➔DOI : 10.15740/HAS/AJBS/12.2/156-164
e ISSN-0976-8343 | ■ Visit us : *www.researchjournal.co.in*
ASIAN JOURNAL OF BIO SCIENCE
Volume **12** | Issue 2 | Oct., 2017 | 156-164

RESEARCH **P**APER

Study of lethal toxicity of Hilban® on freshwater catfish, Singhi (*Heteropneustes fossilis;* Bloch, 1794)

SNEHA VERMA, ANURAG RAWAT **AND** ABHA MISHRA

Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, LUCKNOW (U.P.) INDIA
Email : cute_neelu2006@yahoo.com

Article Info : Received : 04.07.2017; Revised : 13.08.2017; Accepted : 10.09.2017

The objective of the present study was to investigate the behavioural and morphological changes and acute toxicity of Hilban$^{(R)}$in freshwater catfish, *Heteropneustes fossilis*. This study was conducted by exposing the fish to different concentrations of chlorpyrifos (0.5 to 150µl/L) for 96 h along with control groups. The data were subjected to get LC_{50} by probit analysis. The LC_{50} values for chlorpyrifos for 24, 48, 72 and 96 h were 51.98, 18.91, 7.47 and 4.61µl/L, respectively. Fish exposed to higher concentration showed uncoordinated alterations in behavioural responses, especially erratic and jerky swimming, frequent surfacing and ingulping, mucus secretion, an increase in opercular movement and copious secretion of mucus all over the body. It is concluded that Hilban$^{(R)}$ is highly toxic to catfish and severely affect their physiology and behaviour.

Key words : Acute toxicity, Chlorpyrifos, *Heteropneustes fossilis*, LC_{50}, Mortality

How to cite this paper : Verma, Sneha, Rawat, Anurag and Mishra, Abha (2017). Study of lethal toxicity of Hilban® on freshwater catfish, Singhi (*Heteropneustes fossilis;* Bloch, 1794). *Asian J. Bio. Sci.*, **12** (2) : 156-164. **DOI : 10.15740/HAS/AJBS/12.2/156-164.**

INTRODUCTION

Chlorpyrifos (CPF) (O,O–diethyl–O (3, 5, 6-trichlor-2-pyridyl) phosphorothionate; an organophosphorus pesticide was first registered for use in the United States in 1965 (by Dow Chemical Company). It is a widely used and most preferred broad spectrum chlorinated organophosphate pesticide due to their high effectiveness and low persistence in the environment. They are frequently used for the eradication of a wide range of insect pests. Their worldwide indiscriminate use of agricultural and other activities poses great threat to the aquatic environment (Chernyak *et al.,* 1996; Livingstone, 2001 and Matsumoto *et al.,* 2006). CPF directly inhibits acetylcholinesterase enzyme activity in fishes and invertebrates (Fulton and Key, 2001 and Rao *et al.,* 2005) which may lead to decreased mobility of fish (Bretaud *et al.,*2000) which include neurological, behavioural and possibly reproductive efects (Mueller-Beilschmidt, 1990 and Hill, 1995). Aquatic contamination of pesticides causes acute and chronic poisoning of fish and other organisms (Heger *et al.,* 1995 and Velmurugan *et al.,* 2007). The responses of fish towards the toxic chemicals are broad-ranged depending on the toxicant, exposure duration, water quality and the species (Fisher, 1991; Richmonds and Dutta, 1992 and Venkateswara, 2004).

Toxicity test gives first-hand information about the effects of such pesticides on organisms and the ecosystem as a whole. These tests are valuable in creating awareness regarding potential harmful effects of pesticide in the environment (Adedeji *et al.,* 2008 and Onyedineke *et al.,*2010). The mortality of fish is not only the endpoint to consider the toxicity level. There is a growing interest in the development of behavioral markers to assess the toxicant affects because behavioural responses are the most promising and sensitive indicator of ecotoxicology (Drummond and Russom, 1990 and Scherrer, 1992). The behaviour study is becoming

prominent in toxicity assessment in a unicellular organism (Tadehl and Hader, 2001), insects (Martin, 2003 and Venkateswara *et al.,* 2004), fish (Hansen *et al.,* 1999 and Rao *et al.,* 2005) and even rodents (Dell'Omo *et al.,* 1997). Different studies reported that fish exposed to a wide range of pesticides exhibited abnormal behavioural and morphological alterations (Devi and Mishra, 2013). There were various reports on the toxicity of different organophosphate pesticides on fish (Gul, 2005 and Pandey *et al.,* 2005).

The purpose of the present study was to determine toxic level of chlorpyrifos with special emphasis on behavioural and morphological responses on a freshwater catfish, *Heteropneustes fossilis* (Bloch, 1794).

RESEARCH METHODOLOGY

Sample collection:

The fresh water *H. fossilis* of relatively same length (17±2 cm) and weight (185±20g) were collected from commercial fisherman of Lucknow, Uttar Pradesh, India. After formal quarantine treatment with 0.05 per cent potassium permagnate solutions to remove any dermal infection, fish were acclimatized in 120 L glass aquaria containing water having a pH of ≈ 7.5, dissolved oxygen 5-6 mg/l under normal maintained temperature for two weeks. Water was renewed daily to remove faecal matter and waste metabolite of fish during acclimatization. During this period, fish were fed regularly with commercial fish food pellets and goat liver. An experiment was performed in accordance with local/national guidelines of ethical committee for experimentation in animals.

