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COMMUNICATION

Subchronic Exposure to Fenthion Induces Hematological Changes in Liver Tissue of African Catfish *Clarias gariepinus*

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Abstract

In this study, African Catfish (also known as Sharptooth Catfish) *Clarias gariepinus* were exposed to sublethal concentrations of fenthion of 2.0, 4.0, and 8.0 mg/L for 21 d and allowed to recover for 7 d to investigate the potential for hematological changes. Whole blood was sampled on days 1, 7, 14, and 21 postexposure and after a 7-d recovery period. During exposure, fenthion caused a reduction in red blood cell counts, hemoglobin concentration, and packed cell volume. There was an increase in white blood cell counts but no significant difference in mean corpuscular hemoglobin concentration, mean corpuscular volume, and mean corpuscular hemoglobin. Both increases and decreases were observed in white blood cell differentials. After the 7-d recovery period, both increases and decreases were observed in the hematological parameters. These results reveal that sublethal concentrations of fenthion can cause hematological alterations in African Catfish and that the substance should be used with caution.

Hematological alterations allow rapid evaluation of the toxicities of compounds (Jaya and Ajay 2014), with changes induced in the circulating fluid offering valuable parameters for assessing the state of fish health (Lazar and Lazar 2012). Tilak et al. (2007) noted that blood parameters are highly susceptible to changes in the environment and thus important in diagnosing the functional status of pollutant-exposed fish.

Fenthion is one of the organophosphate insecticides–larvicides most widely used in agriculture and public health for controlling sucking and biting pests (Cong et al. 2009; Sevgiler and Uner 2010). Fenthion is known to be a cholinesterase inhibitor as well as a lipophilic compound that is bioaccumulated in the fat tissues of animals (Vlastos and Ganidi 2004). Research has demonstrated that fenthion increases DNA damage in humans and that it should be considered potentially hazardous (Wu et al. 2011). Recent research by Kanter and Celik (2012) has revealed that marsh frogs *Rana*

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ridibunda exposed to fenthion had an increase in lipid peroxidation and antioxidant defense systems. Israel and Sam (2012) reported biochemical changes in some tissues of Mrigal *Cirrhinus mrigala* exposed to fenthion, while Altuntas and Delibas (2002) reported that fenthion caused liver damage in Wister albino rats (a strain of the brown rat *Rattus norvegicus*). Somdare et al. (2015) reported fenthion-induced toxic stress and alterations in the gill architecture of African Catfish (also known as Sharptooth Catfish) *Clarias gariepinus*. All fenthion formulations have been banned in the United States and Canada (USEPA 2003; PMRA 2004). However, it is still produced in some countries, such as China and India, and its application is ongoing in Nigeria.

The African Catfish is one of the predominant indigenous fish species of economic importance in Africa (Adeyemi et al. 2014). It is used in toxicological studies due to its wide availability in rivers, streams, ponds, ditches, beels (lake-like wetlands), swamps, marshes, and lakes throughout the season; its high reproductive potential; its ability to acclimatize easily in the laboratory; and its general hardiness under culture conditions (Nwani et al. 2015b).

Despite the increasing use of fenthion in Nigeria, there is limited information on the hematological parameters of African Catfish exposed to the insecticide.

METHODS

Procurement of fish and chemicals.—A total of 360 healthy African Catfish with a mean ± SD standard length of 27.36 ± 0.23 cm and a weight of 197.39 ± 2.34 g were procured from Freedom Fisheries, Ltd., Nsukka, Enugu, Nigeria. They were transported to our fisheries wet laboratory and subsequently subjected to a 2-min bath with 0.05% potassium permanganate ($KMnO_4$) to safeguard against dermal infections. The fish were held in two plastic tanks (300-L capacity) for 21 d for acclimatization and were fed ad libitum daily a commercially available food (Coppens commercial feed; Coppens International, Helmond, The Netherlands) containing 35% crude protein. A commercial formulation of fenthion (600 g/L) manufactured by Yufull Industry, Ltd., Shenzhen, China, was used in the study.

