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Full Length Research Paper

Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*)

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Pesticides and drugs used in agriculture and veterinary medicine may end up in aquatic environments and bioaccumulate in the food chain, thus posing serious problems for fauna and human health. The lethal concentrations of 50% of sample (LC_{50}) and 25% (LC_{25}) were determined using semi-static method. Eighty-four adult catfish were used for the toxicity study. Seven fish per test concentration in 2 replicates were exposed to varying concentrations of diazinon (0, 1.62, 3.24, 6.48, 12.96 and 25.96 ppm) in water. The effect of diazinon (LC_{50}) 6.6 ppm was further studied to determine the effect on behavioural, blood parameters and plasma biochemistry. The fish in the experimental group showed restlessness, increased reaction to exogenous stimuli, incoordination of movement and postural orientation before death. It is obvious from this study that diazinon negatively affects respiration and produces nervous signs with decrease acetylcholinesterase activities in fishes.

Key words: African catfish, *Clarias gariepinus,* acute toxicity, lethal dose.

INTRODUCTION

Human destructive influence on the aquatic environment is in the form of sublethal pollution, which results in chronic stress conditions that have a negative effect on aquatic life. Pesticides and drugs used in agriculture and veterinary medicine may end up in aquatic environments and bioaccumulate in the food chain, thus posing serious problems for fauna and human health. The acute toxicity of chemicals may be easily evaluated in a short term test and death represents unequivocal end points. From an ecological point of view, survival, growth, reproduction, spawning and hatching success provide reaction and adoption to environment parameter regardless of whether they are natural or man made. Macroscopically, overt signals of toxicity are almost always preceded by changes at the organ, tissue, cellular and molecular levels (Segner and Branubeck, 1990). Diazinon is a common active substance of organophosphorous pesticides (Roberts and Hutson, 1998). Although the aquatic environment is not the main target and the aquatic invertebrates are not the target organisms, presence of diazinon in water has been reported and its negative effect to aquatic organisms has been proven (De-Vlaming et al., 2000). This study determines the LC_{50} and LC_{25} (concentration of the test substance that will be lethal to 50 and 25% of the organism) and the behavioral response of the African catfish to acute effects of diazinon, an organophosphate pesticides.

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MATERIALS AND METHODS

Fish

Live specimens of the Africa catfish (*Clarias gariepinus*) (94, 350 ±15 g, 35 ± 2.0 cm) were purchased from the sale outlet of Oyo State Fish farm In Ibadan.

Acute tocixity test

The 96 h acute toxicity test on *C. gariepinus* with Diazinon followed the OECD (2001a) Direction No. 203 and Methodical Manual ISO 7346/2. The fish were acclimatized for 14 days at room temperature in plastic tanks and fed with commercial fish pellet at 3-5% body weight. No disease treatments were administered within 48 h of test

Table 1. Average mortality pattern for acute toxicity test (24 -96 h).

Conc. (ppm)	24 h		48 h		72 h		96 h	
	No. Alive	No Dead	No. Alive	No. Dead	No. Alive	No. Dead	No. Alive	No Dead
0.00	7	0	7	0	7	0	7	0
1.62	7	0	7	0	7	0	7	0
3.24	7	0	5	2	5	2	4	3
6.48	6	1	2	5	2	5	2	5
12.96	4	3	1	6	1	6	1	6
25.92	0	7	0	7	0	7	0	7

initiation and during testing. Mortality during the period of acclimatization was less than 5%.

Introduction of fish into the tanks

84 catfish of average weight (350 g ± 15 g) in size and 35 cm ± 2.0 cm mean length were added to test chamber within 30 min of addition of diazintol[R]. The test compound, diazinon of a concentration of 162 mg/ml was added to the diluting water. The fish were introduced randomly to individual replicates containing five concentrations of the test substance- 1.62, 3.24, 6.48, 12.96, 25.92 and 0.00 as control. The dilutions were made with the control water.

Replicates

Two replicates per test concentration were used to avoid test repetition due to system failure and to provide a stronger statistical baseline. Each test chamber contains an equal volume of test solution (100 l) and equal numbers of fish (7). Replicate test chambers were physically separated.

