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Effects of Acute Nominal Doses of Chlorpyrifos-Ethyl on Some Haematological Indices of African Catfish *Clarias gariepinus*-Teugels

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Abstract: Static bioassay experiments were conducted to ascertain the acute effects of waterborne chlorpyrifos-ethyl at 0.64, 0.80, 0.96, 1.12 and 1.28 mg L^{-1} on a freshwater fish *Clarias gariepinus* after 96 h. The effects were assessed by examining the haematological profile against control. Exposure to chlorpyrifos-ethyl caused a significant dose dependent ($p<0.05$) inhibition in haematocrit (PVC) and Haemoglobin (Hb) values. There was also a significant decrease ($p<0.05$) of RBCC in 1.28 mg L^{-1} concentration. The WBCC elevation was significantly dose dependent ($p<0.05$) in all concentrations except 0.64 mg L^{-1}. The inhibition of MCH and elevation of MCHC were both Non-Significant (NS), but the inhibition of MCV was significant between control and exposed group ($p<0.05$). The results of this study highlight the stress to which freshwater fish are exposed through the uncontrolled discharge of insecticides in the aquatic environment.

Key words: Toxicology, chlorpyrifos-ethyl, haematology, *Clarias gariepinus*

INTRODUCTION

Pesticides are of great value to agriculture and public health. However when they are used in an unsustainable manner, they impact the environment negatively. Frequently, organo-phosphorus contamination has been found in environments, elements of the food chain and humans. Chlorpyrifos is effective against a wide range of arthropods and insect pests including Coleoptera, Diptera, Homoptera and Lepidoptera species. In 2000, there were 164 registered products containing Chlorpyrifos (NRA, 2000). They enter water bodies through intentional application, run-off from farms, aerial drift and accidental and illegal release (Zelkoff, 1994). Due to its lipophilic nature, fish are able to absorb and bioconcentrate chlorpyrifos to moderate or high levels and variable Bioconcentration Factors (BCF) between 100-5100 have been reported (Racke, 1993). Chlorpyrifos is directly toxic to the nervous system and is also transformed inside animals to chlorpyrifos-oxon and 3, 5, 6-Trichloro-2-Pyridinol (TCP) both of which are many times more toxic to the nervous system than chlorpyrifos itself (Chambers *et al*., 1989). In addition, organophosphates are reactive and may cause damage through direct oxidative damage to membranes (Galloway and Randy, 2003).

Acute LC_{50} values for freshwater and marine fish have been from 2-520 μg L^{-1}, but generally below 100 μg L^{-1} (NRA, 2000). Chronic exposures of aquatic organisms generally results in No-Observable Effect Concentrations (NOEC's) of <1.0 μg L^{-1}. Rainbow trout studies reported a NOEC of 0.5 μg L^{-1}, while there was complete mortality at 2-3 μg L^{-1} (USEPA, 1999). For invertebrates, acute LC_{50} values typically in the 0.1-10 μg L^{-1} range and algal endpoints are typically above 100 μg L^{-1} (NRA, 2000). Chronic exposures of aquatic organisms generally results in NOECs of <1.0 μg L^{-1}. Some toxic effects in aquatic biota are reversible, whereas others are not, leading to organisms mortality especially the non-target ones. In many cases, toxic effects are reversible only if the organisms can escape the toxicant and migrate to an uncontaminated environment (Rand *et al*., 1995). According to Seith and Saxena (2003) blood is a pathophysiological reflector of the body because it is highly susceptible to internal and external environmental fluctuations. Physicomorphological changes in blood indicate the changes in the quality of the environment and therefore blood parameters are important in diagnosing the functional status of the fish exposed to toxicants.

The purpose of this study was to investigate the hematological perturbations in the fresh water catfish *Clarias gariepinus* at nominal concentrations of chlorpyrifos-ethyl during acute exposure.

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MATERIALS AND METHODS

Juveniles of *Clarias gariepinus* was purchased from Maigana fish farm in Zaria, Kaduna State Nigeria. The *Clarias* species averaging 14.33±0.50 cm standard length and body weight of 20.38±1.25 g were used for the study. The fish were conveyed to fisheries laboratory in a portable well-aerated white polythene bag containing water from the fish farm. They were held in large water baths of 160 L capacity at 24.5-25.5°C and acclimatized for two weeks in dechlorinated municipal water. During this period, the fishes were fed with pelleted diet containing 35% crude protein twice per day at 5% body weight. Also, the water in the glass aquaria was changed once every two days. The fishes were accepted as well as adapted to laboratory conditions when less than 5% death was recorded for the 14 days period and feeding was discontinued 24 h before the start of the experimental run (Reish and Oshida, 1987).

