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Toxicity of Lindane (Gamma Hexachloro - CycloHexane) to *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. Five graded concentrations of lindane were prepared as 0.08, 0.18, 0.40, 0.80, 1.80 mg/L and a control experiment (0 mg/L). The 96 h (LC50) value computed on logarithm was 0.36 mg/L. The median LT50 was 26 and 67 h for 1.8 and 0.8 mg/L, respectively. At various concentration of lindane, fish showed uncoordinated behaviour such as incessant gulping of air and increase in opercular ventilation. Mortality was recorded earliest in the highest concentrations of 1.8 mg/L and with increasing period of exposure to lindane. Marked diffuse fatty degenerative hepatocytes, necrosis and heterophilic infiltration and pyknosis and degenerative changes of liver were the major histopathological effects distinctively shown. The results show that lindane is highly toxic to *Clarias gariepinus* juveniles.

Key words: Lindane · *Clarias gariepinus* · toxicity

INTRODUCTION

Agrochemical, such as pesticides especially chlorinated hydrocarbons are routinely employed as part of the integrated farming practice to protect crops and animals from insects, weeds and diseases. Widespread use of pesticide in agriculture is now a worldwide phenomenon [1].

The use of chlorinated hydrocarbon such as DDT, dieldrin and Lindane as pesticides has been documented. Many of the pesticides currently in use are biocides that have high mammalian toxicity and necessitate considerable precautions in their application [1].

The aquatic ecosystem as a greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity due to indiscriminate use of pesticides [2]. Pesticides become readily available in the food chain and subsequent bioaccumualate in both aquatic and terrestrial flora and fauna [3], with possible unquantifiable disastrous consequences on the ecosystem [4]. Due to the residual effects of pesticides, important organ like kidney, liver, gills, stomach, brain, muscle and genital organs are damaged in fish exposed to pesticide [2].

The African catfish (*Clarias gariepinus*) is one of the commercially important species of fish for rapid aquaculture expansion in Nigeria and elsewhere in the developing world. African catfish grow quickly, are omnivores and are desirable as food; they are a valuable species worldwide [5, 6].

Increase pesticides use in most tropical countries has been reported to cause severe toxicities, bioaccumulation and reduction of fish production in rice-fish culture [7-9].

There is therefore the need to investigate the toxicity of lindane, (chlorinated hydrocarbon) often use in pest management in agriculture to juveniles stage of *C. gariepinus* which is often stocked for aquaculture in Nigeria. Very limited work has been done on the histopathological effect of pesticides on fishes [2].

The objective of this study is therefore to determine the 96 h LC50 of lindane to juveniles of *C. gariepinus* and its histopathological effects on the liver.

MATERIALS AND METHODS

Experimental set up, Fish and management: Bioassay was conducted in the laboratory of the Department of Wildlife and Fisheries Management, University of Ibadan, Nigeria. The bioassay method of short-term toxicity in aquatic environment outlined by FAO [10] and APHA [11] with some modification.

Juveniles of *C. gariepinus* (Mean weight 38.07±0.05 g) and length (14.55±0.12 cm) were purchased from a commercial fish farm in Ibadan, Nigeria for this study. The lindane (λ-HCH) 200 g/L, EC 20% (Emulsifable concentrate) used as a toxicant for this investigation was obtained from International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria.

Two hundred juveniles of *C. gariepinus* were randomly assigned into eighteen aquaria tanks with 30 L of tap water in each. The fish were acclimatized for a period of twenty one days. They were fed with commercial fish feed (Pellets) containing 40% crude protein at 3% of their body weight [10]. Unconsumed feed and faecal wastes were removed and water replenished regularly as recommended by

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Oyelese and Faturoti [12]. A range finding test was carried out as described by Solbe [13], Omitoyin *et al*. [14] and Rahman *et al*. [2], respectively using a spacing factor of 10 to determine the concentrations of lindane used in the definite test.