Toxicity bioassay:

For toxicity assessment of Hilban® (20% EC CPF), fish were exposed to varying concentrations (0.5 to 150µl/L) of Hilban®with five replicates. In each group, fish were in ten numbers. The control group was maintained side by side. The control and test solutions were renewed daily and dead fish were immediately removed. The physico-chemical parameters such as dissolved oxygen (DO), temperature, pH and hardness of the water were recorded daily for the 96 h exposure period following the standard methods (APHA, 1998).

The behavioural and morphological parameters *viz.*, schooling behaviour, swimming pattern, excitability and reflex responses, surfacing and ingulping, mucus secretion, opercular movement per minute, avoidance behaviour, escaping tendency, jerky movement, drooping of fins and body colour changes were consistently monitored and recorded Gupta and Dua (2010) and Nimila and Nandan (2010). The frequency of occurrence of different malformations was recorded during first hour of treatment for group of exposed fish.

Data analysis :

The mortality data was recorded after every 24 h upto 96 h to get sub- lethal concentration of Hilban®. Significance level was checked by one way ANOVA. The sub-lethal concentration or LC_{50} with 95 per cent confidence limits of 24, 48, 72 and 96 h was estimated by probit analysis (Finney, 1971) with the IBM SPSS (version 20) software.

RESEARCH FINDINGS AND ANALYSIS

The physico-chemical parameters of the tested water used in toxicity experiment were recorded daily for the 96 h exposure period as follows, dissolved oxygen (DO) (7.5±0.16 mg/l), temperature (26.8±0.12), pH (7.3±0.05) and hardness of the water (116.49±0.37mg/l).

The acute toxicity test is widely used in order to investigate the effective concentration and effective exposure duration associated with the 50 per cent mortality of fish which was expressed as LC_{50}. The present investigation was reported that exposure of different concentrations of Hilban(R) to *H. fossilis* showed varied degree of mortality. The chlorpyrifos caused 100 per cent mortality at 25µl/l during 96 h exposure and LC_{50} for 96 h was 4.61µl/l. The previously studied of acute toxicity of Hilban(R) (20% EC) for *Heteropneustes fossilis* revealed that LC_{50} value for 96 h was found to be 1.2 µl/l (Barbhuiya and Dey, 2014). The value of LC_{50} 36 h of chlorpyrifos was determined as 2.84 ppm for *H. fossilis* in the laboratory condition (Khatun and Mahanta, 2014). LC_{50} value of deltamethrin to *H. fossilis* was 1.86µg/l (Srivastav *et al*., 2002). This is in agreement with that of Sprague (1969) who observed variation in LC_{50} for the same species and toxicant depending on size, age, sex and condition of test species along with experimental factors. Several reports were given for different LC_{50} values of various pesticides on freshwater fish (Chindah *et al*., 2004; Rao *et al*., 2005; Koprucu *et al.*,2006; Patil and David, 2008 and Halappa and David, 2009). The range of 96h LC_{50} of CPF was 0.57-3270 ppb for

mosquito fish, Bluegill, Fathead minnow, Rainbow trout, Nila tilapia, Goldfish (Holcombe *et al.*, 1982; Gul, 2005; Wang *et al*., 2009). The organophosphorus compound coroban (20% EC- CPF) LC_{50} for 96h was 2.2 mg/l of *H. fossilis* (Srivastav *et al*., 1997). 24h, 48h, 72h and 96h LC_{50} value of tricel (20% EC-CPF) for *Labeo bata* as 257.03µg/l, 208.92µg/l, 177.82µg/l and 109.64µg/l, respectively (Samjadar and Mandal, 2015). The LC_{50} value of other organophosphate pesticide, dimethoate for *Clarius batrachus* was 65mg/l (Begum and Vijayaraghavan 1995), 17.9mg/l in *Channa punctatus* (Pandey *et al.*, 2005) and 1.61mg/l for *Cyprinus carpio* (De Mel *et al*., 2005).

The percentage of mortality was significantly increased with the increase in concentrations of the toxicant (Hilban(R)) as well as duration of the experiment ($F=52.27$ at $P<0.05$) (Fig. 1). On the basis of mortality, LC_{50} value was 51.98, 18.91, 7.47 and 4.61µl/l for 24, 48, 72 and 96 h, respectively (Table 1).

The behavioural manifestation can be considered as symptoms of stress on account of the toxic nature of the environment. The observation of behavioral changes was started within 30 min after treatment with Hilban(R). The response time was decreased as the toxicant concentration increased. The behavioural alterations were positively correlated with the toxicant concentration (Table 2). Hilban(R) influence the behaviour pattern of fish by interfering with the nervous systems and sensory receptors (Keizer *et al*., 1995; Pan and Dutta, 1998 and Cong *et al*., 2009) and this incident may impair the identification of situation and development of appropriate response by fish exposed to pesticide. The control group showed a normal behaviour and tend to move together. They came to the surface at intervals to gulp air. In low concentrations of Hilban(R) (0.5 and 1µl/l) fish behave similar to control group.

