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EFFECT OF CHLORPYRIFOS ON THE BIOCHEMICAL COMPOSITION OF THE FISH, MYSTUS GULIO (Hamilton, 1822)

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ABSTRACT

Aquatic toxicology is the study of the effects of environmental contaminants on aquatic organisms, such as the effect of pesticides on the health of fish or other aquatic organisms. Fishes are exposed to pesticides in three primary ways: dermally through direct absorption by skin, direct uptake while breathing through gills during respiration and orally by drinking pesticide-contaminated water. Chlorpyrifos, a pesticide, is moderately toxic to humans, and exposure has been linked to neurological effects, persistent developmental disorders, and autoimmune disorders. In the present study, fingerlings of fish, *Mystus gulio* were chosen as test animals because of their easy availability and their importance as edible fishes. It has been calculated that LC_{50} values of CPF for 24h, 48h, 72h and 96h are 0.090 ppm, 0.080 ppm, 0.073 ppm and 0.070 ppm respectively. Jerks, erratic and uncoordinated movements and the tendency of fish to escape can be attributed to the damages in the brain tissues, suppression of AchE activity and low glycogen content which impair the function of brain. At higher concentrations (0.06 and 0.08ppm) as the exposure periods increases to 96h, the increased opercular movements associated with lethargy in swimming and increased surface activity are the indication of hypoxic conditions in the fingerlings due to pollution stress. In conclusion, studies on the effects of CPF on fish have diagnostic significance as the results obtained can be used to predict probable mechanisms of toxicity in human. Besides, fish have proven to be useful experimental models for the evaluation of the health of aquatic ecosystems exposed to environmental pollution and the associated biochemical changes.

KEY WORDS: Chlorpyrifos, Fish, pesticide, aquatic toxicology.

INTRODUCTION

Occupational exposure to pesticides is a common and alarming worldwide phenomenon. Various industrial and agricultural activities increase pollution, particularly in the aquatic environment, which is contaminated by various toxic chemicals from the discharge of waste waters and agricultural drainage.[1] These are responsible for multiple effects at the organisms, including humans, affecting organ function, reproductive status, species survival, population size, and ultimately biodiversity. Chronic sub lethal effects are also clearly relevant if the concentrations of these chemicals are below the acute threshold. Presently, over 100 organophosphates representing a variety of chemical, physical, and biological properties are being used for agricultural purposes.[2]

In India, pesticides constitute an important component in agriculture development and protection of public health since the tropical climate is very conducive to pest breeding.[3] Contamination by pesticides in aquatic ecosystem is a serious problem and fishes are more frequently exposed to these pollutants and may be taken in through gills, skin and contaminated foods.[4]

Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems.[5] Chlorpyrifos is a widely used organophosphate pesticide, second largest selling in India and used for more than a decade to control pests on cotton, paddy fields and vegetable crops.[6] Its extensive use may increase the toxicity load to aquatic environment, causing adverse effects on non-target organism, fish.[7]

Chlorpyrifos (O,O-diethyl-O-3,5,6-trichlor- 2-pyridyl phosphorothioate; CPF) is a broad spectrum organophosphate insecticide (OP) that is commercially used to control foliar insects that affect agricultural crops[8] and subterranean termites[9] CPF, since it was first introduced into the marketplace in 1965, has been used globally as an insecticide to control pests agriculturally and in the home. It is the second largest selling OP and found to be more toxic to fish than organochlorine compounds.[10] Earlier reports revealed that fish kill incidents in association with Chlorpyrifos in water reaching several hundred parts per billion.[11]

CPF has an average soil half-life of 30 days and two months in less alkaline soils. It also can persist indoors for weeks to months.[12] CPF passes via air drift or surface runoff into surrounding waters and gets accumulated in different aquatic organisms, particularly fish, adversely affecting them.[13]

CPF can enter the body either by inhalation of air containing CPF, ingestion of contaminated food or by dermal contact with CPF. It can cause acute poisoning and well known symptoms include myosis, increased urination, diarrhea, diaphoresis, lacrimation and salivation.[14] It is also reported to be involved in multiple mechanisms like causing hepatic dysfunction,[15] genotoxicity,[16] neurobehavioral and neurochemical changes.[17] CPF intoxication is shown to cause a significant decrease in the reduced glutathione (GSH), catalase (CAT) and glutathione S-transferase (GST) activities.[18]

CPF has been frequently detected in air, food and water. Although various standards exist to minimize its exposure in food and water, CPF is frequently used and bio-accumulates in certain scenarios.[19] The present study explains the probable adverse effects of pesticide Chlorpyrifos in the fish, *Mystus gulio*.

