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Determination of chlorpyrifos residue in water and liver and muscle of *Clarias batrachus* by HPLC with UV detection

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Abstract : The present study was conducted for the determination of chlorpyrifos residues in water and tissue (liver and muscle) of the fish *Clarias batrachus* by HPLC with UV detection. The fish were exposed to 1/5th sublethal concentration of chlorpyrifos i.e., 0.258 mg/L for 96 hr. Water was collected at 0, 24, 48, 72 and 96 hr after the addition of toxicant to correlate the relationship between the concentration added and the concentration left in water. The liver and muscle tissues were collected after 24, 48, 72 and 96 hr for determination of chlorpyrifos residue accumulated in the tissue. Samples were extracted, cleaned up and purified. There was a decrease of chlorpyrifos residue in water and increase in concentration of chlorpyrifos in liver and muscle with increase in time of exposure, which is a sign of bioaccumulation of the toxicant in the fish through uptake. Also, the accumulation was more in the liver than muscle of the fish.

Keywords : Chlorpyrifos, HPLC, *Clarias batrachus*, bioaccumulation

INTRODUCTION

Pesticides are global pollutant of the aquatic environment and have detrimental effects on the living resources. The pesticides which are discharged into the aquatic ecosystem can contaminate fish and in turn man.[1] Aquatic organism's response to insecticides depends on the compounds, exposure time, water quality and species.[2-4] Many pesticides have been banned in India and other advanced countries due to their persistence and toxicity towards non-targeted organisms. Hence, production and use of permitted insecticides could increase considerably.[5] Mortality is obviously not the only endpoint to consider and there is a growing interest in the estimation of residues of the pesticides in the non-target beneficial organism to assess the sub-lethal effects of toxicant. Residue analysis helps to provide information on the action and effects of this toxicant on aquatic life especially in fish. In the present study, an attempt has been made to quantitively evaluate the residue of chlorpyrifos, O, O-diethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate; an organophosphate insecticide on physiologically important tissues of fish, viz. liver and muscle, exposed to sublethal concentration (0.258mg/L; 1/5th of LC_{50}) and also to study the residue of chlorpyrifos left in water after its addition. The fish selected for the present investigation was the walking catfish, *Clarias batrachus* L., an edible fish which is economically important in pisciculture.

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MATERIALS & METHODS

Fish collection and acclimatization

Healthy walking catfish, *C. batrachus* measuring 35.7±7.7 g in weight and 17.1±1.4 cm in length were bought from the local fishermen from Ranchi district, Jharkhand India. The fishes were subjected to a prophylactic treatment by bathing twice in 0.05% $KMnO_4$ for two minutes to avoid dermal infection. The specimens were kept in glass aquaria containing 20L dechlorinated tap water and were left for acclimatization for two weeks under laboratory condition in semi-static system. The experiment was set under the photoperiod of 13hr light and 11hr dark along with temperature of 24.93±1.8ºC, pH 7.07±0.33 and dissolved oxygen 8.08±1.6 mg/L. Fishes were fed with commercial diet twice daily and feeding was stopped 24hr before the experiment.

Test solution

For the present study both the technical grade pesticide and commercial formulation of chlorpyrifos (20% EC) with trade name ELDRIN^{TC} manufactured by Crystal Crop Protection Ltd. was purchased from the local pesticide market from Ranchi and used.

Experimental design

Previous study conducted by Kujur *et al*. (2019)[6] reported LC_{50} 96hr value of commercial grade of chlorpyrifos in *C. batrachus* to be 1.29 mg/L. In the present study 1/5 of $LC5_0$ 96hr value (0.258 mg/L) was taken and added to the aquarium for determination of residue analysis. Liver and muscle were collected after addition of toxicant at 24, 48, 72 and 96hr for conducting the experiment. Water was analyzed for the concentration of chlorpyrifos at 0, 24, 48, 72 and 96 hr.

Residue analysis

Water analysis- Water samples were collected in 500mL amber glass bottles. The amount of chlorpyrifos residue was analyzed using HPLC following the method described by Hasanuzzaman *et al.* (2017)[7] with some modifications.

Tissue analysis- The experimental fish were rinsed with distilled water prior to the dissection to avoid the external pesticide residue. Fish were dissected and liver and muscle were collected. At a time, three fishes were taken and their tissues were pooled for the analysis. Extraction of chlorpyrifos from fish tissue was performed by the method of Rao *et al*., (2003)[5].