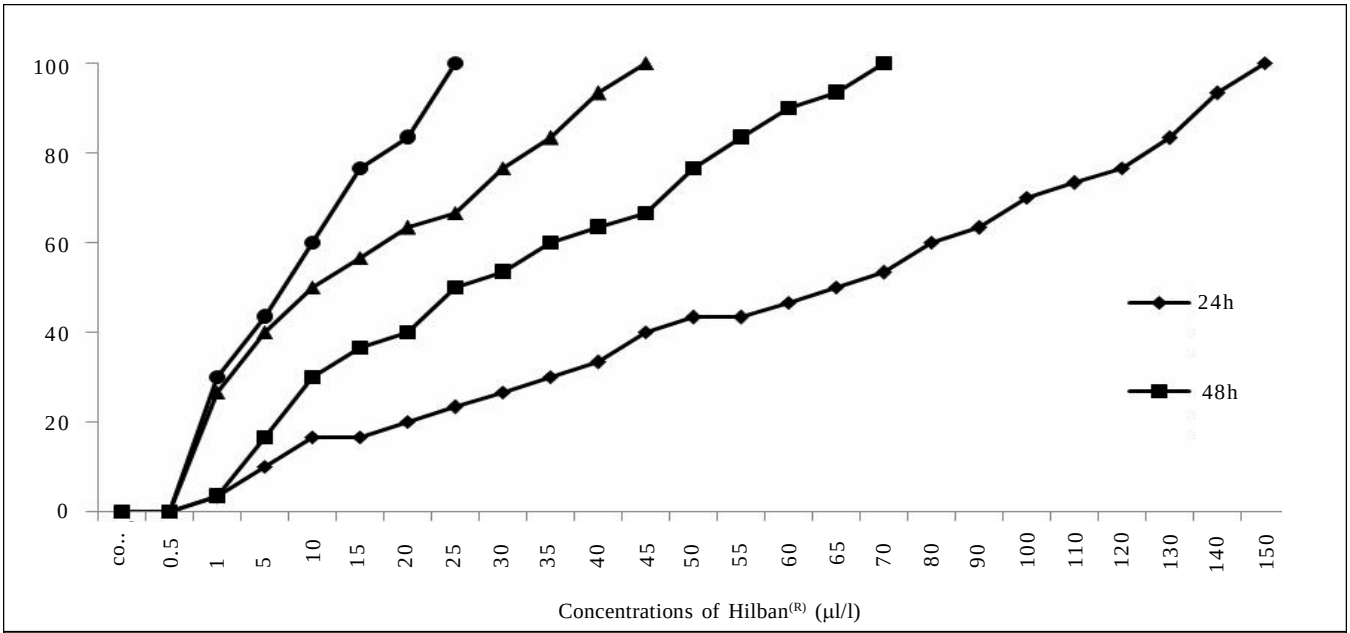

Fig. 1 : Per cent mortality of freshwater catfish *Heteropneustes fossilis* exposed to different concentrations of Hilban® for 24, 48, 72 and 96 hexperimental duration. Values are means ± SEM of percentage of mortality of ten fish in five replicate. Data were analyzed by one way ANOVA ($P < 0.001$)

Table 1: LC_{50} values for Hilban® (with 95% confidence limit) exposed to freshwater catfish *H. fossilis* (Bloch, 1794).

Probit value	24 h	48 h	72 h	96 h
LC_{50} (µl/l)	51.98 µl/l	18.91µl/l	7.47µl/l	4.61 µl/l
95% confidence limits				
Upper limit	62.62	25.25	11.69	9.02
Lower limit	42.79	13.06	4.04	1.82

The LC_{50} values were estimated by finney probit analysis. Experiment was performed with five replicates of ten fish in each concentration

The exposed group of fish were lethargic or motionless in all the concentrations. They showed a number of abnormalities in their swimming pattern. The swimming speed of fish was higher in higher concentration. Fish were calm down in lower concentration after some time, but in higher concentration, they showed restlessness throughout the experimental period. The restlessness and hyperactivity may occur due to the inactivation of acetylcholinesterase, leading to acetylcholine accumulation at synaptic junctions (Fulton and Key, 2001) and stimulation of peripheral nervous system which results in to increased metabolic activities and more oxygen utilization (Rao, 1989).

The schooling behaviour of fish was also disturbed in comparison to control, followed by hyper-excitability, pectoral fin forwarded, frequent surfacing and ingulping, avoidance behaviour, escaping tendency, drooping of fins and loss of buoyancy by vertically hanging in the aquaria after treatment and moreover progressively became sluggish and lethargic and finally lead to death in exposed group of fish, supported by Venkata *et al*. (2008) who studied which showed that abnormal swimming and loss of balance was caused by the deficiency in nervous and muscular co-ordination due to stress by toxicant. Convulsion prior to death were most evident, the severity of which paralleled the concentration of CPF. On initial exposure at higher concentration, the fish exhibited characteristic avoidance behaviour by rapid swimming, stretching half of their body out of the water surface and trying to jump out. The abrupt, erratic and jerky swimming and hanging vertically indicates the loss of physiological equilibrium may be due to inhibition of the acetylcholinesterase in the brain and neuromuscular junctions (Rao *et al*., 2005 and Patil and David, 2008).

