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Research Article
Chlorpyriphos 20% EC Induced Alteration in Haematological Parameter of Freshwater Fish, *Clarias batrachus* (Linnaeus, 1758)

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Abstract

Background and Objective: Indiscriminate use of pesticides may alter the haematological parameters cause's health hazards in fishes which ultimately affects the physiological condition of the human being. Therefore, the objective of the work was to elucidate the Chlorpyriphos 20% EC induced alteration in haematological parameter to a freshwater fish, *Clarias batrachus* (Linnaeus, 1758) at its lowest and highest concentrations during 96 hrs static bioassay. **Materials and Methods:** A 96 hrs full-scale static bioassay test has been performed to the known range of concentrations of Chlorpyriphos 20% EC to calculate LC_{50} values, presumable safe and safe dischargeable concentrations. Blood samples were collected through the caudal vein and haematological analysis were done. Data were statistically analyzed through software, SPSS (Version 16) and Graph Pad Prism software (version-7). **Results:** The 96 hrs LC_{50} values and Confidence Ratio (R) for Chlorpyriphos 20% EC to the *Clarias batrachus* was estimated to be 217.755 and 1.294 ppb, respectively by Finney's Probit Analysis method. However, safe or harmless and safe dischargeable concentration was also calculated as 77.604 and 1.056 ppb, respectively. The percent alteration (%) in Dissolve Oxygen (DO) content and Free CO_2 was observed as 8.108 and 21.621 mg L^{-1} and 15.116 and 76.744 mg L^{-1} respectively for Control and 320 ppb concentrated (tested) toxicant solution after 96 hrs. Furthermore, the decreasing trend in RBC ($\times 10^6$ mm^3), WBC Count ($\times 10^3$ mm^3), PCV (%) and Haemoglobin (g dL^{-1}) were observed whereas MCH (pg/Cell), MCHC (g dL^{-1}) and Glucose Levels (mg dL^{-1}) were found to be increased during an investigation from control to 320 ppb during the course of bioassay. **Conclusion:** Chlorpyriphos 20% EC are extremely toxic to a freshwater fish, *Clarias batrachus* (Linnaeus, 1758) which alter the haematological parameters.

Key words: Chlorpyriphos 20% EC, *Clarias batrachus*, LC_{50} values, haematological parameter, glucose level

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pesticides may enter into the food chain, induced functional damage[1] and thus modulate the performance of the organism[2]. In other words, pesticides silently interrupt the community structure and function of ecosystem[3] and also contaminate ground and surface water[4]. The extensive use of various pesticides for pest control required attention for large scale investigations on the way of toxic actions of pesticides in aquatic animals, particularly[5]. The previous study exhibits the physiological variations in the values of some haematological parameters of aquatic fauna when aquatic quality is affected by contaminants[6,7].

Recently, the use of haematological parameters has become more appropriate during the assessment of the acute toxicity of pesticides in aquatic animals because of maintaining homeostasis and supporting the vital functions in fish species. Alteration in haematological properties also helps to diagnose the structural and functional status of animals exposed to the toxicants[8]. Haematological parameters are effectively used as an important diagnostic tool to evaluate the status of fish health[9]. There is serious disagreement in physiological and biochemical responses that may occur during this condition which creates harmful effects on fish's health[10]. Chlorpyriphos is a broad-spectrum organo-phosphorus insecticide used against plants, animals and humans pests worldwide. If, it enters into the aquatic ecosystem may exert toxic effects on non-target organisms[11]. Chlorpyriphos is reported to be highly toxic to aquatic animals, mobile in the environment and is the most identified pesticide in ponds, rivers, streams and reservoirs[12,13]. However, adequate literature on Chlorpyriphos toxicity regarding the sub-lethal concentration in the haematological parameter alteration on *Clarias batrachus* is scanty. Therefore an effort has been made to elucidate the Chlorpyriphos 20% EC induced alteration in haematological parameter to a freshwater fish, *Clarias batrachus* (Linnaeus, 1758) at its lowest and highest concentrations during 96 hrs static bioassay

MATERIALS AND METHODS

Study area: This study was conducted at Zoology Department Lab, J.P. University, Chhapra from April-July, 2019.

Water quality parameter: Dissolve Oxygen (DO) content (mg L^{-1}) of the toxicant solution were measured at the interval of 0, 24 and 96 hrs by using Winkler Method. Furthermore, the free CO_2 was analyzed at the interval of 0, 24 and 96 hrs by the titrimetric method using phenolphthalein indicator and NaOH titrant.