Experimental design for toxicity bioassays and hematology test.—The acute toxicity test to determine the concentration of fenthion lethal to 50% of the test animals (LC50) at 96 h was conducted in a semistatic laboratory system according to Organization for Economic Cooperation and Development guideline 203 (OECD 1992). Fenthion was dissolved in filtered distilled water and added to the aquarium following the method of Pluta (1989). The water in the experimental setup was changed every 24 h by adding a fresh fenthion solution to counterbalance the decreasing concentration. In the acute toxicity test, sets of 10 fish were randomly exposed to fenthion concentrations of 12, 24, 36, 48, 60, and 72 mg/L. Another set of 10 fish were simultaneously maintained in an equal amount of tap water but without fenthion (the control). The experiments were conducted in triplicate, and fish were not fed during them, as recommended by Ward and Parrish (1982) and Reish and Oshida (1987). Fish were visually examined daily and considered dead when no sudden swimming in response to gentle touch was observed. Dead fish were removed with plastic forceps, and mortality was recorded at intervals of 24, 48, 72, and 96 h. The 96-h LC50 value of fenthion for African Catfish was determined to be 39.97 mg/L by the probit analysis method as described by Finney (1971). During the sublethal hematological test, the fish were randomly divided into four groups of 30 fish each without regard to sex. Each group was further subdivided in triplicates of 10 fish. Fish in the first, second, and third groups were exposed to 2.0, 4.0, and 8.0 mg/L fenthion (corresponding to 1/20, 1/10, and 1/5 of the 96-h LC50), respectively. Fish in the fourth experimental group were exposed to tap water only and served as the control. The experiment lasted for 21 d, after which fish were removed and monitored for another 7 d (i.e., to the 28th day of the experiment) to observe the recovery effect. On each sampling day (days 1, 7, 14, and 21), a total of three fish per concentration, comprising one fish from each replicate of the treatment and control groups, were anesthetized with tricaine methanesulfonate (MS-222) to minimize stress. During the recovery phase of the research, one fish from each replicate was sampled on day 28 for similar analysis. Approximately 2 mL of blood was collected from the caudal peduncle using a 5-mL sterile disposable syringe with a 22-gauge needle and stored in EDTA vials for analysis.

Determination of hematological parameters.—The packed cell volume (PCV) was determined using the microhematocrit method (Nelson and Morris 1989). Hemoglobin (Hb) determination was done by means of the cyanmethemoglobin method (Blaxhall and Daisley 1973). The red blood cell (RBC) and white blood cell (WBC) counts were estimated using a Neubauer-type hemocytometer and Toisson's solution as the diluting fluid for RBCs and Turk's solution as the diluting solution for WBCs (Rusia and Sood 1992). The numbers of different types of leukocytes (neutrophils, monocytes, lymphocytes, eosinophils, and basophils) in the blood smears were identified following the method described by Hibiya (1982) and Chinabut et al. (1991). Hematological indices (mean corpuscular hemoglobin concentration [MCHC], mean corpuscular hemoglobin [MCH], and mean corpuscular volume [MCV]) were calculated from the estimated values for red blood cells, hemoglobin, and packed cell volume according to the method of Dacie and Lewis (1984).

Statistical analysis.—The data obtained were statistically analysed using SPSS 17.0 (SPSS, Chicago, Illinois). Data were subjected to one-way analysis of variance and Duncan's multiple-range test to determine significant differences at the 5% probability level. Results are expressed as means ± SEs.

RESULTS

The changes in the various hematological parameters of African Catfish exposed to sublethal concentrations of fenthion are presented in Table 1. The PCV and Hb of the treated fish were generally lower than those of the control. However, significant differences ($P < 0.05$) were observed only on days 1 for PCV and for 1 and 21 Hb at the 8-mg/L concentration. After the 7-d recovery period, the values of PCV and Hb were higher than those for the control except for the fish exposed to the 8.0-mg/L concentration. The RBC counts of the fenthion-exposed fish were significantly lower than those of the control at all concentrations throughout the exposure period except on days 7 and 21 at 2.0 and 4.0 mg/L, respectively. Further, the RBC value for the 2-mg/L exposure at day 21 was significantly higher than that for the control. During postexposure, the RBC counts in the exposed fish were also significantly lower than that of the control. There were concentration-dependent significant increases in WBCs at 2.0 and 4.0 mg/L on day 1 and at 8.0 and 2.0 mg/L on days 14 and 21, respectively. However, a significant decrease was observed at 8.0 mg/L on day 1. During the 7-d recovery period, there were significant increases in WBCs at 4.0 and 8.0 mg/L but a decrease at 2.0 mg/L.