The opercular movements or beats are major indicators of stress. The opercular beats increased upto 130/min in Hilban(R) exposed fish group from 85-90/min obtained in the control group (Fig. 2). The opercular movement in fish showed increasing trend with the increase in concentration of toxicant. It was showing significant linearity with concentration (r^2= 0.9846). It was first increased after the exposure of Hilban(R) and followed by decreased opercular movement with the exposure time and similar finding was observed by Pandey *et al*. (2005); Omitoyin *et al.* (2006); Yadav *et al*. (2007); Koprucu *et al*. (2006) and Srivastava *et al.* (2010), in *Channapunctatus, Clariasgariepinus, Channastraita, Silurusglanis* and *Heteropneustes fossilis* exposed to mercuric chloride and malathione, lindane, fertilizer, diazinon and dimethoate, respectively. Rapid opercular movement was also confirmed by Wasu *et al*. (2009) in *Clariasbatrachus* treated with carbaryl and malathion.

Table 2 : The behavioural and morphological observations of freshwater catfish *Heteropneustes fossilis* (Bloch, 1794) after 96 h exposure of Hilban®

Behavioural and morphological observations	Control	Concentration of CPF (µl/l)						
		0.5	1	5	10	15	20	25
Opercular movement	-	-	-	+	++	++	+++	+++
Ingulping	-	-	-	+	+	++	+++	+++
Surfacing	-	-	-	+	+	++	+++	+++
Frequent swimming	-	-	-	-	++	++	+++	+++
Avoidance behaviour	-	-	-	+	+	++	+++	+++
Hyperactivity	-	-	-	+	+	++	+++	+++
Pectoral fin forward	-	-	-	+	+	++	+++	+++
Convulsions	-	-	-	+	+	++	+++	+++
Abrupt swimming	-	-	-	+	+	++	+++	+++
Escaping tendency	-	-	-	+	++	++	+++	+++
Loss of buoyancy	-	-	-	+	++	++	+++	+++
Lethargic	-	-	-	+	++	++	+++	+++
Mucus secretion	-	-	-	+	++	++	+++	+++
Discoloration of skin	-	-	-	+	++	++	+++	+++
Fins drooping/ necrosis	-	-	-	+	+	++	+++	+++
Lesions of skin	-	-	-	+	++	+++	+++	+++

Normal (-), Mild (+), Moderate (++), Maximum behaviour (+++)

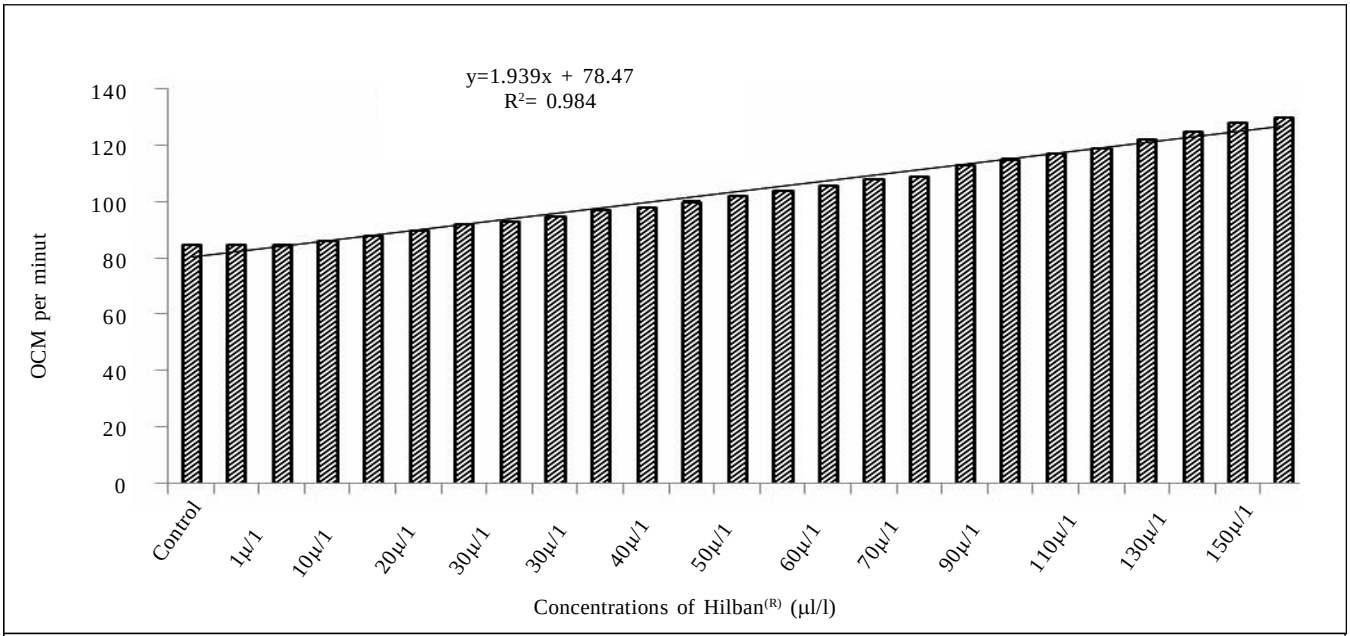

Fig. 2 : Opercular movement (OCM) of freshwater catfish *Heteropneustes fossilis* exposed to different concentrations of Hilban®