Test concentration

Six (6) test concentrations were used in this investigation. The test concentrations were selected to produce a no-observable-effect concentration (NOEC) and, at least two mortalities. Aerators were connected to the tanks to aerate the solution. The preparations were changed every 24 h with fresh preparation of diazinon daily, to prevent evaporation. Temperature and dissolved oxygen of the water in the tanks containing the experimental and control catfish were monitored, and mortalities were immediately removed. Mortalities were collated at 24, 48, 72 and 96 h. Abnormal behaviours of the fish were also recorded. The lethal concentration (LC_{50} was then determined after 96 h of the experiment) using Aritheme-tric Method of Karber (adapted by Dede, 1992) and Probit analysis. Probit analysis (SAS, 1988) was used to calculate the LC_{50}, and the 95% confidence limits (lower and upper limits).

RESULTS

General effect

Abnormal behaviors such as restlessness, sudden quick movements, rolling movements, swimming on the back at higher doses were observed when the media started to act on test species. A neural paralytic syndrome was typical for fish poisoned with diazinon. Strong restlessness started when fish came into contact with the poisoning bath and they tried to jump out of the water. Fish excitation was reflected by an increased reaction to exogenous stimuli and by cramp movements of fins and mouth. Loss of movement coordination began, as well as loss of orientation in water. The fish turned on the flank and swam in half- circles. Reaction to excitation was manifested by sudden movement and fin tremor. Body surface darkening was noticeable in this phase of poisoning, mainly on the dorsal part. Weakening of jerk or a reflexia, paralysis, arrhythmia and block of respiration movement began in the terminal phase of poisoning. At the highest concentration (25.92 ppm) some of the fish fell into pain and died in a short time. Body surface was opaque with slightly increased amount of mucus and with extensive pigmentation mainly on the dorsal part. Gills had straight edges and normal colour (pinkish red). In the body cavity, there was an evident injection of internal organ vessels, mainly hyperaemia. Mortality pattern for 24, 48, 72, and 96 h are as presented in Tables 1 and 2. The LC_{50} was calculated to be 6.6 ppm while the LC_{25} was calculated as 3.3 ppm. Using Probit analysis (SAS, 1988) LC_{50} was calculated to be 6.15 with 95% confidence limits (lower limit 3.91 and 9.48 as upper limit; Figure 1).

DISSCUSION

There were differences in the acute toxicity of diazinon for various fish species. The $96hLC_{50}$ values range in tenths to several tens of mg l^{-1} (Tsuda et al., 1997). In European eel (*Anguilla anguilla*) the $96hLC_{50}$ values ranged even in hundredths of mg l^{-1} (Sancho et al., 1992a,b). The difference in toxicity of diazinon may be demonstrated by the example of two fish species used for ecotoxicological assessment of chemical substances. The $96hLC_{50}$ value of diazinon for guppy (*Poecilia reticulata*) was 0.8 mg l^{-1} but for zebra fish (*Brachydanio rerio)* it was 8 mg l^{-1} (Keizer et al., 1991). Oh et al. (1991) presented three factors causing the selective toxicity of diazinon for various fish species as: different inhibition of acetylcholinesterase, different detoxification and absorption. The above factors were responsible for the different toxic reaction of the fish to varying concentration of the

Table 2. 96hLC$_{50}$ determination using Arithmetic Method of Karber (adapted by Dee, 1992).

Conc. (ppm) (A)	Conc. Diff (B)	No. Alive	No. Dead	Mean Dead (E)	B X E
0.00	----	7	0	----	----
1.62	1.62	7	0	----	----
3.24	1.62	4	3	1.5	2.43
6.48	3.24	2	5	4.0	12.96
12.96	6.48	1	6	5.5	35.64
25.92	12.96	0	7	6.5	84.24
Total					135.27

$LC_{50} = LC_{1OO} - \sum$ Mean death x Conc.Diff
NO OF ORG NISM PER GROUP
25.92 - 135.27 / 7
25.92 - 19.32
6.6ppm