MATERIALS AND METHODS

Fingerlings of the fish, Mystus gulio, were chosen as test animals because of their easy availability throughout the year, survival capacities in the laboratory condition and their importance as edible fishes in India.

Classification

Domain: Eukaryota
Kingdom: Animalia
Phylum: Chordata
Subphylum: Vertebrata
Class: Osteichthyes
Order: Siluriformes
Family: Bagridae
Genus: *Mystus*
Species: *M.gulio* (Hamilton, 1822)[20]

Distribution

Asia: countries bordering the eastern Indian Ocean, from India to Indonesia and Vietnam. It has also been reported from Pakistan.[21]

Morphology

Body color bluish-brown on head and back, dull white below. Head depressed. Body elongated and compressed. Rough and granulated upper surface. Barbels – 4 pairs. Maxillary barbells extending beyond the pelvic fins, often to the end of the anal fin. Mouth terminal. Dorsal spine strong and serrated. Adipose fin small. Caudal fin forked and caudal peduncle equal at height.[21]

Biology

It's a brackish water fish found in swamps and lakes with a mud substrate.[22] Juveniles and adults feed on debris, zooplanktons, zoobenthos, other benthic invertebrates, fish eggs and larvae. It serves a s a food source.[23]

Collection and maintenance of the experimental fish

Live specimen of *M.gulio* fingerlings procured from fish farm at Padappai village, near Chennai, Tamilnadu, India, were collected and brought to the laboratory without any physical injury. The collected fishes were given a dip treatment in 1% $KMnO_4$ solution for few seconds to avoid any dermal microbial infection and then were rinsed with pure water. The fishes were screened for pathological signs, if any and kept in a big sterilized plastic trough containing well aerated and dechlorinated tap water for a period of two weeks at 27˚ ± 2˚C room temperature under the laboratory conditions.[24]

Acclimatization and feeding: During acclimatization, the fishes were fed once a day with egg white, groundnut oil cake, rice bran and minced meat, so as to avoid any possible effect of starvation on enzyme activities. The aquaria water was renewed daily after feeding, to prevent fungal infection due to accumulation of excess food and faecal matter. No mortality was recorded during acclimatization. Feeding was stopped 24h prior to and also during the course of acute toxicity study.[25]

Characteristics of test water

The physico-chemical characteristics of the holding water listed in the following table 1 were analyzed using standard method of APHA, 1995.

Table 1: Physico-chemical characteristics of the test water. (APHA, 1995)

Sr.No	Physico-chemical parameters	Value
1.	Total dissolved solids	1698 mg/I
2.	pH	8.06
3.	Alkalinity total ($CaCO^3$)	248 mg/I
4.	Total hardness ($CaCO^3$)	535 mg/I
5.	Calcium (Ca)	134 mg/I
6.	Magnesium (Mg)	55 mg/I
7.	Iron (Fe)	0.07 mg/I
8.	Free ammonia (NH_3)	0.29 mg/I
9.	Nitrite (NO_2)	0.01 mg/I
10.	Nitrate (NO_3)	4 mg/I
11.	Chloride (Cl)	565 mg/I

Preparation of stock solution: A commercial grade of Chlorpyrifos, 20% EC manufactured by ACCO industries, Haveli, was used in the present investigation. The stock solution was prepared by adding 1.0 ml of Chlorpyrifos 20% EC to 19.0 ml of distilled water, which denoted 10,000 ppm of pesticide dissolved. From this solution, 1.0 ml was pipette out and added to 9.0 ml of distilled water, which denoted 1,000 ppm of pesticide dissolved. From this solution, further 1.0 ml was pipette out and added to 9.0 ml of distilled water, which denoted 100 ppm of pesticide dissolved. Required amount of test solution as described in standard methods of examination of water and waste water were prepared from the above stock solution.[26]

Analytical procedure: Only healthy and active fish fingerlings of particular size of 6-7 cm in length and 8-9 g in weight were collected from the same fish farm and put in use for all the tests to find out the potency of pesticide pollutant Chlorpyrifos and to get information for the design and selection of dose levels. Static but renewal type of acute toxicity study was conducted after feeding the fish, to prevent any fungal infection and also maintain the optimum dissolved oxygen level.[24]

Screening test or Wide range finding test: The preliminary screening test was conducted to assess the highest concentration at which all experimental fishes survived for 24 h and likewise the lowest concentration at which most of the fishes dies simultaneously. Moreover, this test was conducted to avoid delay and to save time and effort. A wide range concentration viz. 0.1, 0.5, 1.0, 1.5, 2.0 ppm of Chlorpyrifos 20% EC solution were prepared from the stock solution and poured separately into the arranged five plastic troughs consisting of 10 fishes with 10 liters of water.[27] From the pilot study, it was that the wide range of

concentration of Chlorpyrifos has been laid between 0.001 ppm and beyond 1.5 ppm. The fingerlings could not withstand the effect of toxicity beyond 1.5 ppm and died within 24 hours. Below 0.01 ppm, no mortality was observed within 24 hours.