No significant differences in MCHC, MCH, and MCV were observed between the fenthion-treated fish and the controls throughout the experiment. During the recovery period, there were higher (but not significantly so) values of MCV in fish exposed to 2.0- and 4.0-mg/L fenthion concentrations than in control fish.

The effects of fenthion on WBC counts are shown in Table 2. Exposed fish had significantly lower ($P < 0.05$) neutrophil counts than control fish at the 8.0-mg/L fenthion concentration throughout the experiment except on day 14; on day 21, however, the value was significantly higher than that for the control. Monocyte counts were similarly lower in exposed fish, but significant differences were observed only on day 14 at the 4.0- and 8.0-mg/L concentrations. However, during the 7-d recovery period monocyte values were not significantly different from those for the control. Compared with the control, lymphocyte values were generally higher in fish exposed to all fenthion concentrations. Lymphocyte values were also higher (though not significantly so) during the recovery period. The numbers of eosinophils and basophils in African Catfish exposed to all concentrations of fenthion were not significantly different from those of the control throughout the experiment.

DISCUSSION

Hematological indices are good indicators of the physiology and health status of organisms (Saravanan et al. 2012). The reductions in RBC and Hb values in the present study may be evidence of an impaired erythropoietic process caused by fenthion. Similar decreases in RBCs and Hb have been reported in Common Carp *Cyprinus carpio* chronically exposed to fenthion (Muralidharan 2012) and in other fish species exposed to other pesticides (Banaee et al. 2011; Vani et al. 2012; Haider and Rauf 2014). The significant reduction in PCV at the highest fenthion concentration in the present study is similar to the results reported by Ahmad (2012) when African Catfish were exposed to the insecticide malathion. Like the present study, Banaee et al. (2011) reported decreases in PCV, RBC counts, and Hb in fish exposed to another organophosphorous insecticide, diazinon, and related it to the destruction of cells and/or the decrease in cell size due to the adverse effects of the pesticide. Nwani et al. (2015a) attributed lower Hb in Sprague Dawley rats (another strain of the brown rat) exposed to carbosulfan to intravascular hemolysis, anemia, or the suppression of hemopoiesis. The reduction in the level of Hb in the fish may also be the consequence of toxic effects of fenthion that disrupt the synthetic pathway by affecting the enzymes involved in the synthesis of hemoglobin. Similar to the present findings, Muralidharan (2014) reported decreases in RBCs in Common Carp exposed to fenthion. The lower RBC counts may be due to inhibitions in RBC production and/or Hb synthesis. The presence of fenthion in the water might have caused a reduction in RBCs, which in turn caused reductions in Hb and packed cell volume (Jaya and Ajay 2014).

