

Acute Toxicity and Biological Responses of *Clarias gariepinus* to Environmentally Realistic Chlorpyrifos Concentrations

Kanu, K.C.*, Ogbonna, O.A., and Mpamah, I.C.

Department of Environmental Management and Toxicology, Michael Okpara
University of Agriculture, Umudike, Nigeria

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ABSTRACT: In this study, the lethal toxicity, behavioral responses and hematotoxicity of formulated chlorpyrifos on *Clarias gariepinus* was evaluated. *C. gariepinus* fingerlings were exposed to 0.2 mg/L, 0.25 mg/L, 0.3 mg/L, 0.35 mg/L and 0.4 mg/L of the active ingredient chlorpyrifos to determine the lethal concentrations and behavioral effects. *C. gariepinus* juveniles (38.84±7.67g) were then exposed to 0.0256 mg/L and 0.0128 mg/L for 14 days to study somatic indices and haematological effects. The 24h, 48h, 72h and 96h LC₅₀ were estimated as 0.292 (0.210 – 0.376) mg/L, 0.275 (0.252 – 0.297) mg/L, 0.263 (0.242 – 0.282) mg/L, and 0.256 (0.235 – 0.275) mg/L respectively. Hyper activity, loss of equilibrium, erratic swimming, trembling, respiratory distress and poor startle response were observed in fingerlings in response to acute toxic stress of chlorpyrifos. Liver somatic index (LSI) of exposed juveniles increased significantly (p<0.05) compared with control, while there was no statistically significant difference in all the haematological parameters of the exposed fishes compared with the control (p<0.05). The results indicate that the chlorpyrifos formulation was highly toxic and induced behavioral changes in *C. gariepinus* fingerlings, while sub-lethal concentrations induced inflammation in the liver but had no effect on haematological parameters of *Clarias gariepinus* juveniles. LSI was sensitive to the sub-lethal concentrations and could serve as indicators or exposure to organophosphate insecticides.

Keywords: Insecticide, hematology, catfish, liver somatic index.

INTRODUCTION

Only an estimated 0.1% of applied pesticides reach the target pests (Hart and Pimentel, 2002). Inland surface waters are sinks for different types of pesticides delivered via run-off or discharges from commercial, agricultural, domestic sources or aerial application. Organochlorine pesticides endrin (0.08 mg/L) and chlordane (0.07 mg/L), heaxachlorobenzene (0.06 mg/L), were reported in water samples from the Amir-kalaye wetland in Iran (Esfahani, et al., 2012), while Wee et al., (2016) reported the

occurrence of quinalphos (0.0178 µg/L), diazinon (0.0094 µg/L) and chlorpyrifos (0.0202 µg/L) in the Langat River, Malaysia. In a study conducted by Arain et al., (2018), chlorpyrifos concentration within a range of 43.46 to 79.7 µg/L was recorded in water samples collected after 24 hours of spraying the insecticide in Pakistan. Higher concentration of 0.67 mg/L was reported by Akan et al., (2015) in water samples from river Benue, in Adamawa State, Nigeria.

Chlorpyrifos, a broad spectrum organophosphate is frequently used to eradicate agricultural pests (Deb & Das 2013) and in the aquatic ecosystem, it poses

* Corresponding Author, Email: kanu.kingsley@mouau.edu.ng

serious threat to non-target organisms. In target organisms, chlorpyrifos affects the nervous system by inhibiting the breakdown of acetylcholine (ACh), a neurotransmitter primarily by binding to the enzyme acetylcholine esterase (AChE). Inactivation of this enzyme by chlorpyrifos or its metabolites leads to ACh accumulation between neurons, hyperstimulation, disrupted neurotransmission and eventually death (Christensen et al., 2009; Čolović et al., 2013). Similar mechanism of toxic action occurs in non-target organisms including fish and mammals due to the presence of acetylcholine in different organs (Szabó et al. 1991; Christensen et al., 2009).