Definitive test or Narrow range finding test: Short-term tests for acute toxicity study over a period of 96h were performed on *M.gulio* fingerlings following the static but renewal bioassay methods. For experimental purpose, randomly selected acclimation healthy fingerlings were taken, divided into eight groups having 10 fingerlings in each and put separately into 8 plastic troughs with 10 liters of water in each. Freshly prepared different doses of Chlorpyrifos solution viz., 0.005 and 0.010 ppm were poured into plastic troughs and the ninth plastic trough with 10 fishes was also kept with them as control without pesticide solution. Normal photoperiod was maintained during the course of toxicity works and the loading of fishes in the test trough was according to the recommendation given by USEPA, 1975.[27]

Calculation of LC_{50}: The LC_{50} value was determined by interpolation of different log dose of selected pesticide, Chlorpyrifos against the probit value of percentage mortality of fish for every 24h up to 96h using probit analysis method.[28] The formula used to calculate LC_{50} was as follows

$Sxx = \Sigma x^2 - 1/n (\Sigma x)^2$

$Sxy = \Sigma xy - 1/n (\Sigma x) (\Sigma y)$

$B = Sxx/Sxy$

$M = (Yp - Y) b + x$

LC_{50} = Antilog m / 100

Where,

Y = Probit value of the percentage of mortality at a particular period of time for a given dose.

X = Logarithm value of administered dose (D) multiplied by 100. (log D X 100)

Yp = Probit value for 50 percent mortality

LC_{50} = Median lethal concentration for that particular period

Calculations of sub-lethal doses: The $1/5^{th}$ and $1/10^{th}$ sub-lethal doses were extrapolated on the basis of calculation of LC50 and were found to be 0.005 ppm and 0.010 ppm respectively. A weekly interval for sub-lethal toxicity study was selected as 7, 14 and 21 days. The toxic

effects of Chlorpyrifos on *M.gulio* were found out biochemically at the end of each exposure period in the control and experimental fishes.[29]

Studies on the behavioral response of *M.gulio* against Chlorpyrifos: The acute toxicity of Chlorpyrifos at different concentration viz., 0.02, 0.04, 0.06, 0.08 and 0.10 ppm on the behavioral changes of the fish *M.gulio* at different time intervals (24, 48, 72 and 96h) were observed and recorded. Beyond 0.10 ppm dose level, no mortality of fish was observed.

Assay of Acetylcholinesterase (AchE) activity: Acetylcholinesterase activity was assayed by the method of Ellman *et al*, 1961.[30] The AchE enzyme activities in brain and significant inhibiton and exposure periods in the treated fish were observed.

Assay of Alkaline phosphatase activity: Alkaline phophatase activity was assayed by the method of King and Armstrong (1934).[31] The alkaline phosphatase enzyme levels in the muscle of the treated fish at sub-lethal toxic stress of Chlorpyrifos were recorded.

RESULTS AND DISCUSSION

Acute bioassay of *M.gulio* exposed to Chlorpyrifos: Acute toxicity tests are used to assess the potential hazards of chemicals in aquatic organisms. In the present study, the LC_{50} values for different exposure hours are presented in the table 2. The percentage mortality in different concentrations is found to be dependent on both time and concentrations. There is no mortality in the control as well as at the lower concentrations below 0.02 ppm up to 96h.

Table 2: Acute bioassay of *M.gulio* exposed to Chlorpyrifos 20 EC

Exposure period (hours)	LC_{16} (ppm)	LC_{50} (ppm)	LC_{84} (ppm)	Log LC_{50}	Regression equation	95% confidence limit		Chi-square value
						Lower	Upper	
24	0.337	0.090	0.012	-1.046	Y-3.17+0.974x	0.040	0.199	8.58
48	0.030	0.080	0.126	-1.086	Y-3.29+0.756x	0.032	0.207	8.86
72	0.024	0.073	0.132	-1.139	Y-3.39+0.694x	0.024	0.216	9.08
96	0.020	0.070	0.142	-1.155	Y-3.41+0.541x	0.090	0.246	9.22

The results have thus clearly indicated that the percentage mortality increases with an increase in toxicant concentrations and also with the increased exposure periods. The LC_{50} values of CPF for 24h, 48h, 72h and 96h were calculated as 0.090 ppm, 0.080 ppm, 0.073 ppm and 0.070 ppm respectively.