The increase in WBC counts may indicate the occurrence of leucocytosis in the treated fish. The observed leucocytosis may have been a normal immune response of the fish to toxic invasion. Muralidharan (2014) observed increases in WBCs in Common Carp exposed to fenthion and attributed it to interference with the development of erythrocytes in the hemopoietic tissues, which could be an adaptive response to the stress induced by the insecticide. Khalid (2012) reported that WBC changes in Nile Tilapia *Oreochromis niloticus* exposed to malathion manifested themselves in the form of leucocytosis with heterophilia and lymphopenia, which are characteristics of leucocytosis in animals exhibiting stress. The lack of alterations in the values of MCH and MCHC may indicate that the fenthion-induced anemia is of the normocytic type (Zhang et al. 2008; Li et al. 2011). The change in differential leukocyte counts is recognized as a sensitive indicator of environmental stress (Cole et al. 2001). The possible increase in lymphocyte counts in the exposed fish may be due to fenthion-induced stress and possible defence against the stressor (Davis et al. 2008). The reduction in the neutrophil counts at the highest fenthion concentration and in monocytes at 4.0 and 8.0 mg/L may be attributed to the destruction of the hemopoietic system due to prolonged exposure to the pesticide. The present study indicates that fenthion has no significant effect on eosinophils and basophils. Similar to the present findings, no significant differences in these leukocyte differentials have been reported in fish exposed to various concentrations of toxicants (Mohammad et al. 2012; Roy and Nath 2012; Nwani et al. 2014). After the 7-d recovery period, Hb values were restored to physiological levels except at the 8.0-mg/L concentration. Other blood parameters were also restored to their normal levels, but irreversible changes were observed in RBCs, WBCs, and PCV (except at the 8.0-mg/L concentration), which suggests the immunosuppressive potential of fenthion.

TABLE 1. Effects of exposure to fenthion on hematological parameters of African Catfish. Abbreviations are as follows: PCV = packed cell volume, Hb = hemoglobin, RBCs = red blood cells, WBCs = white blood cells, MCHC = mean corpuscular hemoglobin concentration, MCH = mean corpuscular hemoglobin, and MCV = mean corpuscular volume. Values with different lowercase letters differ significantly ($P < 0.05$) among concentrations within the same exposure duration; values with different uppercase letters differ significantly among exposure durations within the same concentration.

