damage to animal health (Hinton &lauren,1990) Absorbed toxicant transported by blood circulation to liver for transformation and / or storage, and if it 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). Trifluralin caused many alterations in the tested organs gills, liver and kidney of black olly fish. Gill in control group consist of primary and secondary lamellae ,arranged parallel ,lined by epithelial tissue.Trifluralin caused hypertrophy of chloride cells(epithelial cells found between each secondary lamellae) in the gill exposed with 0.009 mg/L fig (1),while the fish exposed with 0.018 mg/L,gills suffered from lifting of the epithelial cells and oedema fig (2). Hyperplasia of the epithelial cells were occurred and caused fusion of secondary lamellae, were noted in the gills exposed with 0.028 mg/L fig (3).

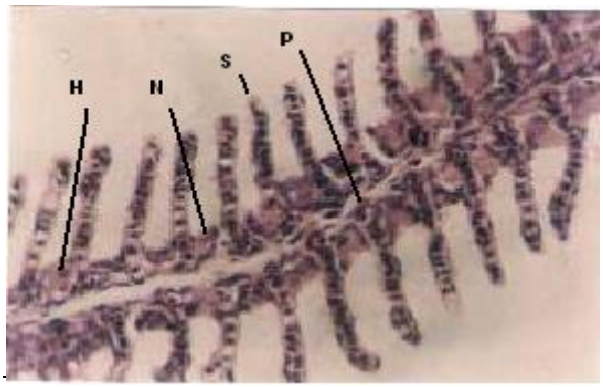
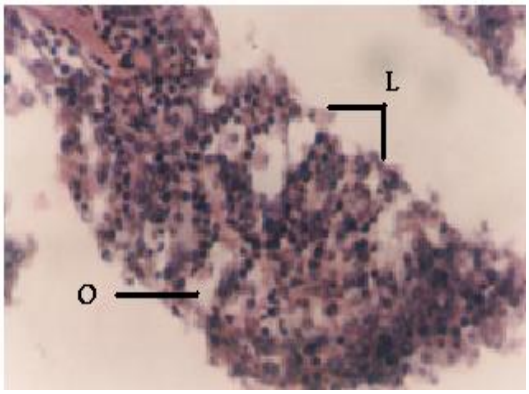


Fig 1 : Showed Hypertrophy (H) of the chloride cells in the gill exposed to Trifluralin 0.009 mg/L
N=Normal chloride cell, P= Primary lamella,
S= Secondary lamella.400X.



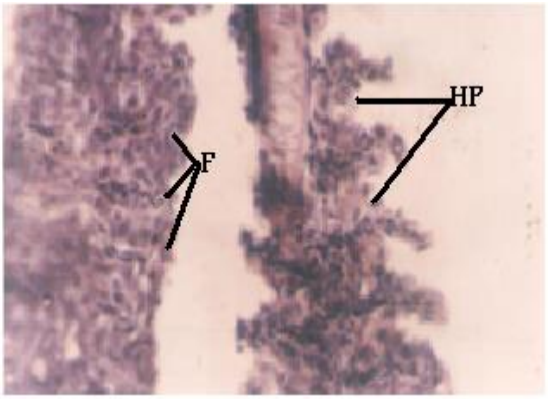
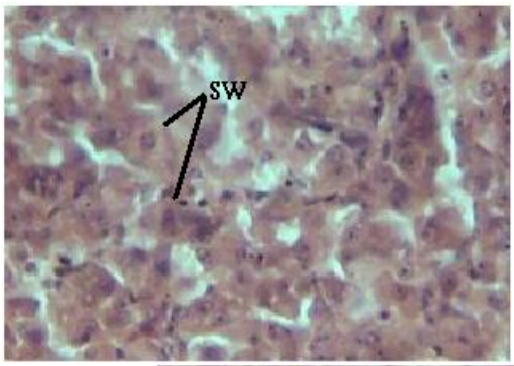


Fig 2: Showed lifting of the epithelial cells (L) and Oedema (O) in gills exposed by Trifluralin 0.018 mg/L.400X.

Fig 3: Showed Hyperplasia (HP),and Fusion of secondary lamellae (F) in gill exposed Ttifluralin 0.018 mg/L.400X.

Because gills come in immediate contact with the environment ,tissue damages brought about by waterborne pollutants can be easily observed, (Dutta *et al*.,1993.).