Acute bioassay: Acute 96 h static bioassays were conducted in the laboratory following the methods of Spragne (1975) and APHA (1985). The nominal concentration for chlorpyrifos-ethyl was 0.64, 0.80, 0.96, 0.12, 0.28 mg L^{-1} and a control with no toxicant. Each concentration was replicated three times. The desired chlorpyrifos concentration was measured and introduced into 25 L of dechlorinated tap water in the glass aquaria. The mixture was allowed to stand for 30 min before introducing test fishes. A total of 180 fish were stocked to give a loading rate of 10 fish per tank. Survival and mortality were recorded from 1to 6, 8, 16, 24, 72 and 96 h. Fishes were considered dead when the opercular movement ceased and there was no response to gentle probing.

Physico-chemical parameters: Temperature, pH, alkalinity, hardness, conductivity and dissolved oxygen levels of control and other treatments were determined and the readings were taken at 12, 48 and 96 h intervals in three replicates. The temperature was determined using the mercury-in-glass thermometer, pH was determined using a pH meter (Model 3015 Jenway), alkalinity was determined by standard methods (APHA, 1985) conductivity was conductivity meter PACM 35 model, for water hardness, E.D.T.A. titration method was used (APHA, 1985) and DO was measured by Winklers method (APHA, 1985).

Haematological analysis: Blood samples were collected at the end of 96 h by severance of the caudal peduncle techniques. Haematocrit (Ht) was analysed using micro capillary tubes filled with blood and centrifuged at 11,000 r min^{-1} for 6 min. The mean value of haematocrit (%) were measured with a micro capillary reader. The Red Blood Cell Count (RBCC) and White Blood Cell Count (WBCC) was determined using improved Neubauer counting chamber as described by Hesser (1960). For RBCC, Hendrick's diluting fluid was used and the multiplication factor of 10^6 employed, while for WBCC, Shaw's diluting fluid was used and the total counts multiplied by 500. Haemoglobin (HB) was estimated using the cyanomethaemoglobin (Blaxhall and Daisey, 1973) at a wavelength of 540 nm. The 'absolute values' made up of Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated from the results of RBCC, WBCC, haemoglobin and PCV (Dacie and Lewis, 1968).

Statistical analysis: For the various biochemical parameters, the GenStat statistical analysis software (GenStat, 2006) was used to run Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to test for differences between different levels of treatment and to separate means, respectively, were applicable (Duncan, 1955). Test of significance was at the 5% level of significance.

RESULTS

The physico-chemical parameters results obtained from the test solutions for the period of 96 h showed that the values were close the physico-chemical parameters of the control (Table 1). It was observed that the exposure of juveniles of *C. gariepinus* to Chlorpyrifos-ethyl for 96 h significantly ($p<0.05$) inhibited haematocrit and haemaglobin concentrations in the fish blood. The percentage reduction from control values of haematocrit and haemoglobin was 64.74 and 49.23%, respectively (Table 2). These values were highest in the highest nominal concentrations of 1.28 mg L^{-1}. The inhibition of these parameters in the exposed fish was significant ($p<0.05$) and dose dependent (Table 2 and 3). The percentage elevations of RBCC in the first three nominal concentrations were 34.72, 24.72 and 11.11%, respectively. These percentages decreased with increasing concentrations (Table 2). However in the last highest nominal concentration, there was 25.56% inhibition in RBCC. Also the ANOVA result indicated a significant elevation ($p<0.05$) of RBCC in the nominal concentrations 0.64 -0.96 mg L^{-1} and significant inhibition in 1.28 mg L^{-1} (Table 3). The WBCC showed a dose dependent percentage elevation from the least to the highest nominal concentration (Table 2).

Table 1: Mean values of physico-chemical parameters of diluting water used in the acute toxicant treatments of *C. gariepinus*. Each value (n = 9) is mean±SE

	Concentrations (mg L^{-1})					
Parameters	Control	0.64	0.80	0.96	1.12	1.28
Temperature (°C)	30.25±0.84	30.35±0.65	30.50±0.69	30.50±0.68	30.45±0.75	30.35±0.75
pH	6.41±0.16	6.36±0.16	6.38±0.13	6.45±0.13	6.51±0.09	6.51±0.24
Conductivity (μscm^{-1})	160.0±5.990	160.50±2.49	169.0±26.99	165.50±27.49	171.0±24.99	163.0±26.99
DO (mg L^{-1})	4.05±0.85	3.35±1.04	3.60±1.19	3.65±1.74	3.85±1.44	4.25±1.05
Hardness (mg L^{-1}CaC03)	38.0±1.990	36.0±3.990	38.0±1.990	38.0±1.980	38.0±9.980	38.0±9.990
Alkalinity (mg L^{-1})	40.50±24.46	41.0±30.97	36.0±25.99	43.0±31.99	39.0±28.99	34.5±24.46

