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Acute Toxicity of Lindane (Gamma Hexachloro-Cyclohexane) to African Catfish (*Clarias gariepinus*, Burchell, 1822)

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Abstract: The acute toxicity of Lindane to African Catfish (*Clarias gariepinus*) juveniles was assessed in a static renewal bioassay for 96 h to examine the effects of Lindane on *C. gariepinus*, ascertain their level of tolerance and their suitability as bio-indicator in freshwater ecosystems. Eight graded concentrations of lindane were prepared as 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 ppm and a control experiment (0.0 ppm). The 96 h median lethal concentration (LC_{50}) value computed using probit method was 1.29 ppm. The median lethal time (LT_{50}) of 0.9, 1.0 and 1.1 ppm was zero while the LT_{50} of 1.2, 1.3 and 1.4 ppm are 4365 min (~73 h), 3548 min (~59 h) and 2884 min (~48 h), respectively. The minimum lethal concentration was 1.40 ppm while the minimum lethal time was 4365 min (~73 h). At various concentrations of lindane, fish showed uncoordinated behaviour such as incessant gulping of air and increase in opercular ventilation. The number of survivors in each concentration differ significantly ($p<0.05$) from others. The results show that lindane is highly toxic to *Clarias gariepinus* juveniles.

Key words: *Clarias gariepinus*, LC_{50}, LT_{50}, Lindane, minimum lethal concentration, minimum lethal time

INTRODUCTION

A pesticide is any substance used to control pests. It is used directly or indirectly for controlling or preventing, destroying, mitigating or repelling any pest or of altering their growth, development and characteristics (Lee, 1992). Pesticide is defined by United Nations Environment Programme (UNEP, 2005) as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides are widely used in the world in the control of pests on crops and on animals for vector control. One of the adverse effects of their use is the contamination of the environment which often results from direct application of pesticides into crops, animals, soil and water. It has been estimated that only about one percent of applied pesticides land on the target and that the rest contaminates the environment.

The development of highly complex chemical methods of pest control started around World War II, with the introduction of the first synthetic organochlorine (OC) insecticides, which included DDT, lindane (HCH), aldrin and dieldrin. The thousands of different pesticides manufactured today fall roughly into the following chemical categories; organochlorines, halogenated hydrocarbons, carbamates, heterocyclic compounds, organophosphates, chlorinated phenoxy substances, amines and ureas, benzonitriles, phenolic compounds and pyrethroids. They consist of a mixture of active ingredients; designed to destroy the pests, together with many other chemical additives, such as solvents, combined into usable products (BMA Guide to Pesticides, 1992).

Since the early development of agricultural practices, people have always sought different ways to increase their crop yield. The early use of pesticides included a variety of substances, such as urine, lime, soap suds, vinegar, tobacco, and similar simple compounds. Agrochemical production began as a relatively simple process, based primarily on combinations of a few chemical substances such as copper, mercury salts, elemental sulphur, arsenic, and cyanide (BMA Guide to Pesticides, 1992).

Gamalin 20 is an organochlorine insecticide and fumigant which contain lindane as the active ingredient. This has been used on a wide range of soil-dwelling and plant eating insects. Lindane is presently used primarily for seed treatment and in lotions, creams and shampoos for the control of lice and mites (Scabies) in humans (Adedeji *et al*., 2008). Benzene Hexachloride (BHC) is the 100% pure form of the product while lindane is slightly less pure (99% pure).

Lindane is highly toxic to fish, bees and aquatic invertebrates. It is very stable in both fresh and salt water environments. It will disappear from the water by secondary mechanisms such as adsorption on sediment, biological breakdown by microflora and fauna and adsorption by fish through gills, skin and food (Ulman, 1972).

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Bioassays are used to determine the toxicity of chemical substances and to indicate which organisms are the most sensitive to such chemicals (Ndimele and Jenyo-Oni, 2009). These data are used to rank chemicals, determine their water quality criteria and set standards for effluent discharges (Finney, 1971).