Alteration like epithelial lifting, hyperplasia, hypertrophy of the epithelial cell, fusion of some secondary lamellae are examples of defense mechanisms, in general, these result in the in increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants, as a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired (Fernandes&Mazon, 2003).Winkaler et al.,2001 mention there is a hypothesis around hyperplasia and hypertrophy is result of contaminated and exposure water make structural damage to the fish gill. In the other hand mention the hyperplasia in some situation represents adaptations by the organism to protect underlying tissues from any irritant (Meissner &Diamandopoulous, 1977). Liver in fish surrounded by connective tissue called capsule, inside it there is a central vein and parenchyma which consist of hepatocytes arranged as two rows leaving capillaries between them called sinusoids lining with un complete epithelial cells . Swelling of hepatocytes observed in the liver tissue as a result of trifluralin effect 0.009 mg/L fig (4). Infiltration of white cells found in the liver exposed for trifluralin 0.018 mg/L fig (5). Every toxic agent would produce vaculation and accumulation of glycogen in hepatocyte cytoplasm was the predominant findings. Glycogen accumulation seems to be more prominent in the earlier period of the experiment whereas lipid accumulation predominated in longer-duration exposures (Visoottiseth *et al*., 1999.)



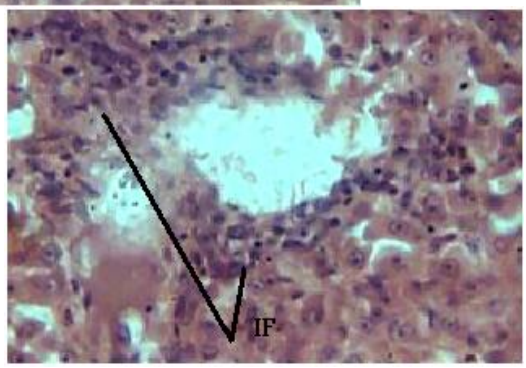
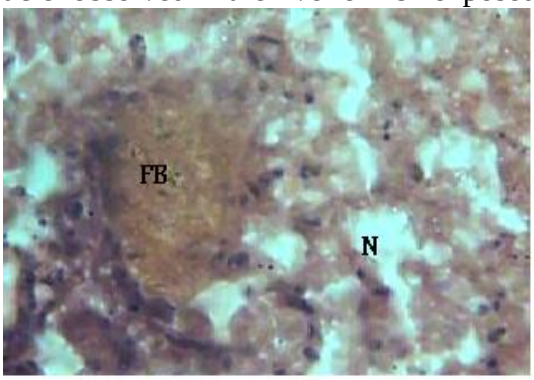


Fig 4:Showed swelling of hepatocytes (SW). (0.009 mg/L). 400X.

Fig 5: Showed infiltration of white blood cells (IF) within liver tissue.(0.018 mg/L).400X.

Severe lesions such as fibrosis and necrosis (pyknotic , karyohexis and karyolysis nuclei observed in the liver of fish exposed with 0.028mg/L fig (6-7).



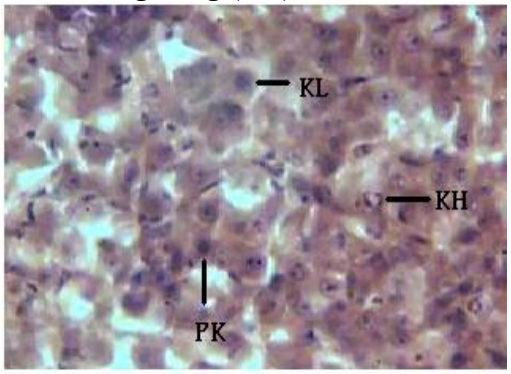


Fig 6: Showed Fibrosis (FB)and Necrosis(N) in the liver tissue.(0.028 mg/L). 400X.

Fig 7: Showed the stages of necrosis in (0.28 mg/L). Pyknotic (PK),Karyohexis(Kh) and Karyolysis (KL).(0.028 mg/L). 400X.

When the liver severely damaged by an exogenous toxin, most of features of frank necrosis, the dead cells have a homogenous eosinophilic cytoplasm when compare with living cells. this cytoplasmic appearance is due to loss of basophilic RNA from the damaged rough endoplasmic reticulum, disorganization of mitochondria and exposure of increase numbers of acidophilic groups from breakdown of structural protein, the nuclei of the dead cells are smaller,more darkly stained and less well defined which is known as pyknosis, is the end result of changes in which the nuclear chromatin becomes progressively clumped, possibly due to the low pH from anaerobic metabolism (Wheater *et al*.,1985). The large vacuoles in the hepatocytes, with pyknotic nuclei and necrosis of hepatocytes with enlarged sinusoid similar with (Olurin *et al*., 2006). Cortex and medulla, two main parts in fish kidney, cortex

includes nephrons (glomerulus and bowman's capsule) and renal tubules. Medulla has collecting tubules. Kidney surrounded by capsule.Histopathological changes in kidney were degeneration fig (8) and hypertrophy with pyknotic nuclei of the cells in the renal tubules as a result of 0.009mg/L trifluralin exposure fig (9) .