Fishes are highly susceptible to aquatic habitats contaminated with insecticides. Insecticide exposure often elicit cellular, physiological, behavioral, biochemical and morphological responses in fish (Heger et al., 1995; Deb & Das 2013; Hussain et al., 2015) depending on the pesticides mode of action, duration of exposure, water quality and fish species (Misha and Verma 2016). Chlorpyrifos induced toxicity via AChE and non-AChE associated mechanisms of neurotoxicity may lead to physiological anomalies including behavioral impairment and eventual death (Tilton et al., 2011). Fish cholinesterase serves as biomarker of exposure to organophosphate and carbamate insecticides and is often employed in the environmental monitoring of these classes of insecticide (Assis et al., 2011).

Aquatic pesticide pollution is exacerbated by unwholesome practices among farmers like use of wrong nozzles or enlarging the nozzles to increase the discharge rate, application of pesticides close to the raining season, and disposal of left over pesticides in water bodies (Asogwa, 2009). All this increase the risk of pesticide toxicity to aquatic organisms. Thus, the study aimed to assess the toxicity of commercial chlorpyrifos formulation using *Clarias gariepinus*, an ecologically and economically important fish species. We evaluated the

behavioural, morphological and hematological responses of the fish species to environmentally relevant concentrations of the pesticide formulation.

MATERIALS AND METHODS

Chlorpyrifos- O, O-diethyl O-3, 5, 6-trichloropyridin-2-yl phosphorothioate (C₉H₁₁Cl₃NO₃) is a crystalline cholinesterase inhibitor used to control cockroaches, fleas, mites, ticks and termites. The formulated product used in the study was Rocket® 20% EC, containing 200 g/L chlorpyrifos as active ingredient. The product was manufactured by Shanxixian Nong Biological Services Co Ltd., China

Clarias gariepinus fingerlings and juveniles were purchased from a fish farm in Umuahia, Abia State, Nigeria and transferred to the laboratory where they were acclimated to laboratory condition for at least one week prior to the commencement of the experiments. The fishes were kept in a plastic aquarium containing borehole water. All the fishes were fed with commercially available fish feeds daily and excess feed and feces was siphoned daily. After the acclimation period, the fishes were randomly transferred into the plastic aquaria for the acute and sub-lethal toxicity tests.

Stock solution of 0.2 g/L was prepared by making up one ml (1 ml) of the pesticide to 1000 ml using borehole water. The concentrations used for the definite toxicity test was informed by the range finding test. Nominal test concentrations of 0.2 mg/L, 0.25 mg/L, 0.3 mg/L, 0.35 mg/L and 0.4 mg/l were prepared. The test concentrations were calculated from the percentage of active ingredients of the commercial formulation. Ten (10) fingerlings were exposed to each concentration in duplicates in a 3 liter plastic aquaria containing 1 liter test solution. The fishes were exposed for 96 hours and mortalities were recorded at 24, 48, 72 and 96 hours in order to estimate the corresponding lethal concentrations. Temperature, pH,

dissolved oxygen, electrical conductivity, total dissolved solids of the test solution was monitored during the experiment using portable meters. Behavioral changes were observed and recorded during the experiments as well as the number of fishes exhibiting the behavioral changes.

Using a semi static toxicity test procedure, five juvenile *C. gariepinus* were randomly distributed to 3 experimental groups comprising 1 control and 2 test groups in a complete randomized design. Fishes in the test groups were exposed to test solutions containing 0.0256 mg/L (1/10th of 96h LC₅₀) and 0.0128 mg/L (1/20th of 96h LC₅₀) chlorpyrifos respectively. The test solutions were renewed every 48 hrs. At the end of 14 days, three samples were randomly selected to assess fish biometrics, while blood was collected for haematological analysis.