The objective of this study is to examine the acute toxicity of lindane (Gamma Hexachloro-Cyclohexane) to *Clarias gariepinus* so as to ascertain their level of tolerance and their suitability as bio-indicator in freshwater ecosystems.

MATERIALS AND METHODS

Collection of test organism: 270 live specimens of juvenile catfish *C. gariepinus* were used in the present study. They were procured from Goshen Fisheries and Aquaculture, Badagry, Lagos, Nigeria. The body weight and total length measurement ranged from 26.89-95.50 g and 16.40-25.30 cm, respectively. The specimens were brought to the laboratory in plastic container filled with oxygenated and cool water to reduce their activity and stress before reaching the laboratory. Collection and transportation were done between 4 pm and 6 pm to further prevent stressful condition.

Acclimatization of test organisms: The acclimatization and the toxicity bioassay were conducted in the Toxicology Laboratory, Department of Fisheries, Faculty of Science, Lagos State University, Ojo, Lagos, Nigeria. In laboratory, the specimens were kept in 27 plastic tanks (20 L) containing dechlorinated water. They were acclimatized to laboratory conditions for 14 days. The dechlorinated water in each experimental tank was stocked with 10 juveniles each and was renewed daily to avoid contamination of wastes and the dissolved carbon dioxide (DCO_2), pH, and dissolved oxygen (DO_2) were recorded. The aquaria (experimental units) during acclimatization and experimental period were aerated with mechanical air pumps. The specimens were fed with commercial fish feed (40% CP) twice daily at 3% body weight (Reish and Oshida, 1987).

Sources of lindane (Gammalin 20): Lindane (Gamma Hexachloro-Cyclohexane) used for this experiment was obtained from reputable shops in Lagos, Nigeria. The Lindane was stored in the laboratory at room temperature.

Dilution water: Dilution\well (dechlorinated) water was used during acclimatization, control tests and in the making of various concentrations of lindane. The water was chemically and biologically certified before it was used for toxicity test and the chemical criteria include - low or undetectable levels of priority pollutants (ASTM, 1980).

The biological criteria include survival of test organism throughout acclimatization and test period with no sign of stress or discoloration of skin or unusual behaviour. For maintenance of the stock solution and serial dilution for the bioassay test, dechlorinated water was used.

Preparation of stock solution: Dilution of lindane for bioassay test was carried out by preparing a stock solution. 1 mL of the toxicants was added to 1 L of dechlorinated water. Then 1 mL of the stock solution into 1L of H_2O (giving 1ppm). Subsequent serial dilution was made from the stock solution.

Acute toxicity studies: Static bioassay techniques APHA/AWWA/WPCF (1980) and Reish and Oshida (1987) were employed in the determination of acute toxicity of lindane (Gamma Hexachloro-Cyclohexane) on African catfish *Clarias gariepinus*. The concentration of the bioassay was renewed every 24 h.

Bioassay procedure: Standard method for bioassay as described by Reish and Oshida (1987) was used. The acute toxicity procedure started with a Range Finding Test, which was conducted for 96-hour period to determine the concentration at which the pesticide was lethal to the fish.

Definitive test: After the range finding test, the definitive test was carried out. The control and the treatments were run simultaneously. 90 acclimated active fish of the specimens were divided in groups of ten separately for each of the concentration. The bioassays concentrations were 0.0 ppm (control), 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5 and 1.6 ppm. The experiment was conducted in triplicates, which gave a total of twenty seven (27) experimental units (test tanks) containing 270 juveniles of *C. gariepinus*.

Using the standard methods of observation by Sprague (1969) and Veirns (1982), fish mortality was recorded on basis of 24, 48, 72 and 96 hours. The water in the experimental and the control tanks were renewed daily and freshly prepared stock was added, and the fishes were unfed during 96 hour period of exposure. The LC_{50} was determined using probit method (Finney, 1971).