Table 2: Changes in haematological parameters of *Clarias gariepinus* exposed to acute nominal concentrations of Chlorpyrifos-ethyl. Each value (n = 3) is mean±SE (%) represents percentage changes over the control

	Concentrations (mg L^{-1})					
Blood parameters	Control	0.64	0.80	0.96	1.12	1.28
Haematocrit (%)	39±2.49	36.5±1.5 (6.4)	20.5±0.50 (47)	18.5±0.50 (52.56)	17.5±0.50 (55.12)	13.75±0.25 (64.74)
Haemoglobin (g 100 mL^{-1})	13±0.99	12.1±0.50 (6.92)	10.2±0.19 (21.53)	10±0.99 (23.07)	9.8±0.79 (24.61)	6.6±21 (49.23)
WBCC (*10^6mm^3)	12.8±0	12.9±0 (0.78)	13.3±0 (3.90)	13.8±0.05 (7.81)	14.3±0 (11.76)	15.9±0.05 (24.21)
RBCC (*10^6mm^3)	180±9.99	242.5±12.49 (34.72)	224.5±0.50 (24.72)	200±9.99 (11.11)	183.5±1.49 (1.94)	134±0.99 (25.56)
MCV (*10^{-6} μm^3)	2.17±0.04	1.51±0.01 (47)	0.91±0.02 (58.06)	0.93±0.06 (57.14)	0.96±0.03 (55.75)	1.03±0.02 (52.53)
MCH (*10^6pgcel L^{-1})	0.73±0.01	0.49±0.01 (32.87)	0.46±0.01 (36.98)	0.49±0.02 (32.87)	0.53±0.04 (27.39)	0.49±0.15 (32.88)
MCHC (g/100 mL^{-1})	33.33±0.01	33.15±0.01 (0.54)	49.81±2.18 (49.44)	54.24±6.86 (62.73)	56.18±6.17 (68.55)	48.28±15.4 (44.85)

Table 3: Means for *C. gariepinus* Haematological parameters after exposure to acute nominal concentrations of Chlorpyrifos-ethyl

Conc. (mg L^{-1})	Haematocrit (%)	Haemoglobin (g 100 mL^{-1})	RBCC (*10^6mm^3)	WBCC (*10^6mm^3)	MCV (*10^{-6}μm^3)	MCH (*10^6pgcel L^{-1})	MCHC (g 100 mL^{-1})
0.00	39.00±3.00^a	13.00±1.00^a	180.00±10.00^c	12.80±0.00^e	2.16±0.04^a	0.73±0.01^a	33.30±0.01^a
0.64	36.50±1.50^b	12.10±0.50^a	242.50±12.50^a	12.95±0.05^e	1.51±0.01^b	0.50±0.00^a	33.10±2.19^a
0.80	20.50±0.50^c	10.20±0.20^{ab}	224.0±0.05^d	13.40±0.10^d	0.91±0.03^c	0.46±0.02^a	49.80±2.19^a
0.96	18.50±0.50^{cd}	10.00±1.00^{ab}	200.00±10.00^{bc}	13.80±0.10^c	0.93±0.07^c	0.50±0.04^a	54.20±6.87^a
1.12	17.50±0.50^{cd}	9.80±0.80^{ab}	183.50±1.50^c	14.40±0.10^b	0.96±0.03^c	0.53±0.14^a	50.20±6.17^a
1.28	13.75±0.25^d	6.60±2.00^b	134.±1.00^d	15.85±0.05^a	1.03±0.02^c	0.50±0.00^a	48.30±15.42^a

Means with the same superscript along columns are not significantly different ($p<0.05$), (Mean values±SE) n = 2

However, the ANOVA result indicated that from 0.80-1.28 mg L^{-1} nominal concentrations, the elevation of WBCC were significantly ($p<0.05$) dose dependent (Table 3). There were varying percentage inhibitions in the values of Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) in the exposed fishes (Table 2). The percentage inhibition of MCV was dose dependent from 0.80-1.28 mg L^{-1} exposed fishes. There was also 0.54% inhibition in MCHC of 0.64 mg L^{-1} exposed fish, but other concentrations recorded varying elevations against control (Table 2). The ANOVA results indicated significant difference ($p<0.05$) between control and exposed fishes for MCV, but No Significant difference (NS) among concentrations except for 0.64 mg L^{-1} (Table 3). Also there were No Significant difference (NS) between control and exposed fishes and among concentrations for MCH and MCHC (Table 3).