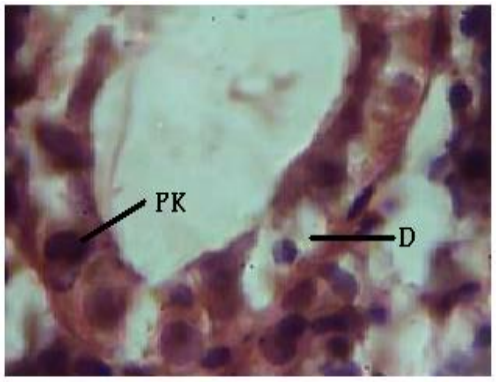


Fig 8: Showed Degeneration(D) and Pyknotic Pyknotic nucleus(PK)in the tubular cel Cells. (0.009 mg/L).1000X.

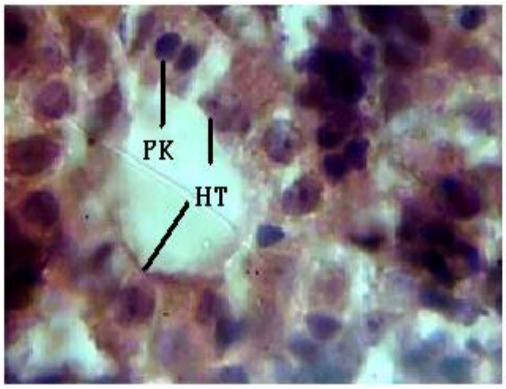


Fig 9: Showed Hypertrophy(HT) and nucleus (PK)in the tubular cells(0.009 mg/L). 1000X.

Trifluralin 0.18 mg/L caused shrinkage of glomerulus and increasing the space between capillaries and bowman's capsule fig (10) and hyperplasia in the epithelial of tubules fig(11),

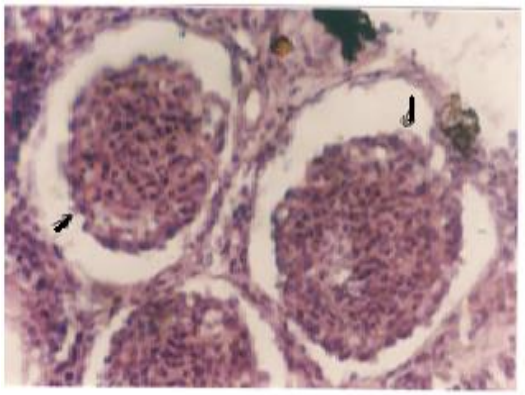


Fig 10: Showed Shrinkage of Glomerulus. (0.018 mg/L). 400X.

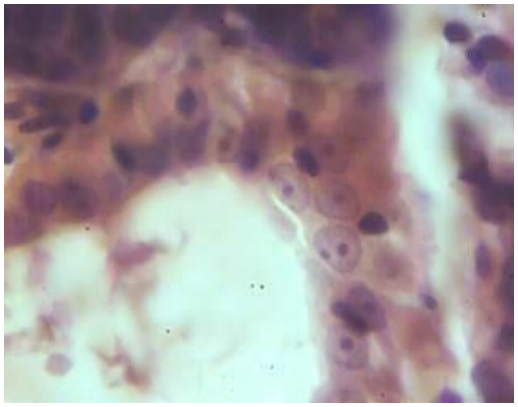


Fig 11: Showed Hyperplasia in the epithelial tubule.(0.018 mg/L).1000X.

Hickman C P and Trump B P.1969,Noted that the renal changes in visceral glomerular and tubular epithelial cells (particularly in the proximal tubules)in fish were the result of filtration and subsequent resorption and lysosomal degradation of macromolecules. kidney suffered from hyalinization ,hemorrhage fig (12),also atrophied of epithelial cells with oedema were observed with Trifluralin 0.028mg/L fig (13). Yildirim *et al*.2006, mentioned that hyaline droplets formation results from tubular reabsorption of plasma protein lost to the urine by glomerular damage.

