Research Article

Evaluation of Triazophos Induced Histopathology and Recovery in a Fish *Anabas testudineus*a.

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ABSTRACT

Histopathology is promising field for research in aquatic toxicology as it provides the real picture of the toxic effects of xenobiotics in vital functions of a living organism (Anees, 1976). The extent of histopathological damage inducing in the test and the amount of cell damage in relation to concentration of toxicants are utilized in assessing the toxicity of pollutants. It is generally evident that structural change are more serious than functional abnormalities, over more structural alternation is irreversible while altered function is considered as a reversible effect. The change in the structure at tissue, cellular and organelle levels can be correlated with the functional alterations.Couch (1975) stated that gill, liver, intestine and kidney of fish species are the best suited organs for histopathological investigations. The present study aims to evaluate the toxic effect of pesticides namely triazophosto fresh water fish Anabas testudineus. Fresh water fingerlings of Anabas testudineus of length 16cm±1cm and weight 72±1g were procured from the fish farm at kolathur, Tamilnadu, Southindia. The collected fish sample were acclimated to laboratory conditions in dechlorinated tap water for 15 days.Impact of Triazophos as mixed in fresh water at 5%, 10%, 15% and for a duration of 46hrs,96hrs recovery, have been assessed. The fish was fed with commercial fish food during acclimation. Susceptibility of the fish Anabas testudineusto the toxic effect of the organophosphorus, Triazophos, observed as percent mortality increased with an increase in concentration of triazophos. Mortality in controls was virtually absent, (Table 1) reveals the LC50 upper and lower confidence limits, and fitted regression equation along with slope function for 96 hrs exposure periods. The pesticide triazophos is found to be more toxic to the fish Anabas testudineus. The acute toxicity studies in Anabas testudineus 96 hr LC50 value for triazophosisis recorded as 0.270 ppm. The histological investigations in Anabas testudineus exposed to Triazophos were found to be highly toxic and the histological alterations were manifested with increase in concentration and duration

Key Words: Anabas testudineus, O,O-diethyl-O-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate (Triazophos).

INTRODUCTION

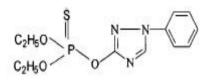
Aquatic ecosystem is the final sink for many chemicals used in industry and agriculture and has become a global problem (Sweet and Zelikoff, 2001; Ghoshet al., 2006). The continuous releases of these chemicals impair water quality and become unsuitable for aquatic organisms due to their persistence, bioaccumulation, toxicity and bio-magnification in the food chain (Diaz 2006; Palaniappan al., and Karthikeyan, et 2009).Aquatic vertebrates originated hundreds of millions of years ago and evolved in different directions (Jarvik, 1980; Bjerring, 1985). In spite of systematic diversity all fish species possess two main types of blood cells, erythrocytes (red cells) and leucocytes (white cells), a property shared by the landliving vertebrates as well, which are derived from early fish like ancestors. Triazophos is a newly introduced organophosphate pesticide and currently being used in a large number and the common name for O,O-diethyl-*O*-1-phenyl-1H-1,2,4-triazol-3-yl phosphorothioate. Triazophos is a broad-spectrum organophosphate insecticide and acaricide with nematicidal properties

(Coats, 1994). It is used on various crops such as cotton and rice to control aphids, fruit borers, leave hoppers and cutworms and is used in large quantities throughout the world. (Worthing and Hanree, 1991; Mingjinget al., 2003).

Histopathology is promising field for research in aquatic toxicology as it provides the real picture of the toxic effects of xenobioticson the histology and physiology of a living organism (Anees 1976).The change in the structure at tissue, cellular and organelle level can be correlated with the functional alterations. Couch (1975) stated that gill, liver, intestine and kidney of fishes are the best suited organs for histyopathological investigations.

Aim and objectives: The present study aims at evaluating the toxic effect of a pesticide namely triazophoson a fresh water fish *Anabas testudineus and to assay an* acute toxicity profile in *Anabastestudineus*.To assess the histological damage in *Anabastestudineus* exposed to Triazophos.