Body and organ weight were measured using a weighing balance. Standard length and total length were measured using a meter rule. Somatic indices including liver somatic index (LSI), and gill somatic index (GSI) were calculated as the ratio of organ weight to body weight × 100.

Blood samples were collected from the fish into an EDTA bottle and analyzed within 4 hours of collection. Haematological parameters – RBC, PCV, Hb and WBC were analyzed using Mindray BC-2300 Hematology Analyzer, India following standard procedures outlined by the manufacturer. The analyzer utilizes electrical impedance method for cell counting and cyanide free method for hemoglobin and

calculates the red blood cell indices automatically from red blood cell count, Hb and PCV.

Lethal concentrations were estimated using probit analysis. The mean values of the weight of the organs and hematological indices of the exposed fishes was compared with control group using one way ANOVA (analysis of variance) while turkeys test poc hoc test was used to obtain the specific significant differences among the different groups. All analysis were performed with SPSS (statistical package of social sciences) version 22.

RESULTS AND DISCUSSION

The current study investigated the lethal and sub-lethal toxicity of a commercial chlorpyrifos formulation on the African catfish *Clarias gariepinus*.

Water quality properties of the test solutions are presented in Table 1. The physicochemical parameters assessed during static acute and sub-lethal toxicity test of formulated chlorpyrifos revealed significant variations in TDS, DO, and EC in the test solutions compared to the control while temperature and pH were relatively constant.

Table 2 shows the mortality pattern of exposed fishes. After 96hrs of exposure, less mortality occurred at 0.2 mg/L, partial mortality at 0.25 mg/L, 0.3 mg/L and 0.35 mg/L and total mortality at 0.4 mg/L of the test solution. 90%, 45%, 25%, 15% of the test organism survived following exposure to 0.2 mg/L, 0.25 mg/L, 0.3 mg/L and 0.35 mg/L respectively.

Table 1. Physicochemical properties of test solutions

Test	Con. (mg/l)	pH	Temp. (°C)	TDS (ppm)	DO (mg/l)	EC (µcm/s)
Control		7.1±0.14	26.5±0.71	16.5±2.12 ^a	5.7±0.28 ^{ab}	33.0±4.24 ^a
Acute toxicity test solution	0.2	7.15±0.07	26.0±0.00	25.5±7.71 ^a	4.35±0.10 ^{bc}	36.0±0.00 ^a
	0.25	7.15±0.07	26.0±0.00	88.0±14.14 ^{bc}	4.5±0.70 ^a	176.0±28.28 ^b
	0.3	7.1±0.00	26.0±0.00	65.0±5.41 ^b	4.9±1.41 ^{ab}	131.0±4.24 ^b
	0.35	7.15±0.07	26.0±0.00	90.5±9.70 ^c	4.6±0.35 ^{ab}	172.5±14.84 ^b
	0.4	7.15±0.07	26.0±0.00	81.5±2.12 ^{bc}	4.8±0.10 ^c	145.0±29.69 ^b
Sub-lethal toxicity test Solution	0.0256	7.2±0.11	25.0±0.00	91±6.65	5.1±0.96	89±18.75
	0.0128	7.1±0.12	25.0±0.00	87±9.86	5.3±0.85	97±11.45

Parameters with different alphabets are significantly different (p<0.05)

Table 2. Mortality rate and percentage Survival of *Clarias gariepinus* fingerlings

Concentration (mg/L)	Total Exposed	Cumulative Mortality Count				Survival (%)			
		24hrs	48hrs	72hrs	96hrs	24hrs	48hrs	72hrs	96hrs
0.2	20	0	1	2	2	100	95	90	90
0.25	20	9	10	10	11	55	50	50	45
0.3	20	11	12	13	15	45	40	35	25
0.35	20	13	15	17	17	35	25	15	15
0.4	20	18	19	20	20	10	5	0	0
Control	20	0	0	0	0	100	100	100	100