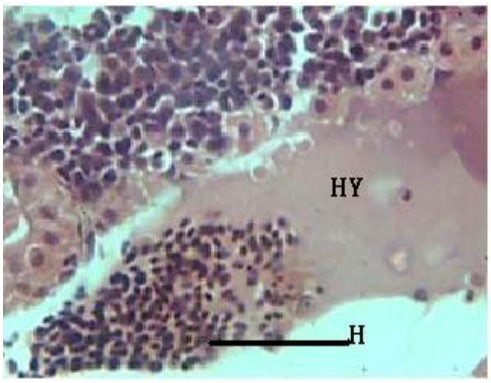


Fig 12: Showed Hyalinization (HY) and epithelial Hemorrhage (H) in Kidney. (0.028 mg/L).400X.

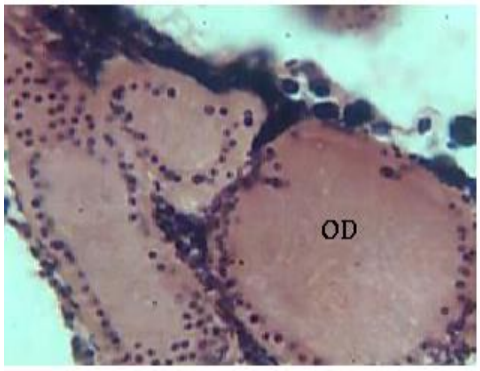


Fig 13: Showed Atrophied Cells with Oedema (O). (0.028 mg/L). 400X.

Most common alterations found in the kidney of fished exposed to the contaminated water are tubule degeneration (cloudy swelling and hyaline droplets) and changes in corpuscles , such as dilation of capillaries in the glomerulus and reduction of Bowman's space . (Thophon *et al*., 2003) .This alteration can be identified by the hypertrophy of the cells and the presence of small granules in the cytoplasm, this 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 the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Takashima & Hibiya, 1995). In more severe cases, the degenerative process can lead to tissue necrosis. 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.

References

Bancroft, J & Stevens, A. (1982). Theory & practice of histological technique. (2nd Ed). Churchill Livingston, London.

Das B K &Mukherjee S Ch.(2000)._A histological study of carp *Labio rohita* exposed to hexachlorocyclohexane ,VETERINARSKI ARHIV 70(4):169- 180.

Dutta H M;Richmonds C R and Zeno T-(1993) .Effect of diazinon on the gills of bluegill sunfish *Lepomis macrochirus* ,Journal of environmental pathology,Toxicologyand Oncology,12(4):219-227.FantaE.,Rios,F.S.,Romao,S.,Vianna,A.C.C.andFreiberger,S.(2003).

Histopathology of the fish *paleatus* contaminated with sublethal levels of organophosphorus in water and food .Ecotoxicology and Environmental Safely, 54:119-130

Fernandes ,M. N. and Mazon,A.F.(2003).Environmental pollution and fish gill morphology . In :Van ,A.L.&B.G.Kapoor(eds).Fish adaptation. Enfield, Science Publishers,203-231.

Gernhofer,M;M.Powet;M. Schramm;E.Muller&R.Triebskorn.(2001).

Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams.Journal of Aquatic Ecosystem,Stress and Recovery,**8**:241-260.

Hickman C P AND Trump B P.1969.The kidney .In ;Fish physiology ,Hoar WS.and Randall D J(eds).New York,Academic Press.New York.pp91-239.

Hinton,D,E and Lauren,D,J.(1990). Liver structural alterations accompanying chronic toxicity in fishes:potentioal biomarkers of exposure.pp 51-65 .In:

McCarthy,J.F.&L.R.Shugart(eds). Biomarkers of Environmental Contamination.Boca. Raton,Lewis publishers.(cited by Marina,et al., 2007)

Hinton,D.E;P.C.Baumann;G.R.Gardner;W.E.Hawkins;J.D.Hendricks;R.A.Murc helano & M.S.Okihiro.(1992).Histopathologic biomarkers In:Hugget.R;R.