MATERIALS AND METHODS



3-(o,o-Diethyl)-1-phenyl thiophosphoryl-1,2,4-triazole

Test species - Fresh water fingerlings of *Anabastestudineus* of length $16\text{cm}\pm1\text{cm}$ and weight $72\pm1\text{g}$ were procured from the fish farm at kolathur, Tamilnadu,South India. The collected fishes were acclimated to laboratory conditions in dechlorinated tap water for 15 days. The fishes were fed with commercial fish feed during acclimation.

Water quality:During the period of acclimatization and experimentation the water used was clear, dechlorinated ground water pumped from a deep well within the college campus. The physiochemical characteristics of the water used throughout the experiments are as follows:

experiments are as rono ws.	
Total dissolved Solids mg/l	: 1598
рН	: 7.06
Alkalinity pH (as CaCO ₃) mg/l	: 0
Alkalinity Total (as CaCO ₃) mg/l	: 228
Total Hardness (as CaCO ₃) mg/l	: 520
Calcium (as Ca) mg/l	:124
Magnesium (as mg) mg/l	: 50
Iron (as Fe) mg/l	: 0.08
Free Ammonia (as NH ₃) mg/l	: 0.31
Nitrite (as NO ₂) mg/l	: 0.01
Nitrate (as NO ₃)mg/l	: 3
Chloride (as Cl) mg/l	: 549
Experimental Toxicant	

Triazophos: Triazophos [3-(o, o-Diethyl)-1-phenyl thiophosphoryl- 1,2,4-triazol], an organophosphate insecticide, is a broad spectrum insecticide and acaricide with some nematicidal properties as well and it is being used paddy in Himalaya. Singh *et al.*, (1996) reported that Triazophos residues were found in trace amounts in paddy grains, straw, and husk when Triazophos was applied at the rate of 0.25 and 0.50 kg a.i. ha⁻¹ after 45 days of transplanting.

Experimental Design: Acute Toxicity Test:Stock solution of triazophos is prepared, using glass distilled water and desired degree of concentrations was prepared. Based on the progressive bisection of intervals on a logarithmic scale, lethal concentrations were selected as experimental concentrations. These concentrations were fixed after conducting the range finding test (APHA, 1998). The LC₅₀ value for triazophos was determined by using the method of (APHA, 1998). The acclimated fish were stocked in 45 liters plastic through of dimensions 60×30 cm being equipped with a continuous air supply.

Feeding of fish was stopped 48h prior to the commencement of the experiment with a view to avoid any possible change *in situ* in the toxicity of pesticide.

After the addition of toxicant into the test tank with 10 litres of water having 20 fishes, mortality was recorded after 24, 48, 72 and 96. Six replicates were maintained simultaneously. Fish showing no respiratory movement and response to tactile stimuli were considered dead and removed immediately. Percent mortality was calculated and the values were transformed into probit scale. Probit analysis was carried out as per Finney (1971). Regression lines of probit against logarithmic transformation of concentrations were made. Slope function (S) and confidential limits (upper and lower) of regression the line with Chi-square test (UNEP/FAO/IAE, 1987) are calculated as follows. Experimental Study:

Sublethal exposure: The Experimental design and calculations for the acute toxicity were based on the procedure given. The acclimated test fishes were divided into four groups each containing 15 fishes. GroupI was used as control reared in toxic free water. The test fish were belonging to groupII was exposed to 0.027ppm. The group III fishes were exposed to 0.081ppm.The water was changed along with waste food and fecal materials periodically by slowly siphoning the water from each container. The containers were refilled and redosed with chemical toxicant daily.

Recovery studies: For recovery, after 3 days of pesticide exposure, the treated fish were transferred to clean tap water and kept for another 3 and 6 day for recovery.