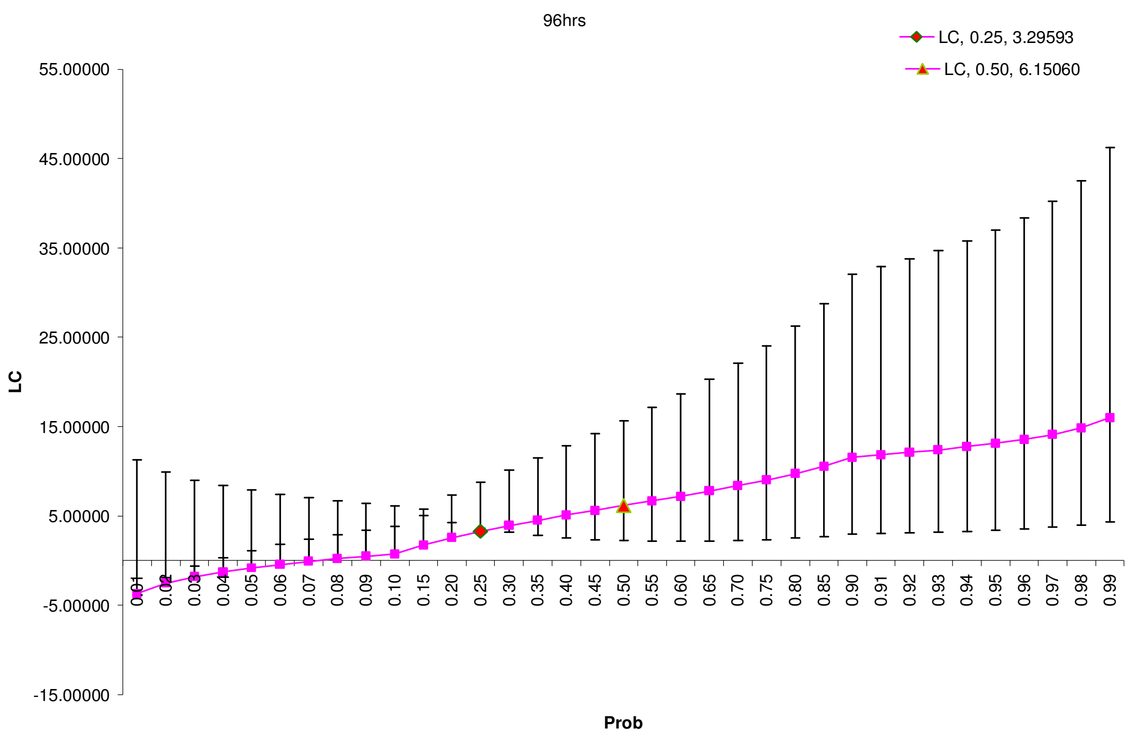

Figure 1. LC $_{50}$ and LC $_{25}$ showing 95% confidence limits (lower and upper).

chemical. The reactions were more pronounced at higher concentration due to increase inhibition of acetylcholinesterase which eventually results in death of the fish.

The exposure of *C. gariepinus* to sublethal concentration of diazinon showed mortality even at low concentrations. From the international classification of toxicity of substances based on their median lethal concentration (LC$_{50}$), diazinon is slightly toxic to *C. gariepinus*. This corroborated with previous reports on the effect of organophosphate on aquatic life (DeVlaming et al., 2000). In the course of 96 h acute toxicity test of diazinon-based organophosphorous preparation Diazintol^{R} (162 mg/ml of diazinon) on *C. gariepinus* fish, there was no mortality in the control tank. However, mortality increased with increase in concentration of the diazinon with time. In the treatment, mortality was first noticed at a concentration of 3.24 ppm and total mortality occurred at a concentration of 25.92 ppm. Oxygen saturation of water did not drop below 60% in any concentration tested, nor in the control treatment. Presence of diazinon (above 80% of the nominal concentration) was provided by means of daily exchange of the testing bath.

Conclusion

This study has shown that diazinon is toxic to the African catfish and that the toxicity varies with the duration of exposure. The LC_{50} for the adult catfish was calculated to be 6.15 ppm with 95% confidence limits (lower limit 3.91 and 9.48 as upper limit).

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