Table 3. Lethal concentrations of Chlorpyrifos

LC _x	LC _x (95% fiducial confidence interval)			
	24 h	48 h	72 h	96 h
LC ₁₀	0.207 (0.042 – 0.255)	0.197 (0.157 – 0.222)	0.195 (0.160 – 0.218)	0.191 (0.156 – 0.213)
LC ₂₀	0.233 (0.077 – 0.278)	0.221 (0.186 – 0.243)	0.216 (0.186 – 0.236)	0.211 (0.181-0.231)
LC ₃₀	0.253 (0.116 – 0.300)	0.240 (0.210-0.261)	0.233 (0.206 – 0.252)	0.227 (0.20-0.246)
LC ₄₀	0.273 (0.162 – 0.329)	0.258 (0.232- 0.278)	0.248 (0.224-0.226)	0.242 (0.218-0.260)
LC ₅₀	0.292 (0.210 – 0.376)	0.275 (0.252 – 0.297)	0.263 (0.242 – 0.282)	0.256 (0.235 – 0.275)
LC ₆₀	0.312 (0.251 – 0.466)	0.294 (0.272 – 0.320)	0.279 (0.259-0.301)	0.271 (0.252-0.292)
LC ₇₀	0.336 (0.282 – 0.634)	0.316 (0.293 – 0.349)	0.297 (0.277 – 0.324)	0.289 (0.269-0.314)
LC ₈₀	0.366 (0.308 – 0.952)	0.343 (0.316-0.391)	0.320 (0.297 – 0.357)	0.310 (0.289 – 0.345)
LC ₉₀	0.412 (0.338 – 1.724)	0.385 (0.348-0.461)	0.354 (0.325-0.411)	0.343 (0.315-0.396)
LC ₉₉	0.546 (0.404 – 7.350)	0.505 (0.431-0.694)	0.451 (0.394-0.586)	0.436 (0.382-0.561)

LC_x (Lethal concentration that kills x% of the exposed fishes).

The lethal concentrations of the formulated chlorpyrifos is presented in Table 3. The 24h, 48h, 72h and 96h LC₅₀ were estimated as 0.292 (0.210 – 0.376) mg/L, 0.275 (0.252 – 0.297) mg/L, 0.263 (0.242 – 0.282) mg/L, and 0.256 (0.235 – 0.275) mg/L respectively. The LC₅₀ decreased as the exposure time increased.

Chemicals are classified on the basis of the LC₅₀ as highly toxic if the LC₅₀ is between 0.1 and 1mg/L, moderately toxic if the LC₅₀ is between 1 to 10 mg/L and slight toxicity if the LC₅₀ is in the range of 10-100 mg/L. The 24hr, 48hr, 72hr, and 96hr LC₅₀ recorded in this study indicate that the formulated chlorpyrifos product (Rocket®) is highly toxic to *Clarias gariepinus* fingerlings. However higher LC₅₀ values,

1.66 mg/l, 1.30 mg/l, 1.03 mg/l and 0.86 mg/l corresponding to 24, 48, 72 and 96 hours of exposure were reported by Nwani et al., 2013 for *Clarias gariepinus* juveniles exposed to chlorpyrifos (Termifos®). Clearly the size (life stage) of the fishes used in both studies may account for the difference in the estimated lethal concentrations as fingerling stage is more sensitive to physical or chemical stressors than the later stages of development. Generally, differences in LC₅₀ values of a toxicant depend on duration of exposure, specie type, life stage and physico-chemical factors (Magesh and Kumaraguru, 2006). The toxicity of the pesticide may also be influenced by other additives in the formulation (Pereira et al., 2009).