Experimental fish: Much healthy freshwater fish, *Clarias batrachus* were collected and acclimatized separately in the cemented container (500 L capacity) for 20 days. Experimental fishes (6.00±0.7 cm) subjected to the bioassay tests, were not provided food materials during the exposure period to avoid any influence on the toxicity of the selected toxicant.

Toxicant solution: The stock solution for Chlorpyriphos 20% EC were prepared as per standard Eq:

$$N_1V_1 = N_2V_2$$

where, N_1 is the Concentration of selected pesticide, V_1 is the Volume of selected pesticide, N_2 is the Required concentration of pesticide to be prepared and V_2 is the Volume of solution required for application

Whereas, the series of different concentrations (in ppb) of Chlorpyriphos 20% EC was prepared by adding stock solution into the measured diluents water by using a micropipette.

Exposure system: The full-scale bioassay test to evaluate the acute toxicity (96 hrs) for Chlorpyriphos 20% EC to a freshwater fish, *Clarias batrachus* were conducted in a 4 L glass container with experimental water of pH 7.62±0.4.

Preliminary or screening tests: The screening tests of Chlorpyriphos 20% EC to a freshwater fish, *Clarias batrachus* were conducted to investigate the critical concentration range. During full-scale bioassay, the test range for Chlorpyriphos 20% EC was taken between the highest and lowest concentrations at which most of the experimental fishes died or survived within a specified exposure duration (24, 48, 72 and 96 hrs).

Full-scale bioassay test: It is based upon the known range of various concentrations of Chlorpyriphos 20% EC as determined in preliminary or screening tests test. The test was carried out in test containers filled with four-litre toxicant solution were designed in three rows. Each test container was labelled with the details of the experiment (viz, date and time of the experiment, replicate number and concentration of selected toxicant). There are ten acclimatized test fishes, *Clarias batrachus* were transferred to these jars after about

30 min of the preparation of toxicant solutions. The experiment was run simultaneously along with controls for 96 hrs duration. The test solutions were changed by fresh toxicant solutions every 24 hrs. The number of tested fishes that died in various concentrations of Chlorpyriphos 20% EC was carefully observed, removed and noted at the intervals of 24, 48, 72 and 96 hrs. The LC_{50}'s and 95 percent confidence limits for selected organophosphate were analyzed at different concentrations and time intervals (viz, 24, 48, 72 and 96 hrs) by Probit Analysis methods utilized by Garakouei *et al.*[14]. Whereas, presumable safe and safe dischargeable concentrations were calculated as per standard formula documented by Nwani *et al.*[15].

Blood collection and haematological analysis: Blood samples were collected through caudal vein from randomly selected (3-5) controlled and tested fish (survived) by using syringes and transferred to EDTA coated tube. Haematological analysis viz, Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Haemoglobin (Hb), Packed Cell Volume (PCV) was carried out as per the standard method described by Jimoh *et al.*[16]. A Blood Glucose level was analyzed by Glucometer Strips (Accu-Chek Active, Ahmedabad, Gujarat, India). However, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated by using the RBC count, Hb and PCV data as per the standard formula cited by Dahiya *et al.*[17]:

$$\text{MCV } (\mu m^3) = \frac{\text{PCV } (\%)}{\text{RBC } (\times 10^6 \text{ mm}^3)} \times 10$$

$$\text{MCH (pg)} = \frac{\text{Haemoglobin (g dL}^{-1})}{\text{RBC } (\times 10^6 \text{ mm}^3)} \times 10$$

$$\text{MCHC (g dL}^{-1}) = \frac{\text{Haemoglobin (g dL}^{-1})}{\text{PCV } (\%)} \times 100$$

Statistical analysis: The data's of Table 1 were subjected to statistical software, SPSS (Version 16) to calculate the LC_{50} values, Confidence Ratio (R) and Probit equation. However, SD and SEM analysis were carried out by using Graph Pad Prism software (version-7).

RESULTS

The percent (%) mortality of *Clarias batrachus* was analysed during 96 hrs exposure of various concentrations of Chlorpyriphos 20% EC in Table 1. The lowest and highest mortality has been observed as 20 and 90% at 180 and 320 ppb concentrated toxicant solution. The LC_{50} value of Chlorpyriphos 20% EC for 24, 48, 72 and 96 hrs to *Clarias batrachus* fishes were estimated as 288.482, 258.682, 232.938 and 217.755 ppb whereas, the confidence Ratio (R) for the same were noticed as 1.371, 1.272, 1.314 and 1.294, respectively in Table 2. The R-value noticed less than 2, illustrates the accuracy of the estimate that should be expected from the replicate. The Safe or harmless and Safe dischargeable concentrations were estimated as 77.604 and 1.056 ppb respectively (Table 2).