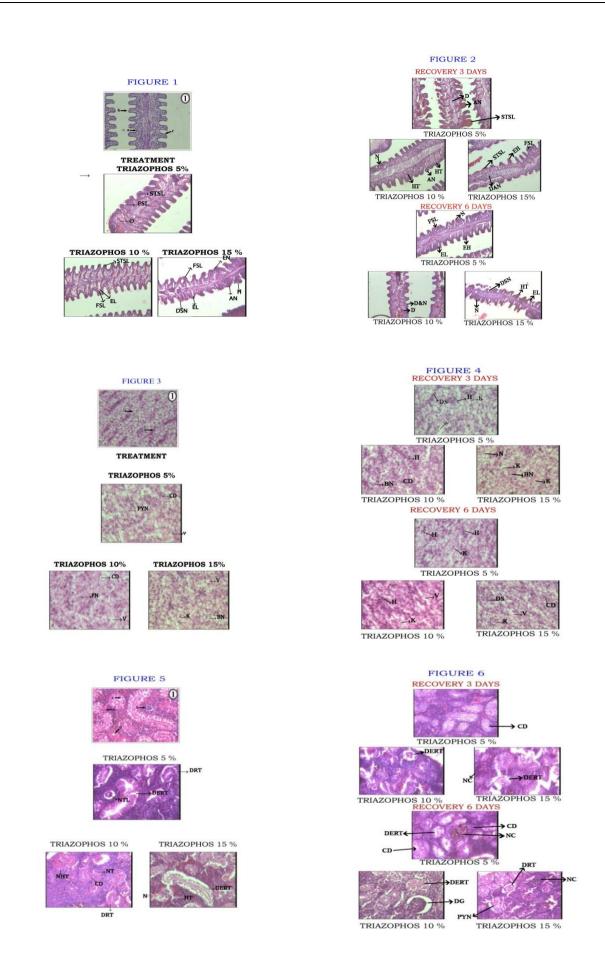
Tissue sample collection: Tissue samples from the triazophos and control fish were collected after 3 days of exposure and 3 and 6 days of depuration respectively. For each sampling 5 fish Samples were sacrificed from control and treated groups.

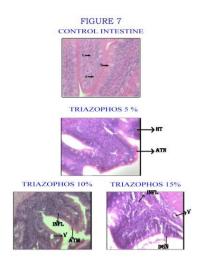
Histological Pursuit: After exposure to sub lethal concentrations the fish samples were dissected out and different tissues like gills, liver, intestine and kidney were fixed in buffered formalin for 24 hours and subjected to standard histological techniques.

RESULTS

Acute Toxicity: Acute Toxicity of Triazophosexposed *Anabastestudineus*

Susceptibility of the fish *Anabas testudineus* to the toxic effect of the organophosphorus, Triazophoswas observed in terms of percent mortality which and it is diversity proportioned to increase in concentration of triazophos. Mortality in controls was virtually absent and ascertains that the LC₅₀ upper and lower confidence limits, and fitted regression equation along with slope function for 96 hrs exposure periods. The LC₅₀ value was determined by following the method of Finney (1971). The 96-h LC₅₀ value of Triazophos in *Anabas testudineus* was found to be 0.270 ppm (95% confidence limit, 0.209 – 0.327ppm) in the present work. There was a reduction in lethal concentration 50 of triazophos to *Anabas testudineus* and Chi-square test





confirmed that all values were well fit at 0.05 probability levels.

Histological Manifestation

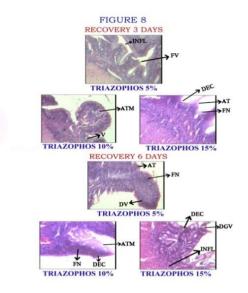
Control Gill: The histological details of the normal gill showed the epithelial lining of the gill, interlamellar region, respiratory lamella and supporting axis intact. Each gill arch showed parallel rows of elongated latterly projecting secondary gill filaments on either side of the primary lamellar. Primary gill lamella consisted of a central one core in which the cartilaginous skeletal rod and blood vessels were seen intact. Each secondary lamella was flattened structure composed of two continuous epithelial sheets separated by an array of pillar cells. The blood lacunae between adjacent pillar cells linked the branches of the afferent and efferent branchial arteries associated with each gill filament.