Shivakumar *et al.* (2006) also observed increased opercular movement in *Cyprinuscarpio* exposed to endosulfan, cypermethrin and fenvalerate. Chindah *et al.* (2004) reported an initial increase in opercular beat frequency in chlorpyrifos exposed tilapia, followed by a marked decline with exposure time and explained the initial increase as sudden response to shock. *Heteropneustes fossilis* showed a unique adaptive feature to avoid toxicant intake by decreasing the opercular movement after the few hour of exposure. The changes in behaviour of *H. fossilis* may be due to a direct manifestation of CPF to the disturbances in physiological mechanism which according to Merler and Hamilton (1996) initiate, maintain and terminate behaviour. The observed behavioural changes showed by the exposed fish to the chlorpyrifos are similar to those observed in other fish exposed to organophosphate pesticides (Ahmed, 1975 and Shukla, 1995). When the oxygen was reduced in toxic medium, fish showed surfacing for demand of higher oxygen level during the exposure period due to hydro toxic condition (Katja *et al.*, 2005) and air ingulping which may help to avoid contact of toxic medium (Katja *et al*., 2005). Similar observation has been reported by Patil and David (2008) in malathion treated fish *Labeorohita* and these results are supported by Cook *et al*. (2005); Halappa and David (2009) and Shivakumar *et al.* (2006).

Abnormal morphological features include pale yellow body colour, discoloration of skin, lesion of skin, eye deformities and fin deformities as compared to control which confirm the presence of stress due to toxicant (Koprucu *et al*., 2006; Wasu *et al*., 2009 and Ree and Paney, 1997). Barbels were also losing their colour. Pandey *et al*. (1990) also reported depigmentation in toxicant exposed fish and attributed it to reduction in number and size of chromatophores. All those observed abnormalities may directly affect the fish growth. High mucus secretion and its coagulation all over the body was reported during the study. Researchers have reported the same alterations in *Oryziaslatipes, Cyprinus carpio, Labeorohita, Oncorhynchus tshawytshe, Crrihinus mrigala, Oreochromis niloticus, Clarius gariepinus* treated with CPF (Rice *et al*., 1997 and Halappa and David, 2009), malathion (Patil and David, 2008), diazinon (Scholz *et al*., 2000), endosulfan (Gormley and Teather, 2003), fenvalerate (Mushigeri and David, 2005), Fenitrothion (Benli and Ozkul, 2010). The fish were found to be lying down motionless at the bottom before death and died by opening their mouth. Same behaviour were found by Koprucu *et al*. (2006); Patil and David (2008) and Susan *et al*. (2010).

Conclusion :

The present study of chlorpyrifos toxicity of air-

breathing catfish *Heteropneustes fossilis* concluded that this fish is very sensitive to the organophosphorus insecticide. The observations indicated that CPF caused many behavioural alterations, such as those observed in this study may result in severe physiological problems, ultimately leading to the death of fish. Therefore, this investigation demonstrated a relation among pesticidal stress, behavioural disorders, survival and mortality rates. Thus, this study can be used as a tool for creating awareness among the local farmers so that the use of the highly toxic pesticide in agriculture can be minimized.

LITERATURE CITED

Adedeji, O.B., Adedeji, A.O., Adeyemo, O.K. and Agbede (2008). Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*). *Afr. J. Biotechnol.*,**7** : 651-654.

Ahmed, A.M. (1975). Acute toxicity of four organophosphorous insecticides to freshwater telcost *Channel punctalus* (Bloch). *Pak J.ooi.*, **7**: 133-140.

APHA, AWWA and WPCF (1998). Standard methods for the examination of water and wastewater, 20th Ed. American Public Health Association, Washington.

Barbhuiya, H.B. and Dey, M. (2014). Histopathological changes in liver tissue of *Heteropneustes fossilis* exposed chlorpyrifos (20% EC). *Indian J. Appl. Res.*, **4** (7) : 237 - 240.

Begum, G. and Vijayaraghavan, S. (1995). *In-vivo* toxicity of dimethoate on protein and transaminase in the liver tissue of freshwater fish *Clarius batrachus* (Linn). *Bull. Environ. Contam. Toxicol*, **54**: 370-375.

Benli, A.C.K. and Ozkul, A. (2010). Acute toxicity and histopathological effects of sublethalfenitrothion on Nile tilapia, *Oreochromis niloticus*. *Pest Biochem. Physiol.*, **97**: 32–35.

Bloch, M.E. (1974). Naturgeschichte der auslandischen fische. Berlin. *Naturgeschichte der Auslandischen Fische*. **8**: (1) : 361-396.

Bretaud, S. Toutant, J.P. and Saglio, P. (2000). Effects of carbofuran, diuron and nicosulfuron on acetylcholinesterase activity in goldfish (*Carassiusauratus*). *Ecotoxicol. Environ. Saf.*,**47**, 117–124.