Moreover, a decline in Dissolve Oxygen (DO) content and an increasing trend in free CO_2 was noticed in the current investigation from 0-96 hrs during bioassay in Table 3. The DO content was analyzed as 7.40 and 6.80 mg L^{-1} at 0 and 96 hrs respectively for control however, the percentage (%) alteration was noticed as 8.108 mg L^{-1}. Furthermore, DO content was estimated for 0 and 96 hrs as 7.40 and 5.80 mg L^{-1} at 320 ppb concentration of selected toxicant. Similarly, free CO_2 were noticed for control as 4.30 and 4.95 mg L^{-1} and at 320 ppb concentration of Chlorpyriphos 20% EC these values were noticed as 4.30 and 7.60 mg L^{-1} at 0 and 96 hrs respectively. However, the percentage (%) alteration was calculated for control and 320 ppb concentrated toxicant solution as 15.116 and 76.744 mg L^{-1}, respectively.

Eventually, the alteration of haematological parameters was observed in Chlorpyriphos 20% EC exposed test fishes at lowest (180 ppb) and highest concentrations (320 ppb) as compared to control during 96 hrs of static bioassay. The mean value of RBC ($\times 10^6$ mm^3) was observed for Control, 180 ppb and 320 ppb concentrated toxicant solution as 2.72, 2.40 and 2.16 however, the mean value of WBC Count ($\times 10^3$ mm^3) were reported to be 18.70, 18.10 and 16.80, respectively. Further, the mean value of PCV, Haemoglobin and MCV were documented for Control, 180 and 320 ppb concentrated toxicant solution as 24.00, 22.80 and 18.32% and 7.80, 7.50 and 7.10 g dL^{-1} and 88.23, 95.00 and 84.81 μm^3, respectively. However, the mean value of MCH (pg/Cell) and MCHC (g dL^{-1}) were analyzed as 28.67, 31.25 and 32.87 and 32.50, 32.89 and 38.75 for Control, 180 and 320 ppb concentrated toxicant solution respectively. In addition, Glucose Levels estimated to be 60.50, 65.20 and 86.60 mg dL^{-1}) for Control, 180 and 320 ppb concentrated toxicant solution respectively in Table 4.

DISCUSSION

Fishes are utilized as a model animal for eco-toxicological assessment mainly due to sensitiveness towards a very low

Table 1: Number and percent (%) mortality of *Clarias batrachus* (Linnaeus) in various concentrations of chlorpyriphos 20% EC during 96 hrs bioassay test

Chlorpyriphos 20% EC concentrations (ppb)	No. of exposed fishes	Duration of exposure				Mortality (%) (96 hrs)	Survival (%) (96 hrs)
		24 hrs	48 hrs	72 hrs	96 hrs		
Control (0.00)	10					00	100
180	10	0	0	1	2	20	80
210	10	2	4	5	5	50	50
240	10	3	4	6	7	70	30
280	10	5	6	7	8	80	20
320	10	6	8	9	9	90	10

*Each observation is a total of three replicates (n = 3)

Table 2: Median lethal concentrations (LC_{50} Value) of chlorpyriphos 20% EC (ppb) for 24, 48, 72 and 96 hrs to *Clarias batrachus* (Linnaeus)

Duration (hrs)	Chlorpyriphos 20% EC (ppb)	LCL	UCL	R	Probit equation (P = intercept+BX)	Safe or harmless (ppb) concentration
24	288.482	259.454	355.873	1.371	-3.914+0.014X	77.604
48	258.682	231.074	294.091	1.272	-3.897+0.015X	Safe dischargeable concentration (ppb)
72	232.938	198.428	260.690	1.314	-3.525+0. 015X	1.056
96	217.755	185.487	240.123	1.294	-4.120+0. 019X	

UCL: Upper confidence limits, LCL: Lower confidence limits and R: Confidence ratio (UCL/LCL)

Table 3: Dissolve oxygen (DO) content (mg L^{-1}), free CO_2 (mg L^{-1}) and percentage (%) alteration during 96 hrs static bioassay test