HPLC condition

The amount of chlorpyrifos residues in water, liver and muscle tissues were analyzed using HPLC (Shimadzu class VP.V6.10) fitted with UV detector. The mobile phase used was a water- acetonitrile gradient (70% acetonitrile +30% water in 0.1% 1M acetic acid). The flow rate was 1.5 mL/minute and the run time was 15 minutes. C_{18} column (Chiralpak IB- 250 mm x 4.6 mm, with particle size of 5µm) programmed at 25°C was used. The eluents were monitored by UV detection at wavelength of 280 nm.

Identification and quantification

The compound was identified by comparing its retention time with respect to technical grade chlorpyrifos standard. Quantification of chlorpyrifos residues was done by calibration curve drawn from chromatographic experiments with standard solution of the pesticide.

RESULTS

Figure 1 shows the standard HPLC chromatogram of chlorpyrifos. The retention time of chlorpyrifos was 4.992 minute. The calibration curve was generated using linear regression of the measured peak areas vs. the concentrations of the calibration standards (in µg/mL) (Fig 2). The correlation coefficient (R^2) was 0.9949

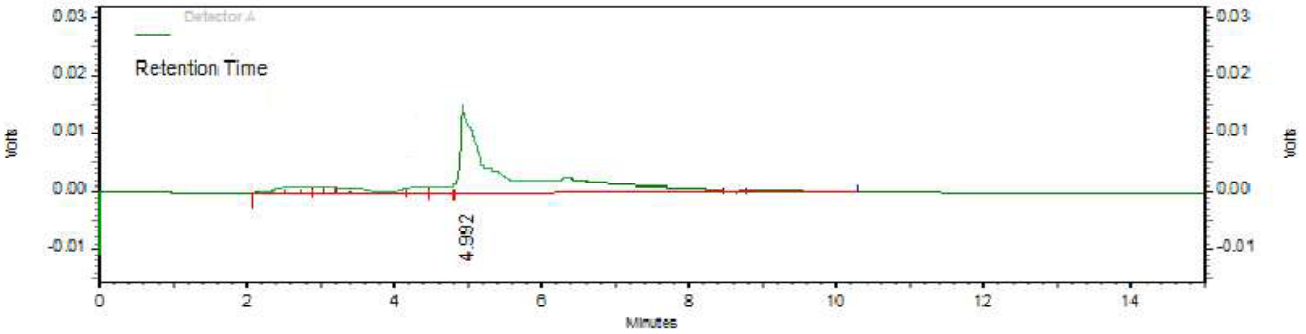

Figure 1: Standard HPLC chromatogram of chlorpyrifos.

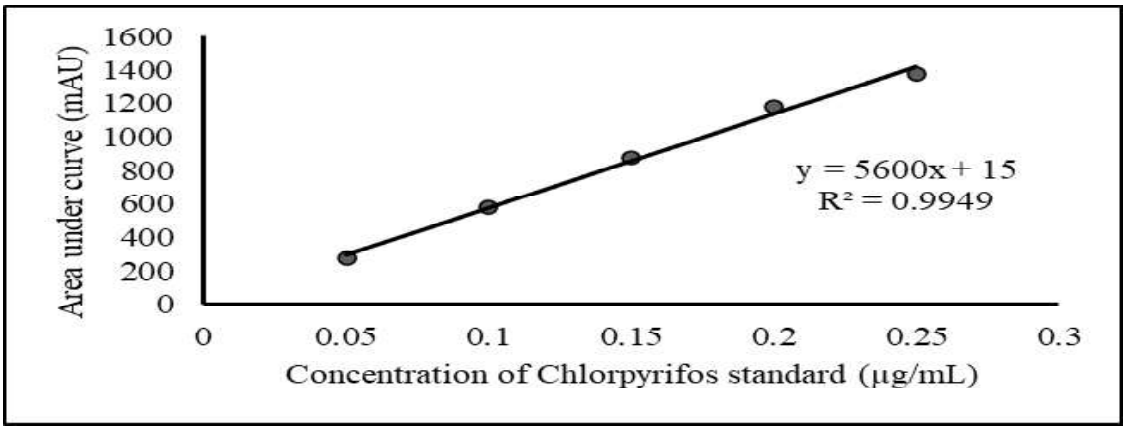

Figure 2: Calibration curve of standard chlorpyrifos.

Table 1: Bio-accumulation of chlorpyrifos in water, liver and muscle of fish *Clarias batrachus*, during sublethal exposure to 0.258 mg/L (1/5 of LC_{50}) for a period of 96 hr.