Gill treated

Anabas testudineus - exposed to triazophos 3 day treatment - gill

Shortening of secondary lamella, fusion of secondary lamellae, degeneration are observed in the present study in anabas testudineusexposed to sublethal concentration of 5% treatment.Anabas testudineusexposed to 10% of sublethal concentration of triazophos for 3 days shows histological alterations such as epithelial lifting and fusion of secondary lamellae.Fusion of secondary lamellae, epithelial necrosis, desquamation and necrosis, epithelial lifting, aneurism, hypertrophy, were observed in the present investigation in fish anabas testudineus exposed to 15% of sublethal concentration of triazophos for 3 days exposure.

Anabas testudineus exposed to triazophos – 3, 6 day recovery- gillanabas testudineus exposed to 5% of sublethal concentration of triazophos for 3, 6 days recovery showed pathological manifestative in the form of desquamation(D), aneurism, shortening of secondary lamella(STSL), necrosis(N), fusion of



secondary lamellae(FSL), epithelial lifting(EL), epithelial hyperplasianecrosise(EHN), hypertrophy(H), aneurism(a), desquamation and necrosis(D&N), oedemaa were observed in 10 % sublethal exposure of triazophos for 3, 6 days.Shortening of secondary lamella, epithelial hyperplasia, fusion of secondary lamellae, desquamation and necrosis, hypertrophy, epithelial lifting, necrosis were recorded in gill of anabas *testudineus*exposed to 15% sublethal concentration of triazophos.

Control Liver - Liver is an important organ of various metabolic process. The normal liver of a teleost fish is characterised by large polyhedral cells which are arranged as a minute network of canalculi; between the liver cells. The nuclei are vesicular with large nucleolus and irregular distribution of bite ducts.

Treated Liver (Anabas Testudineus Exposed To Triazophos3, 6 Day Treatment - Liver): Cloudy pycnotic nuclei, degeneration, vacuolization, wererecieved observed in Anabas testudineusexposed to 5% triazophos exposure. Anabas testudineus exposed to10% triazophos exposure disclosed histological alteration in liver tissues such as cloudy degeneration(CD), focal necrosis(FN), vacuolization(V)., karyolyis(K) and binucleated hepatocyte(BH)are observed in 15% sublethal concentration of triazophos exposure.

Anabas testudineus exposed to triazophos -3, 6 Day Recovery- Liver: Similarly dilation of sinusoid(DS), haemorrage(H), karyolysis(K), vacuolization(V)were the histological alterations observed in the 5% triazophos treated Anabas testudineusin 3, 6 days recovery.haemorrage(H). binucleated hepatocyte(BH). degeneration(CD). cloudv acuolization(V), karyolysis(K)were recorded in 10% triazophos treated testudineus.necrosis(N), Anabas karyolysis, binucleated hepatocyte, dilation of sinusoid, vacuolization, cloudy degeneration(CD)were noticed at15% sublethal concentration of triazophos exposure.

Page 70

Control Kidney - The kidney is the functional excretory unit composed of nephrons. Each nephron is made up of a renal capsule and a well-developed renal tubule and the renal capsule is composed of a glomerulus surrounded by Bowman's capsule.

kidney(Anabas testudineus Treated exposed to triazophos Day treatment 3 kidney): vacuolization(V), necrotic nubule(NT), degeneration of renal epithelial cells(DREC), distortion of renal tubule(DRT), cloudy degeneration(CD) were noticed atAnabas testudineusexposed to 5% triazophos exposure.Anabas *testudineus*exposed to 10% triazophos exposure shows histological alteration in intestine such as necrosis of haematopoietictissue(NHT). dilation of glomeruli(DG), necrotic changes(NC), dilation of renal tubule(DRT)

narrowing of tubular leumen(NTL), pycnotic nuclei(PN), hypertrophy(H), degeneration of renal epithelial cells(DREC), degeneration of glomerular cells(DGC), inter cytoplasmic vacuolization(INV), and necrosis(N)were noticed at 15% sublethal concentration of triazophos exposure.