DISCUSSION

The significant ($p<0.05$) elevation of RBCC in 0.64 and 0.80 mg L^{-1} nominal concentrations may be due to the release of red blood cells into the blood stream. This agreed with observations of Alkahem *et al.* (1998) and Rottman *et al.* (1992) when Trichlorefon (organophosphate pesticide) was exposed to fish for 96 h. They attributed this to a stress-mediated condition, which trigger the release of new erythrocytes from the erythropoietic tissue to improve the oxygen carrying capacity of exposed fish blood with resultant higher values of erythrocyte count. This increase at these lower concentrations may equally suggest a compensatory response of *C. gariepinus* to increase, not only their oxygen demand, but also their metabolic rate and energy production to meet up with the challenge of insecticide detoxification. Similar observation and explanation had been reported by (Atmanalp and Yanik, 2002). However there was also a significant inhibition ($p<0.05$) of RBCC in the highest nominal concentration of 1.28 mg L^{-1}. Hemoglobin concentrations and hematocrit were also inhibited significantly ($p<0.05$) when compared with control. This suggests that the insecticide exerted an effect similar to the production of acute anaemia in this fish. This is consistent with the report of Mgbenka *et al.* (2005) in *Clarias albopunctatus* exposed to sub-lethal actellic concentrations. Anaemia associated with erythropenia has also been reported for several freshwater fish species (Auta, 2001; Svoboda *et al.*, 2001; Gbem *et al.*, 2003). The significant dose dependent inhibition in the Hb concentration observed in the

present investigation may be due to either an increase in the rate at which the Hb is destroyed, or to a decrease in the rate of Hb synthesis. The implication of this finding is that acute Chlorpyrifos-ethyl exposure to *C. gariepinus* may impair its oxygen supply to various tissues, thus resulting in slow metabolic rate and low energy production. Similar findings and explanations had been reported by (Atamanalp and Yanik, 2002). Since it has been reported that hypoxia promote erythropoiesis (Nussey *et al*., 1995) the hypoxic condition of the tissues could have stimulated enhanced erythropoiesis in *C. gariepinus* exposed to chlorpyrifos-ethyl. Thus, the observed reduction in both the hematocrit and the erythrocyte count in *C. gariepinus* suggest that chlorpyrifos-ethyl may have impaired erythropoietic activity in the fish, especially in the highest nominal concentrations. The MCV has been reported to provide information on the size and status of erythrocytes (Nussey *et al*., 1995) thus the inhibition in PVC and MCV as observed in the current investigation indicate that Chlorpyrifos-ethyl may have interfered with the normal physiology of RBC. Anees (1978) had earlier reported that Methyl Parathion and Dimethoate caused a consistent decrease in cellular and nuclear diameter of erythrocytes. The significant ($p<0.05$) elevation of RBCC in 0.64 and 0.80 mg L^{-1} nominal concentrations and concomitant decrease in PVC may show the extent of the shrinking cell size due intoxication by Chlorpyrifos-ethyl.

In the current investigation, no significant differences were observed in the levels of MCHC and MCH. Giron-Perez *et al*. (2006) also reported that chlorpyrifos had no effect on MCH and MCHC of Nile tilapia (*Oreochromis niloticus*). Similarly Svododa *et al*. (2001) observed that the values of MCH and MCHC of Common carp registered after 96 h exposure to Diazinon based pesticide were comparable with control group. The increase in the WBCC can be correlated with an increase in antibody production which helps in survival and recovery of the fish exposed to pesticide (Joshi *et al*., 2000). The present findings also showed hypersensitivity of leukocytes to Chlorpyrifos-ethyl and these changes may be due to immunological reactions leading to antibody production to help fish cope with stress induced by Chlorpyrifos-ethyl. Similar findings were reported by Seth and Saxena (2003).

CONCLUSION

The result of this investigation showed that the entire physiology of the fish was disturbed due chlorpyrifos-ethyl exposure. The toxicant caused haematological disturbances which could lead to impairment of the fish ability to combat diseases, reduce its chances for survival and potential for growth and reproduction. Also adequate data build-up on the haematological parameters of our local fish species might be helpful in the selection of healthy stock for breeding purposes, in the possible early diagnosis of a disease before it becomes an epidemic and also in the assessment of possible toxic effects of various environments when the toxins are in acute concentrations.

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