Exposure Period (Hours)	Bioaccumulation in different fish tissue						Residue present in water	
	LIVER			MUSCLE				
	Area (mAU)	Concentration (ug/g)	BCF*	Area (mAU)	Concentration (ug/g)	BCF	Area (mAU)	Concentration (ug/mL)
0 hr							1147025	204.82
24 hr	47025	8.394	0.032	3379	0.6007	0.002	967025	172.6380
48 hr	87025	15.5375	0.060	3711	0.66	0.002	747025	133.3946
72 hr	957025	170.89	0.662	5084	0.9051	0.003	477275	85.225
96 hr	997025	178.035	0.690	6332	1.1280	0.004	21786	3.88
Average BCF values ± SD			0.361 ±0.364			0.002 ±0.0009		

The presence of chlorpyrifos in water was measured after 0, 24, 48, 72 and 96 hr and in liver and muscle chlorpyrifos residues was measured after 24, 48, 72 and 96 hr (Table 1). The amount of chlorpyrifos added to the water initially was 1/5 of LC_{50} 96 hr (0.258 mg/L). Chlorpyrifos residues in water, liver and muscle of fish *Clarias batrachus* is given in Table 1. Table 1 clearly shows that the pesticide residue in the water decreases, while in liver and muscle of fish pesticide residue increases with the lapse of time during the experiment. It is also clear from the results that the accumulation of chlorpyrifos is more in the liver than in muscle.

DISCUSSION

The present study reports the chlorpyrifos residues detected in water, liver and muscle of *C. batrachus* after sublethal exposure of 1/5 of LC_{50} 96 hr (0.258 mg/L) for 96 hr. chlorpyrifos residue in water refers to its concentration left in the water after it has been added to the water. Concentration of chlorpyrifos remained is analysed to correlate the relationship between the concentration added and the concentration remained.[8] The presence of chlorpyrifos residue in water after 0, 24, 48, 72 and 96 hr were shown in Table 1. A quick degradation of the pesticide was noticed. Manjunatha *et al*. (2015)[8] also observed a gradual decrease in the chlorpyrifos residues in water during the length of exposure. Saad *et al*. (1982)[9] also showed that organophosphorus compounds quickly degrade in the aquatic environment. According to Dow Agro Sciences (2008)[10] chlorpyrifos degrade by hydrolysis and photolysis occurring at moderate rates under neutral conditions with half -lives of about a month at a neutral pH and 25ºC.

According to Tilak *et al.* (2004)[11] residues of organophosphate insecticides in fish species and water depend on the physicochemical characteristics of water, time of consumption, pH of the water and ambient temperature. Various studies have been done indicating the direct toxic effects of chlorpyrifos on various tissues of non- target organisms especially fish. However, chlorpyrifos also indirectly effects by its accumulation in various tissues. In the present study accumulation of chlorpyrifos in liver and muscle of *C. batrachus* was observed after addition of toxicant at 24, 48, 72 and 96 hr. Continuous exposure of fish to chlorpyrifos resulted in bioaccumulation of pesticide in the body of the fish and accumulation was found to be more in liver than in muscle (Table 1). Variation in residue analysis is attributed to factors such as differences in the uptake rate and lipid content of the respective animal tissue. Liver serves as the main detoxifying organ containing relatively high levels of detoxifying enzymes. It is also the first organ to face the effect of pesticides being carried through the portal circulation,[12] which might have been the cause of the greater accumulation of chlorpyrifos in liver than in the muscle.

Liver rapidly synthesizes monooxygenase enzymes during exposure to organic contaminants. Monooxygenase enzymes are found in high concentration in liver, gonads, kidney, intestine, gill and heart.[13] The monooxygenase enzyme facilitates excretion of the pollutants by decreasing the lipid solubility of organic contaminants.[14] The study conducted by Begum *et al.* (1994)[15] on dimethoate bioaccumulation in liver and muscle tissue on *Clarias batrachus* by GC-MS also observed a similar result of higher accumulation in liver than in muscle. In the study conducted by Nagaraju and Rathnama (2014)[12] on residue analysis of carbamate in *Labeo rohita* by GLC-FID, they found that order of carbosulfan residue in different tissues is gill> kidney> liver> muscle at the end of 15 days.

The factor of bioconcentration in liver and muscle were calculated based on the concentration added in the medium (water) and noticed gradual increase in the length of exposure. It may be due to constant vulnerability of fish to chlorpyrifos and subsequent reduction of chlorpyrifos residues in water and their uptake by the fish tissues.

CONCLUSION

The present study correlated the relationship between the amount of chlorpyrifos added to the water and the amount remained or accumulated in the tissue. The decrease of chlorpyrifos concentration in water and the relative increase in liver and muscle with increase in time of exposure is an indicator of the accumulation of the toxicant in the organism through uptake. This could be hazardous as it could make its way into the food chain. In the present study, the individual secondary metabolites were not considered and further studies are necessary for quantification of the metabolites. A regular surveying must be done on fishes and other aquatic species before the extensive use of this pesticide is permitted to provide a hazard free environment to the aquatic biota and to provide a safe and healthy supply of fish for human consumption.

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