Quantal responses (Mortality): In this study, the specimens were considered dead when it become immobile, ceased all respiratory movement (Oronsaye, 1990) and failed to respond to a slight prod with a rod or when they turn upside down or float or when their body/operculum/mouth or tail show no form of movement even when probed with a rod.

Control test: Control is an integral part of toxicity test and was done to ascertain if the death of organisms were

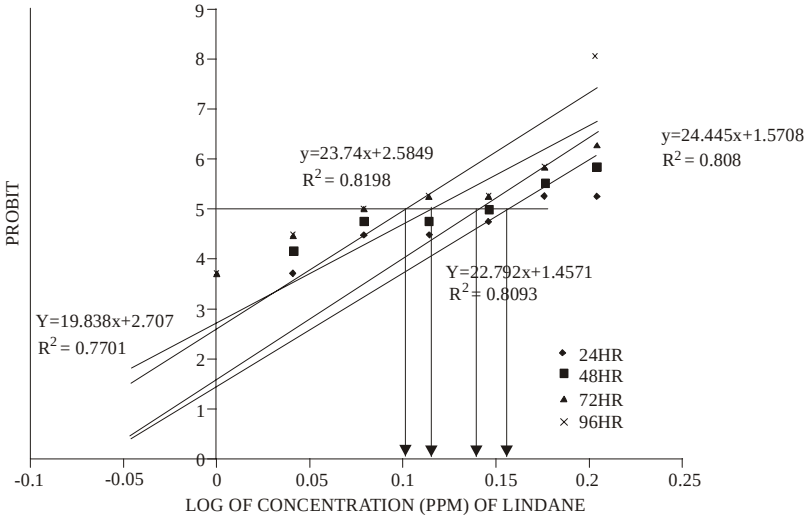

Fig. 1: Median Lethal Concentration (LC_{50}) of Lindane to *Clarias gariepinus*

due to the toxicant or some other factors. Control test were typically conducted by placing the organisms in dechlorinated well water with no toxicant. As a rule, a toxicity test is valid if control mortality was less than 10% (Odiete, 1999).

Physico-chemical parameters: Comprehensive analyses of the four important physic-chemical parameters (temperature, O_2, pH and CO_2) were carried out. Temperature and pH values were determined in the laboratory using a mercury-in-glass thermometer and a Griffin pH meter (Model 400) respectively, while dissolved oxygen and carbon dioxide were determined according to Boyd (1979).

Statistical Analysis: Each test concentration was converted into a logarithm and the corresponding percentage mortality was transformed into probit (Sprague, 1969). The median lethal toxicity (LC_{50}), median lethal time (LT_{50}), minimum lethal concentration and minimum lethal time were determined according to the method described by Finney (1971). Analysis of variance (ANOVA) was used to test for significant differences in the number of survivors in different concentrations of the toxicants (lindane).

RESULTS AND DISCUSSION

The results of the water quality of the media used in the present study are within the optimal range reported by Viveen *et al*. (1985) as optimal requirement for *C. gariepinus*. Temperature, pH, and dissolved oxygen obtained in this study are 28-30ºC, 6.5-7.0, and 2.0-5.0 mg/L, respectively (Table 1), while the recommended range are 20-20ºC (FEPA, 1991), 6-9 (FEPA, 1991) and

Table 1: Physico-chemical parameters of the experimental units

Parameter	Range
Temperature (ºC)	28-30
pH	6.5-7.0
Carbon dioxide (mg/L)	3.2-4.5
Dissolved oxygen (mg/L)	2.0-5.0

Table 2: Median lethal concentrations (LC_{50}) of Lindane to *Clarias gariepinus*

Time (HR)	LC_{50} (ppm)
24	1.45
48	1.38
72	1.32
96	1.29

5 mg/L (Boyd, 1979). The values of the water quality of the test media indicated that lindane did not adversely affect the water quality. Therefore, mortality recorded was probably due to direct effect of lindane.