Anabas testudineus exposed to triazophos – 3, 6 Days Recovery – kidney: cloudy degeneration, degeneration of renal epithelial cells, necrotic changes observed in *anabas testudineus*exposed to 5% triazophos exposure.Dilation of glomeruli, degeneration of renal epithelial cells recorded in 10% triazophos treated *anabas testudineus*.

Degeneration of renal epithelial cells, necrotic changes, pycnotic nuclei, dilation of renal tubule were noticed at 15% sublethal concentration of triazophos exposure.

Control Intestine - Histology revealed the following architecture with the wall of the intestine being composed of four layers such as the mucosa, submucosa, muscularis and serosa the serosa muscularis is composed of linner circular and outer longitudinal muscle fibressubmucosa is poorly developed and it shows only a few granular cells distributed in its meshes.

Intestine Treated (Anabas testudineus exposed to triazophosof 3Days treatment)Hypertrophy, autolysis of mucosa were observed in Anabas testudineusexposed to 5% triazophos exposure. Anabas testudineusexposed to 10% triazophos exposure confirmed histological alteration such as vacuolization(V), infiltration of leucocytes(IL), autolysis of mucosa(AM).infiltration of leucocytes(IL), vacuolization(V), degeneration villi(DV)were noticed at 15% sublethal concentration of triazophos exposure. Anabas testudineus exposed to triazophos - 3, 6Days Recovery - Intestine: Infiltration of leucocytes, degeneration of villi atrophy. focal necrosis. wereobserved in Anabas testudineusexposed to 5% triazophos exposure. Similarly vacuolization(V), Autolysis of Mucosa(AM), Degeneration of Epithelial Cells(DEC), Focal necrosis(FN) were recorded in 10%triazophos treated *Anabas testudineus*. Further mode Atrophy(A), Degeneration of Villi(DV), Focal necrosis(FN), degeneration of epithelial Cells(DEC), degeneration of villi(DV), infiltration of leucocytes(IL)were noticed at 15% sublethal concentration of triazophos exposure.

DISCUSSION

In the present study the LC₅₀ values of 96 hrsfor Triazophosexposed for Anabas testudineuswas found to be 0.270ppm. Percent of mortality increase with an increase in exposure time to Triazophos. The calculated Chi-square value for the fitted regression values are well below the table value indicating a good fit of the regression line. Thus acute toxicity test in the present study evidently indicates the toxicity of Triazophos to the experimental fish. Dramatical changes in the physical behaviour of fish *testudineus* were recorded Anabas in treated triazophosspecimans (Dinesh Kumar et al., 2000). Acute toxicity of broad spectrum pesticide triazophos has been reported in fishHeteropneustesfossilis (Maheshwarietal., 2001).Histological changes associated with pesticides in fish have been studied by many authors (King 1962, Cope 1966, Eller 1971, Razani et al., 1986; Mukhopadhyay et al., 1987, Bruno and Ellis 1988;Narayan and Sigh 1991; Mercy et al., 1996).

The histological studies on fish revealed that various toxicants produce pathological changes such as necrobiotic changes in the liver, tubular damage of kidney, gill lamellar abnormalities (Ramalingam 1985). Flat fish with liver and kidney lesions was found frequently in areas associated with urban pollution (Malins et al 1980). Liver and kidney damages were correlated with development of histopathological lesions dependent on does as well as duration of exposure.

Histopathological manifestation have been observed in the tissue of gill, liver and kidney of the fish *Ctenopharyngodonidellus* (Valenciennes) when exposed to technical and sublethal concentration of 20% EC of fenvalerate, a synthetic pyrethroid. The tissue damages like necrosis, vacuolar degeneration and atrophy were observed which are attributed to the effect of the pesticide (Tilak and Yacobu, 2001).

Gills: Fish gills were chosen for this investigation because they were in direct contact with the aquatic environment and therefore, could be a good indicator of water quality. In the present study gills of *Anabas testudineus*exposed to 6 days of triazophos confirmed histological changes such asshortening of secondary lamella, fusion of secondary lamellae and degenerationare observed in the present study in *Anabas testudineus*exposed to sublethal concentration of 5% treatment.