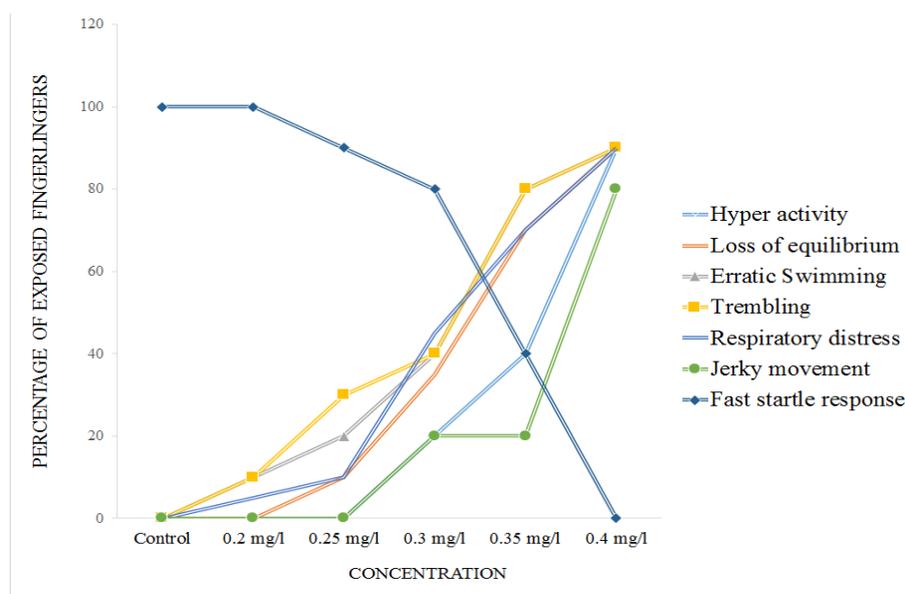


Fig.1. Behavioral response of *Clarias gariepinus* to Chlorpyrifos

Disruption of an organisms biochemical and physiological process by toxicants often result in behavioral dysfunctions (Radhaiah et al., 1987), which when it occurs in fish could be a sensitive indicator of aquatic pollution (Xia et al., 2018). Behavioral dysfunctions in exposed organisms could be useful in differentiating the mode of action of toxicants (Drummond et al. 1986). The behavioral responses elicited by *Clarias gariepinus* shortly after exposure to chlorpyrifos are presented in figure 1. The behavioral alterations were observed within twenty four hours of exposure to the pesticide. Within 1 hour of exposure, erratic swimming (swimming in circles followed by sudden collapse), jerky movements and hyper activity were observed in fishes exposed to 0.35 mg/L and 0.4 mg/L. While

startle response was fast in the control and fishes exposed to 0.2 mg/L and 0.25 mg/L, it was virtually absent in fishes exposed to 0.4 mg/L. Surfacing (gaspings for air) was also observed in fishes exposed to 0.4 mg/l. Behavioral dysfunctions observed in the current study may be attributed to the neurotoxicity of chlorpyrifos. Chlorpyrifos inhibits the breakdown of acetylcholine, a neurotransmitter which eventually builds up (Colović et al., 2013) and leads to behavioral dysfunctions before death occurs. Toxicant induced behavioral impairment interferes with ecologically relevant behaviors of fish such as predator avoidance, reproductive, and social interactions which are essential to wellbeing and survival of fishes in natural ecosystems (Scott and Sloman 2004).

Table 4. Mean biometrics and somatic indices of *Clarias gariepinus*

Morphological parameters	Treatment		
	Control	0.0128 mg/L	0.0256 mg/L
Body weight (g)	38.70±8.14 ^a	38.48±6.20 ^a	39.40±10.94 ^a
Standard length (cm)	17.77±0.75 ^a	17.93±1.97 ^a	17.74±0.72 ^a
Total length (cm)	19.67±0.76 ^a	19.93±2.27 ^a	19.88±0.78 ^a
Weight of gill (g)	1.83±0.12 ^a	1.77±0.28 ^a	1.88±0.59 ^a
Weight of liver (g)	0.17±0.06 ^a	0.28±0.08 ^a	0.32±0.11 ^a
Gill somatic index	4.30±0.61 ^a	4.64±0.57 ^a	4.76±0.73 ^a
Liver somatic index	0.38±0.07 ^a	0.73±0.22 ^b	0.81±0.12 ^b