The 24, 48, 72 and 96 h median lethal concentration (LC_{50}) of lindane to *Clarias gariepinus* are 1.45, 1.38, 1.32 and 1.29 ppm, respectively (Table 2). The correlation coefficient (r^2) between log concentration of the toxicant (lindane) and probit mortality showed that there were strong and positive correlations between correlation and mortality values for 24, 48, 72 and 96 h. The correlation coefficient (r^2) for 24, 48, 72 and 96 h are ($r^2 = 0.81$; $N = 6$; $\alpha = 0.05$); ($r^2 = 0.81$; $N = 6$; $\alpha = 0.05$); ($r^2 = 0.82$; $N = 6$; $\alpha = 0.05$) and ($r^2 = 0.77$; $N = 6$; $\alpha = 0.05$), respectively (Fig. 1). Table 3 shows the number of survivors of *C. gariepinus* exposed to different concentrations of lindane. The number of survivors in each concentration differ significantly ($p<0.05$) from others.

Figure 2 showed the minimum concentration (1.40 ppm) and minimum time (73 h) of lindane to *C. gariepinus*. Minimum concentration was taken as the lowest concentration of toxicants that can cause death of test organism (*Clarias gariepinus*) while minimum time

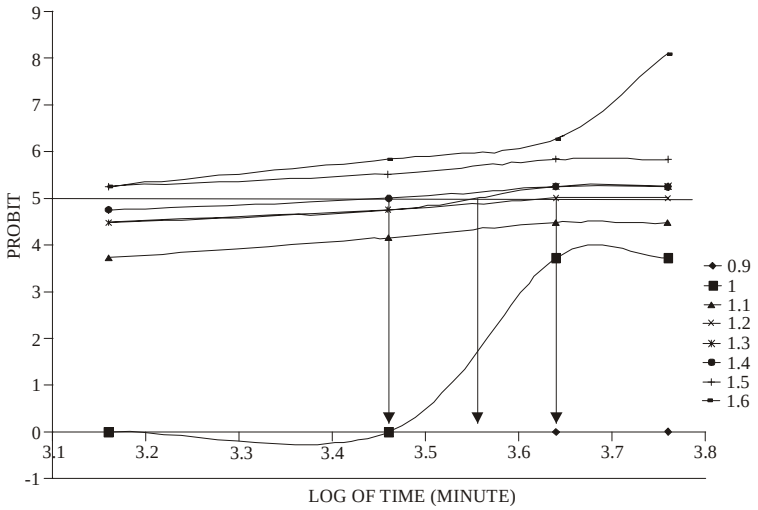

Fig. 2: Median Lethal Time (LT_{50}) of Lindane to *Clarias gariepinus*

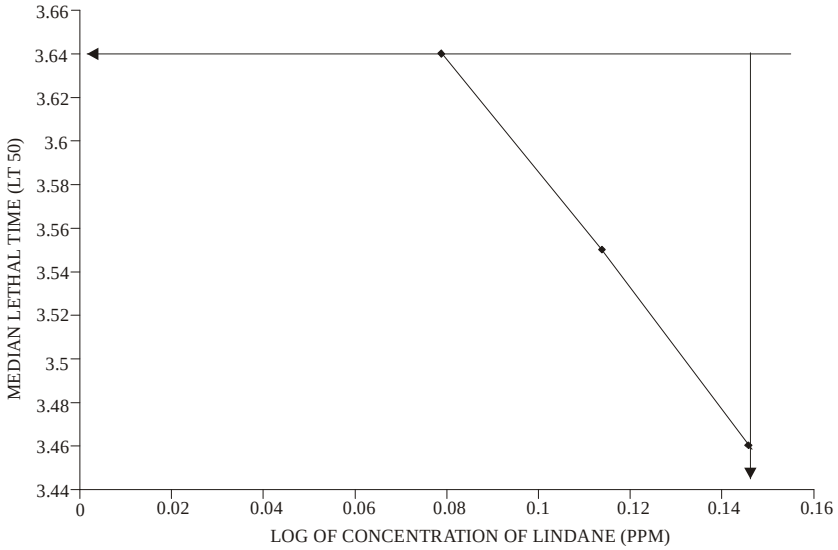

Fig. 3: Minimum lethal concentration and minimum lethal time of Lindane to *Clarias gariepinus*