Similarly*Anabas testudineus*exposed to 10% of sublethal concentration of triazophos for 3 days shows histological alterations such as epithelial lifting, fusion

^{age}71

of secondary lamellae. Fusion of secondary lamellae, epithelial necrosis, desquamation and necrosis, epithelial lifting, aneurism, hypertrophy, has been observed in the fish *Anabas testudineus*. In the same way, when it was exposed to 15%. *Anabas testudineus*exposed to 5% of sublethal concentration of triazophos for 3, 6 days recovery shows pathological changes in gill such as desquamation, aneurism, shortening of secondary lamella, necrosis, fusion of secondary lamellae, epithelial lifting, epithelial hyperplasia.

Furthermodenecrosis. hypertrophy, aneurism. desquamation and necrosis, oedemaare observed in 10 % sublethal exposure of triazophos for 3, 6 days. Similarly shortening of secondary lamella, epithelial hyperplasia, fusion of secondary lamellae. desquamation, necrosis, Hypertrophy, Epithelial lifting, are recorded in gill of Anabas testudineusexposed to 15% sublethal concentration of triazophosafter 6 days recovery. However, it should also be taken in to consideration that in the case of Triazophos one could observe two subsequent effects. The first effect was a result of the direct contacts of the OP with the epithelial cells, including those of the respiratory epithelium, through the blood flow in the form of triazophos.

According to Malatt.,(1985) lifting of epithelia could serve as a mechanism of defense, because separation of epithelia of the secondary lamellae increases the distance across which water-borne irritants must diffuse to reach the bloodstream. Several authors have reported histopathological changes in the gill tissue of fish exposed to miscellaneous pesticides (Lowe, 1964; Eller, 1971; Jauch, 1979; Nowak, 1992; Rijijohn and Jayabalan, 1993). Caliskan*et al.*, (2003) reported lifting of epithelial layer from gill lamellae, exudation, hyperplasia, shortening of secondary lamellae and necrosis in the gills of *Lebistes reticulates* exposed to zeta cypermethrin.

Liver: In the present study liver of *Anabas testudineus*exposed to sublethal concentration of triazophostreatment for 3 daysshows cloudy degeneration, pycnotic nuclei, vacuolization, have been observed in *Anabas testudineus*exposed to 5% triazophos exposure. *Anabas testudineus*exposed to 10% triazophos exposure shows histological alteration in liver tissues such as cloudy degeneration, focal necrosis andvacuolization.

vacuolization. Similarly karyolyis, binucleated 15% hepatocyte are observed sublethal in of triazophos concentration exposure.are observed.dilation of sinusoid, haemorrage, karyolysis, vacuolization were the histological alterations observed in the 5% triazophos treated Anabas testudineusin 3, 6 days recovery. Besides haemorrage, binucleated hepatocyte, cloudy degeneration, vacuolization, karyolysiswere recorded in 10% triazophos treated Anabas testudineus.necrosis, karyolysis, binucleated hepatocyte, dilation of sinusoid, vacuolization, cloudy Degeneration have been observed in 15% sublethal concentration of triazophos exposurein the liver of *Anabas testudineus*after 6 days recovery.

Histopathological changes in the liver of freshwater fishes caused by pesticides/insecticides intoxication have been recorded by Gill et al., (1990). Eller, (1971) observed swollen hepatocytes and progressive degeneration of liver of the cutthroat trout, Salmoclarkii, exposed to high levels of endrin. Histopathology on, A. testudinesus, C. punctatus and B.gonionotus in relationto7 days of exposure to sublethal concentrations of 1.13, and 3.75 ppm; 1.13 and 2.26 ppm; and 1.13 and 2.26 ppm of Diazinon 60 EC respectively resulted in hypertrophy, necrosis and pyknosis of hepatocytes, pyknosis and degenerative changes such as necrosis of tubular and haematopoietic cells of kidney were the major manifestation (Rahmanet al., 2002).