Exposure duration	Dissolve oxygen (DO) content (mg L^{-1})		Free CO_2 (mg L^{-1})	
	Control	Highest concentration of chlorpyriphos 20% EC (320 ppb)	Control	Highest concentration of chlorpyriphos 20% EC (320 ppb)
0 hrs	7.40±0.0503	7.40±0.0917	4.30±0.0721	4.30±0.1311
24 hrs	7.20±0.0451	6.20±0.1250	4.60±0.1000	7.00±0.1457
96 hrs	6.80±0.0721	5.80±0.1604	4.95±0.1082	7.60±0.1908
Percentage (%) alteration (after 96 hrs)	8.108	21.621	15.116	76.744

Table 4: Alteration of haematological parameters in chlorpyriphos 20% EC exposed *Clarias batrachus* (Linnaeus) at lowest (180 ppb) and highest concentrations (320 ppb) after 96 hrs during bioassay test

Haematological parameter	Control (0.00) mean (SD±SEM)	Chlorpyriphos 20% EC concentration (ppb)		Percentage (%) alteration
		180 ppb mean (SD±SEM)	320 ppb mean (SD±SEM)	
RBC (×10^6 mm^3)	2.72 (0.02080±0.0120)	2.40 (0.09170±0.0529)	2.16 (0.14420±0.0833)	20.588
WBC count (×10^3 mm^3)	18.70 (0.60000±0.3464)	18.10 (0.79750±0.4604)	16.80 (2.13180±1.2308)	10.160
PCV (%)	24.00 (0.44000±0.2540)	22.80 (0.65020±0.3754)	18.32 (2.00010±1.1548)	23.666
Haemoglobin (g dL^{-1})	7.80 (0.32000±0.1848)	7.50 (0.42702±0.2466)	7.10 (0.72000±0.4157)	8.974
MCV (µm^3)	88.23 (2.14430±1.2380)	95.00 (3.76001±2.1708)	84.81 (6.78167±3.9154)	3.876
MCH (pg/cell)	28.67 (1.03201±0.5958)	31.25 (1.67434±0.9666)	32.87 (1.34610±0.7771)	+14.649
MCHC (g dL^{-1})	32.50 (1.93011±1.1143)	32.89(0.93602±0.5404)	38.75(4.31358±2.4904)	+19.230
Glucose levels (mg dL^{-1})	60.50 (0.30000±0.1732)	65.20 (2.60000±1.5011)	86.60 (4.20360±2.4269)	43.14

concentration of toxicants and its ability of bioaccumulation[18]. However, the differences in acute toxicity of any chemicals are affected by the physiological conditions of the particular species, their habitats, purity of the chemicals and a few physicochemical quality of water particularly dissolved oxygen and pH. Further, the findings of mortality patterns in the current investigation are may be due to the creation of stress on the immune system of tested fish. However, acute and sublethal toxicity tests have been conducted to evaluate the toxicity of chemicals on non-target organisms[19]. Furthermore, the 96 hrs LC_{50} value of Chlorpyriphos 20% EC for *Poecilia reticulata* (juveniles, males, females and mixed population) were evaluated in the range of 7.009-130.777 ppb whereas presumable safe and safe dischargeable concentrations were ranges between 1.987-41.821 ppb and 1.103-1.063 ppb, respectively[20]. But the 96 hrs LC_{50} value for the combined formulation of Chlorpyriphos 50%+Cypermethrin 5% EC to the juveniles, males, females and mixed population *Poecilia reticulata* (Peters 1859) were estimated in the ranges of 13.396-261.866 ppb. In addition, the safe or harmless concentration and safe dischargeable concentrations of Chlorpyriphos 50%+Cypermethrin 5% EC were ranges between 4.381-32.216 and 1.044-1.069 ppb, respectively[21]. However, the 96 hrs LC50 values for Chlorpyriphos exposed *C. chanos* were reported to be 3.73 µg L^{-1} [22]. During the study of Chlorpyriphos toxicity concerning haematological

examination, the 96 hrs LC_{50} value was reported as 0.30 mg L^{-1}[7]. A more or less similar pattern of observation has been recorded in the context of mortality and LC_{50} value for Chlorpyriphos exposed test fishes during the current investigation.