Kimerle;P.Mehrle &H.Bergman(Eds).Biomarkers-biochemical,physiological and histological markers of anthropogenic stress.Boca Raton,Lewis Publishers,pp.155-195

Lindstoma-Seppa,P;V,Koivussri;D,O,Hanninen.(1981).Extrahepatic xenobiotic metabolism in north European freshwater fish. Comp. Biochem. Physiol. 69:291. Macelo L ; Rodreguez E ;Pacheco F J and Ranzani-Piva M J T.(2002)

Histopathologic changes in the kidney tissue of *Prochilodus lineatus*

Vaenciennes,1836 (Characiformes,Prochilodontidae) Induced by sublethal concentration of Trichlorfon Exposure.—.Brazilian Archives of Biology and Technology. **45**:171-175.
Meissner,M,A and Diamandopoulos,G,Th.1977.Neoplasia. In. Pathology (WAD Anderson,J M Kissane,Eds),**1**:640-691.

Meletev,V,V;A,S Kanaev and N,G,Dzasokhova.1971.Water toxicology. American Publishing Co.Pvt.Ltd.216.

Mayer,F,L and Ellersieck,M,R (1986). Manual of acute toxicity:Interpretation and database for 410 chemicals and 66 species of freshwater animals . U.S.Fish

Wild,Serv,Resour,Publ.160.U.S.Department of the Interior,Fish and Wildlife Service,Washington,DC.

Olurin ,K.B.,Olojo,E.A.A.,Mbaka,G.O. and Akindele,A.T.(2006).

Histopathological responses of the gill and liver tissues of *Clarias gariepinus* fingerlings to the herbicide, glyphosate. J of Biotechnology . **5**:(24) 2480-2487.

Poleksic,V and Karan,V.1999 .Effect of trifluralin on carp:biochemical and histological evaluation ,Ecotoxicology and Environmental Safety , **43**,(2), pp213-221.

Rahman m,z;Hossan z;Mollah m,f,a and Ahmed g,u.2002. Effect of diazinon on Anabas testudineus,Channa punctatus and Barbodes gonionotus. Naga,The ICLARM Quarterly,vol **25**(2): 8-12.

Schwaiger,J;RWanke;S,Adam;M,Pawert;W,Honnen & R,Triebskorn.1997.The use of histopathological indicator to evaluate contaminant-related stress in fish.Journal of

uatic Ecosystem,Stress and Recovery,**6**:75-86.

Takashima F and T Hibya.1995.An atlas of fish histology :normal and pathological features ,2nd ed.Tokyo,Kodansha.

Teh,S.J;S.M.Adams & D.E.Hinton.1997.Histopathological biomarkers in feral freshwater fish populations exposed to different types of contaminants stress.Aquatic Toxicology,**37**:51-70.

Thophon,S;M.Kruatrachue;E.S.Upathan;P.Pokethitiyook;S.Sahaphong;S.

Jarikhuan.2003.Histological alterations of white seabass Lates calcarifer in acute and subchronic cadmium exposure .Environmental Pollution, **121**:307-320.

Velmurugan B;Selvanayagam M;Cengiz E I and Unlu E.(2007). The effect of monocrotophos to different tissue of fresh water fish Cirrhinus mrigala. Bulletin of environmental contamination and Toxicology.**78**(6)pp450-454.

Visoottiseth P;Thamamaruitkun T;Sahaphong S;Riengrojpitak S and Kruatrachue M.(1999). Histopathological effects of triphenyltin hydroxide on liver ,kidney and gill of nile tilapia Oreochromis nilotica— Appl,Organometal, Chem.**13**,749-763.

Wester,P,W& J,H,Chanton.1991.The usefulness of histopathology in aquatic toxicity studies . Comparative Biochemistry and Physiology,**100**:115-117.

Wheater,P.R.;Burkitt, H.G.; Stevens, A. and Lowe, J.S. (1985). Basic 34-Histopathology. A colour Atlas and text. Foreword by Dawson, I.M., Churchill livingstone, Edinburgh, London Melbourne and NewYork.

Winkaler,E,U;A,G,Silva;H,C,Galindo & C,B,R,Martinez.(2001).Biomarcadores histologicos efisiologicos para o monitoramento da saude de peixes ribeiroes de Londrina,Estado do Parana.Acta Scientiarum,**23**:507-514.

WSSA Herbicide Handbook Committee. Herbicide Handbook of the Weed Science Society of America, 6th Ed. theWSSA, Champaign, IL. 1989.

Yildirim M Z ;Beni C K ;Selvi M;Ozkul A;Erkoc F and Kocak O. (2006). Acute toxicity,behavioral changes,and histopathological effects of deltamethrin on tissues (gill,liver,brain,spleen,kidney, muscle,skin)of nile tilapia Oreochromis niloticus L.fingerlings-Environmental Toxicology.**21**(6)pp 614- 620.