Parameter	Concentration (mg/L)	Exposure duration (d)				
		1	7	14	21	7-d recovery
PCV (%)	Control	28.33 ± 1.76 zY	21.00 ± 2.31 zX	30.67 ± 3.71 zY	34.00 ± 1.73 zY	40.67 ± 4.06 zZ
	2.0	27.33 ± 2.91 zY	20.33 ± 0.88 zX	26.00 ± 4.93 zY	33.67 ± 2.96 zY	42.67 ± 4.37 zZ
	4.0	27.33 ± 2.85 zY	20.00 ± 2.08 zX	26.33 ± 0.88 zY	29.00 ± 2.08 zY	42.33 ± 2.19 zZ
	8.0	15.67 ± 2.40 yX	17.00 ± 3.61zX	22.00 ± 4.16 zY	26.33 ± 1.86 zY	36.00 ± 2.65 zZ
Hb (g/dL)	Control	9.43 ± 0.59 zX	7.00 ± 0.75 zX	10.23 ± 1.25 zY	11.30 ± 0.78 zY	13.53 ± 1.36 zZ
	2.0	9.10 ± 0.99 zY	6.77 ± 0.29 zX	8.67 ± 1.66 zX	11.23 ±1.01 zY	14.20 ± 1.46 zZ
	4.0	9.10 ± 0.99 zY	6.67 ± 0.71 zX	8.77 ± 0.29 zY	9.67 ± 0.71 zY	14.13 ± 0.72 zZ
	8.0	5.23 ± 0.79 yY	5.67 ± 1.20 zY	7.33 ± 1.37 zZ	5.77 ± 0.62 yY	8.03 ± 0.88 zZ
RBCs (10^6 cells/mm^3)	Control	146.67 ± 17.64 zZ	130.00 ± 11.55 zZ	108.33 ± 4.41 zZ	126.67 ± 21.86 yZ	121.67 ± 1.67 zZ
	2.0	123.33 ± 33.33 yY	126.67 ± 20.28 zY	88.33 ± 22.05 yW	150.00 ± 15.28 zZ	98.33 ± 6.01 yX
	4.0	108.33 ± 50.87 xY	110.00 ± 15.28 yY	88.33 ± 25.87 yX	130.00 ± 15.28 yZ	106.67 ± 12.02 yY
	8.0	98.33 ± 24.55 xZ	100.00 ± 11.55 xZ	66.67 ± 8.82 xY	100.00 ± 7.64 xZ	100.00 ± 35.12 yZ
WBCs (10^3 cells/mm^3)	Control	3,466.67 ± 290.59 yZ	2,633.33 ± 272.85 zY	2,000.00 ± 115.47 yX	933.33 ± 120.19 yW	1,066.67 ± 66.67 xW
	2.0	4,166.67 ± 578.31 zZ	2,266.667 ± 240.37 yY	2,100.00 ± 251.66 yY	1,066.67 ± 66.67 zX	803.33 ± 347.87 wW
	4.0	4,200.00 ± 721.11 zZ	2,433.33 ± 371.18 zY	2,133.33 ± 266.67 yY	1,033.33 ± 185.59 yX	1,233.33 ± 284.80 yX
	8.0	2,800.00 ± 702.38 xZ	2,266.67 ± 240.37 yZ	2,666.67 ± 176.38 zZ	1,066.67 ± 266.67 zY	1,400 ± 115.47 zY
MCHC (%)	Control	33.29 ± 0.08 zZ	33.35 ± 0.10 zZ	33.36 ± 0.08 zZ	33.24 ± 0.00 zZ	33.27 ± 0.03 zZ
	2.0	33.28 ± 0.08 zZ	33.28 ± 0.11 zZ	33.32 ± 0.07 zZ	33.35 ± 0.07 zZ	33.28 ± 0.03 zZ
	4.0	33.29 ± 0.04 zZ	33.31 ± 0.10 zZ	33.29 ± 0.09 zZ	33.32 ± 0.07 zZ	33.39 ± 0.03 zZ
	8.0	33.44 ± 0.15 zZ	33.33 ± 0.00 zZ	33.37 ± 0.10 zZ	33.29 ± 0.04 zZ	32.77 ± 0.65 zZ
MCH (pg/cell)	Control	0.65 ± 0.04 zZ	0.54 ± 0.01 zZ	0.96 ± 0.16 zZ	0.94 ± 0.16 zZ	1.11 ± 0.12 zZ
	2.0	0.80 ± 0.14 zZ	0.56 ± 0.10 zZ	1.01 ± 0.06 zZ	0.78 ± 0.15 zZ	1.47 ± 0.22 zZ
	4.0	1.12 ± 0.30 zZ	0.65 ± 0.16 zZ	1.13 ± 0.23 zZ	0.77 ± 0.13 zZ	1.34 ± 0.17 zZ
	8.0	0.58 ± 0.09 zZ	0.57 ± 0.08 zZ	0.74 ± 0.32 zZ	0.89 ± 0.12 zZ	1.42 ± 0.31 zZ
MCV (fL/cell)	Control	1.96 ± 0.12 zZ	1.61 ± 0.03 zZ	2.87 ± 0.47 zZ	2.84 ± 0.49 zZ	3.34 ± 0.35 zZ
	2.0	2.41 ± 0.41 zY	1.70 ± 0.30 zY	3.03 ± 0.17 zY	2.34 ± 0.44 zY	4.42 ± 0.66 zZ
	4.0	3.37 ± 0.90 zZ	1.95 ± 0.49 zZ	3.39 ± 0.70 zZ	2.32 ± 0.40 zZ	3.93 ± 0.54 zZ
	8.0	1.72 ± 0.26 zX	1.61 ± 0.21 zX	3.24 ± 0.22 zY	2.68 ± 0.36 zY	4.25 ± 0.92 zZ

TABLE 2. Effect of fenthion on leukocyte differentials (%) in African Catfish. See Table 1 for additional details.