Results indicate Mean ± standard deviation. Values of each parameters in the same row with different alphabets are significantly different (p<0.05)

Table 5. Mean hematological indices of *Clarias gariepinus*

Haematological indices	Treatment		
	Control	0.0128 mg/l	0.0256 mg/l
Hb (g/dl)	7.5±0.71 ^a	8.65±1.58 ^a	8.35±1.48 ^a
PCV (%)	23±4.24 ^a	28±1.63 ^a	25.5±3.87 ^a
RBC×10 ¹² /l	3.68±0.68 ^a	4.48±0.26 ^a	4.08±0.62 ^a
WBC (×10 ⁹ /l)	122.7±39.1 ^a	141.8±13.29 ^a	147±39.02 ^a
MCV (fl)	68.09±2.01 ^a	66.66±1.15 ^a	67.04±3.16 ^a
MCH (Pg)	20.91±5.78 ^a	19.24±2.63 ^a	20.63±3.81 ^a
MCHC (g/dl)	33.46±9.25 ^a	30.79±4.2 ^a	33.02±6.09 ^a

Results indicate Mean ± standard deviation of 4 fishes. Values of each parameters in the same row with different alphabets are significantly different (p<0.05)

All the morphological parameters assessed in the exposed fishes were not significantly different from the control fishes except the liver somatic index of the exposed fishes. Liver somatic index increased by 92% and 113% in fishes exposed to 0.0128 mg/L and 0.0256 mg/L respectively compared with the control.

Morphological indices are useful tools in assessing the health conditions of fish. In our experiment, the liver somatic index of fishes exposed to 0.0128 mg/l and 0.0256 mg/l increased after 14 days. Increase in LSI is suggestive of liver enlargement due to increased metabolic activity and possibly hyperplasia ((Hoque et al., 1998). The liver is the main site for biotransformation of xenobiotics, and cytochrome P450 enzymes catalyze the metabolism of xenobiotics including pesticides into intermediates (Das and Gupta, 2013) to improve water solubility and facilitate excretion. LSI may thus be a useful indicator of exposure to insecticides in field conditions particularly when specie and age specific standard LSI values has being established for comparison.

Results of the haematological profile of the exposed fishes is presented in Table 5. There was no statistically significant difference in all the haematological parameters of the exposed fishes compared (p<0.05). Haematological analysis provide a useful and easy method to assess the toxicity of pesticides in fish (Groff and Zinkl 1999). The results of this study indicates that the haematological system of

C. gariepinus (with wet weight of 38.84±7.67g) may have coped with the stress of exposure to 0.0128 mg/l and 0.0256 mg/l after 14 days even though there were slight increases in Hb, PCV, RBC and WBC count. This suggests that environmentally relevant pesticide concentrations may not be hazardous to matured fishes. In a similar study, no hematological effect was observed by Oropesa et al., (2009) in common carp exposed to 45 µg/l of simazine and by Ashade et al., (2011) in *C. gariepinus* exposed to 0.0223ml/l of chlorpyrifos and 0.0184ml/l of DDVP for 14 days.

CONCLUSION

The outcome of this study indicate that the commercial chlorpyrifos formulation was highly toxic to *C. gariepinus* fingerlings. The liver somatic index of *C. gariepinus* juvenile was sensitive and may be useful indicator of exposure to environmentally realistic concentrations of chlorpyrifos and other organophosphates with similar mechanism of action. Furthermore, short time exposure to low chlorpyrifos concentration neither affected the gill somatic index nor was it haematotoxic. Nevertheless, further studies assessing the effect of chronic exposure to pesticides at very low concentrations on the somatic index and hematology of fish is recommended to validate the use of LSI for biological monitoring and the risk of haematotoxicity in fish in natural waters which are sink for pesticides.

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