The other causes of death of fish owing to lethal action of CPF are perhaps due to the toxic effect particularly on the biochemical processes related to cellular metabolic pathways, thus leading to mortality of the test fish.[32]

Behavioral response of *M.gulio* against Chlorpyrifos: Behavioral responses of *M.gulio* during acute toxicity tests are presented in table 3. In control as well as in the lower concentrations, no abnormal signs are observed. However, a number of behavioral changes were shown on exposure to higher concentrations.

Table 3: Behavioral responses of *M.gulio* during acute toxicity tests

Sr.No	Concentration (ppm)	Exposure period (hours)			
		24	48	72	96
1.	Control	no abnormal signs observed	no abnormal signs observed	no abnormal signs observed	no abnormal signs observed
2.	0.05	jerky and irregularly erratic movements	loss of equilibrium and negative geotaxis	Increased opercular movements	high levels of excitation
3.	0.10	lateral tilting of the body and lethargic swimming	black pigmentation and over secretion of mucous in gill and body region	Light convulsion in caudal region	rests at the bottom of the trough and shows hyperactivity

The erratic swimming, rapid jerky movements, lateral lying position and loss of equilibrium shown by the treated *M.gulio* in the experiment can be attributed to the impairment or disturbed nervous system and emission of abnormal impulses and obstructed functions of neurons due to the neurotoxic effect of Chlorpyrifos.[33]

Effect of AchE in brain: Table 4 gives the data of AchE in the brain of the treated fish at sub-lethal toxic stress of Chlorpyrifos after 7th, 14th and 21st day's exposure to sub lethal concentration of 0.005 ppm and 0.010 ppm of Chlorpyrifos in the experimental fish. On exposure to Chlorpyrifos toxicity, a decreasing trend in AchE activity in brain is recorded during the long terms exposures. When exposed to 0.005 and 0.010 ppm of Chlorpyrifos, a maximum reduction in AchE activity over to control was observed.

Table 4: Estimation of Chlorpyrifos on the AchE in brain of *Mystus gulio*

Concentration (ppm)	Exposure period (days)			Mean
	7	14	21	
Control	4.626	4.702	4.710	4.679
0.005	2.516 (27.35)	2.122 (46.81)	3.534 (63.52)	2.724
0.010	2.208 (47.31)	2.812 (65.78)	3.518 (84.36)	2.846

Values in the parenthesis represent percentage decrease over the control.

A decrease in AChE activity causes acetylcholine accumulation within synapses, leading to the impairment of important functions such as swimming, feeding and general behavior.[34] Further, AChE inhibition may be associated with cell damage and lower AChE expression in the nervous system.[35, 36]

Effect of Alkaline phosphatase enzyme in muscle: Table 5 gives the data on alkaline phosphatase enzyme level of muscle in the treated fish at sub-lethal toxic stress of Chlorpyrifos after 7th, 14th and 21st day's exposure to sub-lethal concentrations of 0.005 ppm and 0.010 ppm of Chlorpyrifos in both control and experimental fish. On long term exposures to Chlorpyrifos toxicity, a decreasing trend in the liver alkaline phosphatase enzyme content is recorded. The pesticide poisoning altered the activity of alkaline phosphatase which indicates changes in the enzyme activity due to toxicity stress.[37]

Table 5: Estimation of Chlorpyrifos on the alkaline phosphatase activity (moles/min/mg) in myscle of *Mystus gulio*

Concentration (ppm)	Exposure period (days)			Mean
	7	14	21	
Control	0.0312	0.0302	0.0314	0.0309
0.005	0.0294 (-1.68)	0.0273 (-3.39)	0.0262 (-7.89)	0.0276
0.010	0.0278 (-7.18)	0.0269 (-9.56)	0.0258 (-15.32)	0.0268

Values in the parenthesis represent percentage decrease over the control.

CONCLUSION

It is evident that Chlorpyrifos presented in aquatic ecosystems can affect aquatic fauna in different ways. Alterations in physico-chemical properties of water, destruction of delicate balance in the environment, entry in to the food chains and physiological damage to the vital tissues of aquatic fauna are the threatening issues of the modern day pesticides. Long term exposure to these products causes countless abnormalities and reduces the life span of organisms.[38] Thus, it can be concluded that Chlorpyrifos is highly toxic to the fishes and causes adverse effects and even mortality of the fish at both lethal and sub-lethal concentrations.

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