Parameter	Concentration (mg/L)	Exposure duration (d)				
		1	7	14	21	7-d recovery
Neutrophils	Control	70.00 ± 0.00 zZ	70.00 ± 1.15 zZ	64.67 ± 4.37 yZ	64.00 ± 5.03 yZ	70.67 ± 0.67 zZ
	2.0	64.67 ± 3.33 yZ	61.33 ± 4.81 yZ	72.67 ± 1.76 zZ	64.00 ± 2.31 yZ	65.33 ± 3.71 yZ
	4.0	67.33 ± 2.40 yZ	72.00 ± 1.16 zZ	66.00 ± 3.06 yZ	65.33 ± 5.70 yZ	67.33 ± 2.40 zZ
	8.0	62.67 ± 3.71 yZ	61.33 ± 4.81 yZ	64.67 ± 2.40 yZ	70.00 ± 1.16 zZ	64.67 ± 3.33 yZ
Lymphocytes	Control	27.33 ± 0.67 zZ	26.33 ± 2.10 zZ	32.00 ± 5.13 zZ	32.67 ± 4.67 zZ	27.33 ± 0.67 zZ
	2.0	34.00 ± 3.06 zZ	35.67 ± 4.63 zZ	24.00 ± 1.73 zY	34.00 ± 2.08 zZ	33.67 ± 3.67 zZ
	4.0	31.33 ± 1.76 zZ	26.33 ± 1.33 zZ	32.00 ± 3.00 zZ	36.00 ± 9.08 zZ	31.33 ± 1.76 zZ
	8.0	33.67 ± 3.67 zZ	35.67 ± 4.63 zZ	34.00 ± 1.53 zZ	28.33 ± 0.88 zZ	34.00 ± 3.06 zZ
Monocytes	Control	2.00 ± 0.00 zZ	2.00 ± 0.00 zZ	1.67 ± 0.33 zZ	1.33 ± 0.33 zZ	0.50 ± 0.00 zZ
	2.0	0.67 ± 0.33 zZ	1.67 ± 0.33 zZ	1.33 ± 0.33 zZ	1.33 ± 0.67 zZ	0.33 ± 0.33 zZ
	4.0	1.33 ± 0.33 zZ	1.33 ± 0.88 zZ	0.50 ± 0.00 yY	0.33 ± 0.33 zZ	0.67 ± 0.33 zZ
	8.0	0.67 ± 0.67 zZ	1.67 ± 0.33 zZ	0.33 ± 0.33 yY	1.00 ± 0.58 zZ	0.33 ± 0.33 zY
Eosinophils	Control	0.00 ± 0.00 zZ	1.33 ± 0.33 zZ	1.33 ± 0.33 zZ	0.33 ± 0.33 zZ	1.33 ± 0.67 zZ
	2.0	0.33 ± 0.33 zY	2.00 ± 0.00 zZ	2.00 ± 0.00 zZ	0.33 ± 0.33 zY	0.67 ± 0.67 zY
	4.0	0.33 ± 0.33 zY	1.33 ± 0.88 zY	2.00 ± 0.58 zZ	0.00 ± 0.00 zY	0.67 ± 0.33 zY
	8.0	0.00 ± 0.00 zY	2.00 ± 0.00 zZ	1.00 ± 0.58 zY	0.00 ± 0.00 zY	0.67 ± 0.33 zY
Basophils	Control	0.00 ± 0.00 zZ	0.00 ± 0.00 Z	0.33 ± 0.33 zZ	1.00 ± 1.00 zZ	0.00 ± 0.00 zZ
	2.0	0.33 ± 0.33 zZ	0.00 ± 0.00 Z	0.00 ± 0.00 zZ	0.33 ± 0.33 zZ	0.00 ± 0.00 zZ
	4.0	0.67 ± 0.33 zZ	0.00 ± 0.00 Z	0.00 ± 0.00 zZ	1.00 ± 0.58 zZ	0.00 ± 0.00 zZ
	8.0	0.33 ± 0.33 zZ	0.00 ± 0.00 Z	0.00 ± 0.00 zZ	0.67 ± 0.67 zZ	0.33 ± 0.33 zZ

Conclusions

The changes observed in the blood parameters of African Catfish exposed to sublethal concentrations of fenthion indicate that the pesticide is harmful to these fish. Although our test concentrations (2.0, 4.0, and 8.0 mg/L) exceeded the freshwater aquatic life standard of 1.0 µg/L (USEPA 2006), in view of the repeated application, drifting, drainage, and leaching of the pesticide in most developing countries, the concentrations in the aquatic ecosystems of those countries are probably higher than the standard, supporting the relevance of our test concentrations. Further studies investigating the toxicokinetics of fenthion in freshwater fishes are recommended to obtain greater insights into the underlying mechanism of this insecticide.

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