Chernyak, S.M., Rice, C.P. and Mc Connell, L.L. (1996). Evidence of currently-used pesticides in air, ice, fog, seawater and surface microlayer in the Bering and Chukchi seas. *Marine Poll.Bull.*, **32**(5): 410-41.

Chindah, A.C., Sikoki, F.D. and Vincent-Akpu, I. (2004). Toxicity of an organophosphate pesticide (chloropyrifos) on a common niger delta wetland fish - *Tilapia guineensis* (Blecker 1862). *J. Appl.* Sci. *Environ. Manag.*, **8** (2): 11-17.

Cook, L.W., Paradise, C.J. and Lom, B.(2005). The pesticide malathion reduces survival and growth in developing zebra fish. *Environ.Toxicol. Chem.*, **24**: 1745-1750.

Cong, N.V., Phuong, N.T. and Bayley, M. (2009). Effects of repeated exposure of diazinon on cholinesterase activity and growth in snakehead fish (*Channastriata*). *Ecotoxicol. Environ. Saf.*,**72**: 699–703.

Dell'Omo, G., Bryenton, R. and Shore, R.F. (1997). Effects of exposure to an organophosphate pesticide on behaviour and acetylcholinesterase activity in the common shrew, *Sorexaraneus*. *Environ. Toxicol. Chem.*, **16** : 272–276.

De Mel, G.W.J.L.M.V.T.M. and Pathiratne, A. (2005). Toxicity assessmnet of insecticides commonly used in rice pest managemnt to the fry of common carp, *Cyprinus carpio* a food fish culturable in rice fields. *J. Appl. Icthyol.*, **21**: 146-150.

Devi, Y. and Mishra, A. (2013). Study of behavioural and morphological anomalies of fry fish of fresh water teleost, channa punctatus under chlorpyrifos intoxication. *Internat. J. Pharm. Bio. Sci.*, **4** (1): 865-874.

Drummond, R.A. and Russom, C.L. (1990). Behavioural toxicity syndromes, a promising tool for assessing toxicity mechanisms in juvenile fat-head minnows. *Environ. Toxicol. Chem.*, **9** : 37–46.

Finney, D.J.(1971). *Probit analysis*.2nd Ed.Cambridge University Press, Cambridge, UK, pp. 333.1953.

Fisher, S.W. (1991). Changes in the toxicity of the three pesticides as a function of environmental pH and temperature. *Bull. Environ. Contam. Toxicol.*, **46**: 197-202.

Fulton, M.H.and Key, P.B. (2001). Acetylcholinesterase inhibition in estuarine fish and invertebrates as an indicator of organophophorus insecticide exposure and effects. *Environ. Toxicol. Chem.*, **20** : 37-45.

Gormley, K.L. and Teather, K.L. (2003). Developmental, behavioural and reproductive effects experienced by Japanese medaka (*Oryziaslatipes*) in response to short-term exposure to endosulfan. *Ecotoxicol. Environ. Saf.*, **54** : 330–338.

Gul, A. (2005). Investigation of acute toxicity of chlorpyrifos-methyl on Nile tilapia (*Oreochromis niloticus* L.) larvae. *Chemosphere*, **59** : 163-166.

Gupta, N. and Dua, A. (2010). Mercury induced behavioural alterations in *Channa punctatus. Pollut. Res.*, **29** (4) : 721-723.

Halappa, R. and David, M. (2009). Behavioural responses of the fresh water fish, *Cyprinus carpio* (Limmaeus) following sublethal exposure to chlorpyrifos. *Turk J. Fish Aquat. Sci*, **9** : 233-238.

Hansen, J.A., Marr, J.C.A., Lipton, J., Cacela, D. and Bergman, H.L. (1999). Difference in neuro behavioural response of Chinook salmon, *Oncorhynchus tshawytscha* and rainbow trout, *Oncorynchus mykiss* exposed to copper and cobalt, behavioural avoidance. *Environ. Toxicol. Chem.*, **18**: 1972– 1978.

Heger, W., Jung, S.T., Martin, S. and Peter, H. (1995). Acute and prolonged toxicity to aquatic organism of new and existing chemicals and pesticides.*Chemosphere*, **31**: 2702 - 2726.

Hill, Frances M. (1995). Managing service quality in higher education: the role of the student as primary consumer. *Quality Assurance in Education*, **3** (3): 10-21, https://doi.org/10.1108/09684889510093497.

Holcombe, G., Phipps, G. and Tanner, D.(1982). The acute toxicity of kelthane, dursban, disulfoton, pydrin and permethrin to fathead minnows *Pimephales promelas* and rainbow trout *Salmogairdneri*. *Environ. Pollut.*, **29** : 167-178.

Katja, S., Georg, B.O.S., Stephan, P. and Christian, E.W.S. (2005). Impact of PCB mixture (Aroclor 1254) and TBT and amixture of both on swimming behaviour, body growth and enzymatic biotransformation activities (GST) of young carp (*Cyprinus carpio*). *Aquat.Toxicol.*,**71**: 49-59.