was the time that this minimum concentration will use to cause the death of test organism (Ndimele *et al*, 2010). The median lethal time (LT_{50}) of 0.9, 1.0 and 1.1 ppm was zero (Fig. 2) indicating that at these concentrations of lindane, the mortalities were less than 50%. The mean lethal time of 1.2, 1.3 and 1.4 ppm are 4365 min (~73 h), 3548 min (~59 h) and 2884 min (~48 h) respectively. The minimum lethal concentration was 1.40 ppm while the minimum lethal was 4365 min (~73 h) (Fig. 3).

The LC_{50} of lindane, an organochlorine and other organophosphates vary considerably when previous reports are compared. The 96 h LC_{50} (1.29 ppm) of lindane to *C. gariepinus* obtained in this study is higher than the value (0.38 ppm) reported in a previous study by Omitoyin *et al*. (2006) where the toxicity of lindane was also tested against *C. gariepinus*. It is also higher than the 96hr-LC_{50} value (0.8 ppm) of diazinon, an organophospahate to guppy (*Poecilia reticulata*). However, it is lower than the values reported in

Table 3: Survivors of African catfish (*Clarias gariepinus*) exposed to different concentrations of lindane (Gammalin 20)

Concentration (ppm)	No of survival (Mean±S.D)
(control)	20.00±0.00[a]
0.9	19.33±0.33[b]
1.0	17.69±0.33[c]
1.1	14.00±0.58[d]
1.2	9.67±0.33[e]
1.3	8.00±0.58[f]
1.4	8.33±0.33[g]
1.5	3.33±0.33[h]
1.6	0.33±0.33[i]

previous studies for some other organophosphates: Keizer *et al*. (1991) reported a 96 h LC_{50} of 8 ppm for diazinon to zebra fish (*Brachydanio rerio*) and Adedeji *et al*. (2008) reported a 96 h LC_{50} of 6.6 ppm for diazinon to *C. gariepinus*. The differential toxicity of lindane to *C. gariepinus* can be attributed to the differences in susceptibility and tolerance related to its accumulation, biotransformation and excretion (Omitoyin *et al*., 2006).

Differences in metabolic pathways among species may result in different patterns of biotransformation, leading to more or less toxic metabolites (Johnson and Toledo, 1993). The magnitude of toxic effects of pesticide also depends on length and weight, corporal surface/body weight ratio and breathing rate (Murty, 1986). Metabolic differences between different animal classes may also be responsible for differential toxicity of chemicals.

Some of the early symptoms of lindane poisoning observed in this study are respiratory distress, increased physical activity, convulsions, difficulty in breathing, erratic swimming behaviour, swimming on lateral and ventral side and occasional darting up and down the water column. These behavioural signs were reported in study of the acute toxicity of lindane to *C. gariepinus* by Adedeji *et al* (2008). Rate of gill ventilation and oxygen consumption increases in fishes treated with sub-lethal concentration of insecticides (Waiwood and Johnson, 1974). Moreover, disrupted structural integrity of fishes gill by pesticide (Kumaragura *et al*., 1982) and deposition on them would reduce gaseous exchange. Meletev *et al*. (1971) reported that pesticides affect the gas exchange of fish and other aquatic organisms. Thus, a hypoxic condition may be induced at tissue level due to high demand and reduced supply of oxygen.

CONCLUSION

The study showed that lindane is toxic to *Clarias gariepinus* and its use as pesticide in agriculture should be strictly regulated to prevent its adverse consequences on aquatic ecosystem and man.

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