Kidney: In the present study kidney of *Anabas testudineus*exposed to sublethal concentration of triazophostreatment for 3 daysshows vacuolization, necrotic tubule, degeneration of renal epithelial cells, distortion of renal tubule, cloudy Degeneration are observed in *Anabas testudineus*exposed to 5% triazophos exposure.

Anabas testudineusexposed to 10% triazophos exposure shows histological alteration in intestine such as necrosis of haematopoietic tissue, dilation of glomeruli, necrotic changes, dilation of renal tubule. narrowing of tubular leumen, pycnotic nuclei, hypertrophy, degeneration of renal epithelial cells, degeneration of glomerular cells, inter cytoplasmic vacuolization, and necrosis are observed in 15% sublethal concentrations of triazophos exposure are observed.cloudy degeneration, degeneration of renal epithelial cells, necrotic changesobserved in Anabas testudineusexposed to 5% triazophos exposure. Similarly dilation of glomeruli, degeneration ofrenalepithelial cells recorded in 10% triazophos treated Anabas testudineus.

degeneration of renal epithelial cells, necrotic changes, pycnotic nuclei, dilation of renal tubule in 15% sublethal concentration of triazophos exposureare observed in the present study in the liver of Anabastestudineusafter 6 days recovery. Amminikutty and Rege (1978) described vacuolation in renal tubular cells, loss of glomerular shape, vascular congestion in widowtetraGymnocorymbusternetziexposed to agallol thiodan.Mandal and Kulshrestha and (1980)demonstrated nephropathy in Clariasbatrachus exposed to sumithion.

The changes observed in the kidney of the treated fish include vacuolation of epithelial cells of uriniferous tubules and degeneration of glomeruli. Elsan treatment in *Channapunctatus* resulted in a significant decrease in the dimension of Bowman's capsule and glomerulus, and the tubules lost their regular shape due to precipitation of cytoplasm and karyolysis (Banerjee

Page 72

and Bhattacharya, 1994).Das and Mukherjee (2000), reported dilation of tubules, necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of affected cells of *Labeorohita* exposed to hexachlorocyclohexane.

Intestine: Hypertrophy, autolysis of mucosa are observed in Anabas testudineusexposed to 5% triazophos exposure. Anabas testudineus exposed to 10% triazophos exposure shows histological alteration in intestine such as vacuolization, infiltration of leucocytes, autolysis of mucosa, infiltration of degeneration leucocvtes. vacuolization, villiare observed in 15% sublethal concentration of triazophos exposure.fusion villi, infiltration of leucocytes, atrophy, focal necrosis, of degeneration of villi observed in Anabas testudineusexposed to 5% triazophos exposure.vacuolization, autolysis of mucosa, degeneration of epithelial cells, focal necrosis recorded in 10% triazophos treated Anabas testudineus.atrophy, degeneration of villi, focal necrosis, degeneration of epithelial cells.

Similarly degeneration of villi, infiltration of leucocytes are observed in 15% sublethal concentration of triazophos exposureare the histological alterations observed in intestine of Anabas testudineusexposed to triazophos for 3,6 days recovery.

Histology of intestine from both the polluted-water fishes (Channastriatus and Heteropneustesfossilis) revealed degenerative changes in the serosa, mucosa and sub mucosal layers. Focal necrosis, proliferation and desquamation of the superficial parts of villi were more frequent. Also seen were the oedematous epithelial and connective tissues, dilated and damaged blood vessels, vacuolization and lymphocyte migration in the polluted water fishes when compared to the control fishes (AnithaKumari and Sree Ram Kumar, 1997). Fresh water fish Nandusnandus was exposed to 0.05 ppm of carbaryl for the period of one month. The outer most layer serosa and muscle layers were severely damaged. Necrosis in intestinal villi and increase in the number and size of mucous cells were seen (Sudha Singh and AshaMehrotra, 1999).