Keizer, J., D'Agostino, G., Nagel, R., Volpe, T., Gnemid, P. and Vittozzi, L.(1995). Enzymological differences of AChE and diazinon hepatic metabolism: correlation of *in vitro* data with the selective toxicity of diazinon to fish species. *Sci. Total Environ.*,**171**: 213-220.

Khatun, K. and Mahanta, R. (2014). A study on the effect of chlorpyrifos (20% EC) on thyroid hormones in freshwater fish, *Heteropneustes fossilis* (Bloch.) by using EIA technique. *Sci. Probe*, **2** (2): 8-16.

Koprucu, S.S., Koprucu, K., Ural, M.S., Ispir, U. and Pala, M. (2006). Acute toxicity of organophosphorus pesticide diazinon and its effects on behaviour and some hematological parameters of fingerling european catfish (*Silurus glanis* L.). *Pest. Biochem. Physiol.*, **86** : 99-105.

Livingstone, D.R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Bull. Marine Pollut.*,**42**: 656-666.

Martin, J. (2003). A portrait of locomotor behaviour in Drosophila determined by a video-tracking paradigm. *Behav. Process.*, **67**(2) : 207-219.

Matsumoto, S.T., Mantovani, M.S., Malagutti, M.I.A., Dias, A., Fonseca, I.C. and Marin-Morales, M.A. (2006) . Genotoxicity and mutagenicity of water contaminated with tannery effluents,as evaluated by the micronucleus test and comet assay using the fish, *Oreochromis niloticus* and chromosome aberrations in onion root-tips.*Genet. Mol. Biol.*, **29**: 148-158.

Merler, P.R. and Hamilton, W.J. (1996). Mechanism of animal behaviour. John Wiley, NEW YORK, U.S.A.

Mueller-Beilschmidt, Doria (1990). Toxicology and environmental fate of synthetic pyrethrois. *J. Pesticide Reform.*, **10** (3): 32-37.

Mushigeri, S.B. and David, M. (2005). Fenvalerate induced changes in the Ach and associated AchE activity in different tissues of fish *Cirrhinus mrigala* (Hamilton) under lethal and sub-lethal exposure period. *Environ. Toxicol. Pharmacol.*, **20** : 65–72.

Nimila, P.J. and Nandan, B. (2010). Effect of organochlorine pesticide lindane (γ-HCH) on behavior of *Etroplus maculatus* (Bloch, 1795). *Pollut. Res.*, **29** (4): 577-582.

Omitoyin, B.O., Ajani, E.K., Adesina, B.T. and Okuagu, C.N.F. (2006). Toxicity of lindane (Gamma Hexachloro-Cyclo Hexane) to *Clariusgariepinus* (Burchell 1822).*North-Western J. Zool.*, **1**:57-63.

Onyedineke, N.E., Odukoya, A.O. and Ofoegbu, P.U. (2010). Acute toxicity tests of cassava and rubber effluents on the Ostracoda *Strandesiaprava* Klie, 1935 (Crustacea, Ostracoda).*Res. J. Environ. Sci.*, **4** : 166-172.

Pandey, A., Kumar, G.K. and Munshi, J.S.D. (1990). Integumentary chromatophores and mucus glands of fish as indicator of heavy metal pollution. *J. Freshwater Biol.*, **2** : 117-121.

Pandey, S., Kumar, R., Sharma, S., Nagpure, N.S., Srivastava, S.K. and Verma, M.S. (2005). Acute toxicity bioassay of mercuric chloride and malathion on airbreathing fish *Channapunctatus* (Bloch). *Ecotoxicol. Environ. Saf.*, **61**: 114-120.

Pan, G. and Dutta, H.M. (1998). The inhibition of brain

acetylcholinesterase activity of juvenile largemouth bass, *Micropterussalmoides* by sub-lethal concentrations of diazinon. *Environ. Res. Sec. A*, **79** : 133-137.

Patil, V.K. and David, M. (2008). Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). *Turk. J. Fish. Aquat. Sci.*, **8**: 233-237.

Rao, D.M.R. (1989). Studies on the relative toxicity and metabolism of endosulfan to the Indian major carp, *Catlacatla* with special reference to some biochemical changes induced by the pesticide. *Pest. Biochem. Physiol*., **33** : 220-229.

Rao, J.V., Begum, G., Pallela, R., Usman, P.K. and Rao, R.N. (2005). Changes in behavior and brain acetylcholinesterase activity in mosquito fish *Gambusiaaffinis* in reference to the sublethal exposure of chlorpyrifos. *Internat. J. Environ. Res. Public Health.*, **2** (3):478-483.

Ree, G.E.and Paney, J.F. (1997). Effect of toxaphene on reproduction of fish. *Chemosphere,***34**: 855-867.

Rice, J.P., Drews, C.D., Klubertans, T.M., Bradbury, S.P. and Coats, J.R. (1997). Acute toxicity and behavioural effects of chlorypyrifos, permethrin, phenol, strychnine and 2,4- dinitrophenol to 30 day old Japansemedaka (*Oryziaslatipes*). *Environ. Toxicol. Chem.*, **16** : 696-704.