In addition, there is a decline in Dissolve Oxygen (DO) content has been observed significantly from 0-96 hrs during this study (Table 3). It may be due to more consumption of dissolved oxygen by experimental fish under stress conditions, created due to toxicant. A similar trend with the highest percent (%) alteration in DO content was reported as 17.56 ppm at 21.0 ppm (Highest Concentration at pH 8.32±0.3) for Dimethoate 30% EC exposed *Heteropneustes fossilis*[23]. A previous study suggested a decrease in the gaseous exchange through gills due to excessive secretion of mucous which reduces the capability of fish to consume dissolved oxygen from the water. Furthermore, a hypoxic condition in fish may arise due to increased utilization of oxygen and reduced supply[24], damage of gills causes reduced oxygen uptake[25] and also may be due to increasing surfacing. Further, a significant increase in free CO_2 (mg L^{-1}) has been noticed from 0-96 hrs during static bioassay (Table 3). It may be due to an illustration of very high metabolic activities under extreme pressure of selected organophosphate. However, alteration in DO content and free CO_2 in the current investigation are also following a decline in DO content (6.96±0.85-5.83±0.21 mg L^{-1}) and enhancement in free CO_2 (4.00±0.00-9.33±2.31 mg L^{-1}) for sumithion (an organophosphate) exposed striped catfish, *Pangasianodon hypophthalmus*[26].

There is a decrease in RBC, WBC Count, PCV (%) and Haemoglobin (Hb) content was reported in the current investigation at both the selected concentration as compared to control (Table 4). However, a significant decrease in the number of RBC was noticed in diazinon exposed rainbow trout[27] and also a decline in RBC and haemoglobin (Hb) content was analyzed due to exposure to various pesticides and other toxicants[28-30]. A significant decline value of WBC Count was analyzed for Chlorpyriphos exposed African catfish, *Clarias gariepinus*[31]. Furthermore, a significantly lower value of RBC, WBC count and PCV were reported in Termifos (Chlorpyriphos-based pesticide) exposed African catfish, *Clarias gariepinus*[15]. Consequently, a significant decline in RBC, PCV and WBC has observed in Dimethoate exposed *Onchorhyncus mykiss*[32] and malathion exposed *Clarias gariepinus*[33]. A significantly decreased value of RBCs and Hb were estimated in sumithion (an organophosphate) exposed striped catfish, *Pangasianodon hypophthalmus* as compared to control[26]. In the current investigation, the significant decline in RBC content might be due to a decline in oxygen content of experimental water or oxygen depletion in the fish body under Chlorpyriphos induced pressure.

A significantly increased value of Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) whereas irregular manner of Mean Corpuscular Volume (MCV) was reported in the present investigation (Table 4). Furthermore, the values of MCV, MCH and MCHC were found to be increased significantly as compared to the control during the experiment[15]. Consequently, a similar pattern of observation has been reported in *O. mykiss* and other freshwater fishes exposed to various pesticides[34,35]. Further, an ascending trend in blood glucose levels has been seen in present investigation upon exposure of increasing concentration of toxicant. The hyperglycaemic condition may be due to reactions of hormones under the pressure of toxicants. It occurs due to increased reaction for gluconeogenesis in stressed fish to get additional energy[36]. However, the hyperglycaemic condition was observed in malathion exposed *C. gariepinus*, caused by hypersecretion of glucocorticoids and catecholamine which leads to glycolysis in the liver and muscle[33]. In the present study, the trend of elevation in blood glucose levels has concurred with the findings of previous authors. The alterations in haematological parameters may depend upon fish species, age, diseases and the cyclic event in the context of sexual maturity of spawners. From previous studies, it is clear that the physiological changes are reflected in the values of some haematological parameters if the water quality is subjected to exposure to toxicants. Anyway, the results withdrawn from this investigation, in terms of changes in haematological factors, are also corroborating with the literature cited by the foregoing authors.

CONCLUSION

Chlorpyriphos 20% EC are extremely toxic and induced the alteration in haematological parameters, which are used as potential biomarkers to monitor the aquatic fauna, specifically a freshwater fish, *Clarias batrachus* (Linnaeus, 1758) in this study.

SIGNIFICANCE STATEMENTS

The present investigation helps the aquacultures to make the people conscious about the health hazard of fish and other aquatic fauna, in the context of normal physiological

development, caused due to indiscriminate use of Chlorpyriphos pesticide in the field. This investigation has great importance from an aquaculture point of view since it helps to manage the health of aquatic bodies by using the controlled concentration of toxicants to reduce the health hazard of fishes and human being ultimate. Moreover, further study should be needed to minimise the concentration of pesticides in the contaminated aquatic ecosystem to prevent the deterioration of the surrounding environment and to reduce public health hazards.

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