SUMMARY AND CONCLUSION

The pesticide triazophos is found to be more toxic to the fish *Anabas testudineus*. The acute toxicity studies in *Anabastestudineus* at96 hr LC_{50} value for triazophosis0.270 ppm.The histological investigations in *Anabastestudineus* exposed to Triazophos were found to be highly toxic and the histological alterations were increasing with increase in concentration and duration. The recovery studies of triazophos exposed to *Anabastestudineus*shows damages in tissues and recovery pattern was less when compared to Triazophos treated fishes.

REFERENCES

- Atienzar, F. A. and Jha, A. N., (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques alied to genotoxicity and carcinogenesis studies: A critical review. *Mutation Research*, 613: 76 – 102. doi:10.1016/j.mrrev.2006.06.001
- Diaz, S., Gonzalez, A.M. and Gutierrez, J.C. 2006.Evaluation of heavy metal acute toxicity and bioaccumulation in soil ciliated protozoa. Environ. Int. 32: 711-717.
- Ghosh, D., Bhattacharya, S. and Mazumder, S. 2006. Perturbations in the catfish immune responses by arsenic: organ and cell specific effects. Comp. Biochem. Physiol. C. Comp. Pharmacol. Toxicol., 143: 455-463.
- 4. Jarvik, E. 1980. "Basic Structure and Evolution of Vertebrates." Academic Press, London.
- 5. Jyothi, B. and G. Narayan, 1997. Effect of Phorate on certain protein profiles of serum in fresh water fish, *Clariasbatrachus* (Linn). *J. Environ. Biol.*, 18(2): 137-140.
- 6. Sweet, L.I. and Zelikoff, J.T. 2001. Toxicology and immunotoxicology of mercury: a comparative review in fish and humans. J. Toxicol. Environ. Health B. Crit. Rev. 4:161-205.
- 7. Worthing, C.R. and R.J. Hanree, 1991. The pesticide manual: a world compendium, ninth ed., *British Crop Protection Council*, Surrey, pp. 838.
- Rong, Z. and Yin, H.(2004). A method for genotoxicity detection using random amplified polymorphism DNA with *Daniorerio*. Ecotoxicology and Environment safety 58: 96-103.
- Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V.(1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.
- N. Fiedler, H. Kipen, K. Kelly-McNeil, R. Fenske, Long-term use oforganophosphates and neuropsychological performance, Am. J. Ind. Med. 32(1997) 487–496.
- L. Rosenstock, M. Keifer, W.E. Daniell, R. McConnell, K. Claypoole, Chroniccentral nervous system effects of acute organophosphate pesticideintoxication, Lancet 338 (1991) 223–227.
- Monograph of toxicological evaluation 601, Joint FAO/WHO meeting onpesticide residues in food: 1982 evaluations. FAO Plant Production andProtection Paper 49, 1983, Nos. 569–604 on INCHEM.
- 13. M. Kumar, A. Kumar, Report on application and health effects of pesticidescommonly used in India, Centre for Science and Environment, New Delhi,India, 2007.
- S. Rani, V.K. Madan, T.S. Kathpal, Persistence and dissipation behavior oftriazophos in canal water under Indian climatic conditions, Ecotoxicol.Environ. Safety. 50 (2001) 82–84.

 $P_{age}73$

- 15. D.J. Echobiochon, Pesticides use in developing countries, Toxicology 160(2001) 27–33.
- S. Jain, S. Mythily, R.S. Ahmed, V.K. Arora, B.D. Banerjee, Induction of oxidative stress and histopathological changes by sub-chronic doses of Triazophos, Indian J. Biochem. Biophys. 47 (2010) 388–392.
- 17. OECD (Organization for Economic Co-operation and Development) Guidelines for the Testing of Chemicals 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents, updated on December 2007.
- O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with theFolin phenol reagent, J. Biol. Chem. 193 (1951) 265– 275.
- 19. W.H. Habig, M.J. Pabst, W.B. Jacob, Glutathione STransferase: the firstenzymatic step in mercapturic acid formation, J. Biol. Chem. 249 (1974)7130–7139.
- 20. K. Satoh, Serum lipid peroxide in cerebro spinal disorder determined by a new colorimetric method, Clin. Chem. Acta. 90 (1978) 3