Richmonds, C.R. and Dutta, H.M. (1992). Effect of malathion on the brain acetylcholinesterase activity of bluegill sunfish *Lepomismacrochirus*. *Bull. Environ. Contam. Toxicol.*, **49**: 431-435.

Samajdar, I. and Mandal, D.K. (2015). Acute toxicity and impact of an organophosphate pesticide, chlorpyrifos on some haematological parameters of an Indian minor carp, Labeo bata (Hamilton 1822). *Internat. J. Environ. Sci.*, **6** (1) :106 -113.

Scherrer, E.(1992). Behavioural responses as indicator of environmental alterations: approaches, results, developments. *J. Appl. Ichthyol.*, **8**: 122–131.

Scholz, N.L. Truelove, N.K. French, B.L. Berejikian, B.A. Quinn, T.P. Casillas, E. and Collier, T.K. (2000). Diazinon disrupts antipredator and homing behaviours in chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish Aquat. Sci.*, **57**: 1911–1918.

Shivakumar, R., Mushigeri, S.B. and David, M. (2006). Toxicity of endosulfan, cypermethrin and fenvalerate to freshwater fish, *Cyprinus carpio. J. Ecobiol.*,**19** (4): 313 - 316.

Shukla, M.(1995). Toxicological assessment of some common pollutants on a freshwater fish. Ph.D. Thesis, University of Gorakhpur, Gorakhpur, U.P. (INDIA).

Sprague, J.B. (1969). Measurement of pollutant toxicity to fish: I Bioassay methods for acute toxicity. *Water Res.*,**3**: 793 - 821.

Srivastav, A.K., Srivastav, S.K. and Srivastav, A.K. (1997). Response of serum calcium and inorganic phosphste of freshwater ctafish, *Heteropenustes fossilis*, to Chlorpyrifos. *Bull. Environ. Contam. Toxicol.*, **58**: 915 - 921.

Srivastav, V.K. and Singh, A. (2001). Studies of seasonal variation in toxicity of frequently used commercial organophosphate, carbamate and synthetic pyrethroids pesticides against freshwater fish Channa punctatus and behavioural responses to treated fish. *Malaysian Appl. Biol.*, **30** :17-23.

Srivastav, A.K., Srivastav, S.K., Mishra, D., Srivastav, S. and Srivastav, S.K. (2002). Ultimobranchial gland of freshwater catfish, *Heteropneustes fossilis* in response to deltamethrin treatment.*Bull. Environ. Contam. Toxicol.*, **68**: 584-591.

Srivastava, A.K., Mishra, D., Shrivastava, S., Srivastav, S.K. and Srivastav, A.K. (2010). Acute toxicity and behavioural responses of *Heteropneustes fossilis* to an organophosphate insecticide, dimethoate. *Internat. J. Pharma Bio. Sci.*, **1**(4): 359-363.

Susan, A.T., Sobha, K. and Tilak, K.S. (2010). A study on acute toxicity, oxygen consumption and behavioural changes in the three major carps, Labeo rohita (ham), Catla catla(ham) and Cirrhinus mrigala (ham) exposed to Fenvalerate. *Bioresearch Bull.*, **1** : 33-40.

Tadehl, H. and Häder, D.P. (2001). Automated biomonitoring using real time movement analysis of Euglena gracilis. *Ecotoxicol. Environ. Saf.*, **48**: 161-169.

Velmurugan, Selvanayagam, B.M., Cengiz, E.I. and Unlu, E. (2007). The effects of monocrotophos to different tissues of freshwater fish *Cirrhinusmrigala*. *Bull. Environ. Contam. Toxicol.*, **78** (6): 450-454.

Venkata, R.V., Vijayakumar, M. and Philip, G.H. (2008). Acute toxicity and behavioural changes in freshwater fish *Labeo rohita* exposed to deltamethrin. *J. Aquat. Biol.*, **23** (2) : 165-170.

Venkateswara, R.J. (2004). Effects of monocrotophos and its analogs in acetylcholinesterase activity's inhibition and its pattern of recovery on euryhaline fish, *Oreochromis mossambicus*. *Ecotoxicol. Environ. Saf.*, **59** : 217-222.

Venkateswara, R.J., Parvathi, K., Kavitha, P., Jakka, N.M. and Pallela, R. (2004). Effect of chlorpyrifos and monocrotophos on locomotor behaviour and acetylcholinesterase activity of subterranean termites, *Odontotermesobesus*. *Pest Manag. Sci.*, **61** : 417-421.

Wang, C., Lu, G., Gui, J. and Wang, P. (2009). Sublethal effects of pesticide mixtures on selected biomarkers of *Carassiusauratus*. *Environ. Toxicol. Pharmacol.*, **28**: 414 - 419.

Wasu, Y.H., Gadhikar, Y.A. and Ade, P.P. (2009). Sublethal and chronic effect of carbaryl and malathion on *Clarias batrachus* (Linn). *J. Appl. Sci. Environ. Manag.*, **13** (2) : 23-26.

Yadav, A., Neraliya, S. and Gopesh, A. (2007). Acute toxicity levels and ethological response of *Channastriatus* to fertilizer industrial wastewater. *J. Environ. Biol.*,**28** (2) : 159-162.