The following concentrations in weight per volume of lindane were used for the range finding test: 0.0008, 0.008, 0.080, 0.8 and 8 mg/L, respectively. They were tested on 3 *C. gariepinus* juveniles per concentration of lindane, with three replications including the control with 0% Lindane. Based on the results of the range finding test, the following definitive concentrations of the pesticides were prepared as: 0.00(control), 0.08, 0.18, 0.40, 0.80 and 1.80 mg/L and were replicated thrice. Ten acclimatized fish were released into each aquarium containing the above different concentrations of lindane. All tests were carried out at room temperature (28±1°C). The behaviour, mortality and other external changes in the body of the test fish were observed and recorded accordingly.

Dead fish were promptly removed and mortality was specifically recorded at 3, 6, 12, 24, 48, 72 and 96 h of exposure time as described by Odiete [15], Omitoyin *et al*. [14] and Rahman *et al*. [2], respectively. The LC50 values for the juveniles *C. gariepinus* were calculated for 96 h of exposure time by logarithm analysis.

Water quality analysis and experimental set up: The water quality parameters such as Dissolved oxygen, temperature and the pH of the test media were recorded daily for the 96 h exposure period following the standard method on water quality assessment by APHA [11].

Histopathological examination: Histopahological examination was carried out on the liver samples (1 cm^3) from each concentration and preserved in small plastic vials with ten times 10% neutral buffer formalin fixatives as described by Omitoyin *et al*. [14] and Rahman *et al*. [2].

The numbers at section of samples was prepared using a microtome, stained and observed under a light microscope [2, 16].

Statistical analysis: The results obtained were analysed with the use of version 5.1 of the 1995 edition of statistical software (excel). Logarithm and arithmetic graph method according to Finney [17] was used to determine the median lethal toxicity and concentration.

RESULTS

Water quality, Toxicity and behavioural effects: The water quality analysis of the water used in this experiment showed that pH was 7.06, dissolved oxygen, 6.3 mg/L and temperature, 27°C±0.5. Mortality were recorded from 3 h and above, especially with higher concentrations of the insecticides, such that by 6 h, 2 (14.2%) mortality was recorded in each of the aquaria with 1.8 and 0.8 mg/L concentration of lindane.

There were changes in the frequency of movement of the fish subjected to different concentrations of lindane. Behavioral changes such as uncoordinated movements, somersaulting, convulsion, excess secretion of mucus, erratic swimming and increase in operculum ventilation, respiratory distress, strong spasm, paralysis, sudden quick movement and prior to the death, darkening of fish were observed during the exposure of fish to lindane. The colour of the skin of fish exposed to the toxicant changed from normal darkly pigmentation in the dorsal and lateral parts.

The 26 h LC50 value of the lindane computed from the logarithm graph for *C. gariepinus* was 0.3 mg/L (Fig. 1), while 96 h median LT50 computed from logarithm graph was 9 and 26 h for 0.8 mg/L and 1.8 mg/L lindane, respectively (Fig. 2).

Histopathological effects: Histopathological changes were well pronounced in the liver of the *C. gariepinus* juveniles exposed to different concentrations of lindane. The liver of the control fish showed no significant lesion (plate a). At 0.08 mg/L exposure of *C. gariepinus* to lindane, there was moderate fatty change (F) in the liver focal lymphocyte (L) and infiltration (I) seen in the portal areas of the liver (plate b). At 0.18 mg/L of lindane, there was moderate fatty degeneration (F) and infiltration (I) in the liver seen in the portal areas (plate c).

As the concentration of the lindane increased, there were more degeneration of portal cells as shown in the photomicrograph of liver of *C. gariepinus* after 96 h exposure to 0.4 mg/L (plate d). More severe moderate diffuse fatty degeneration (F) in hepatocytes with focal lymphocyte (L), infiltration (I) as seen in the liver at 0.8 mg/L of exposure to lindane (plate e). At the concentration of 1.8 mg/L (the highest concentration) exposure of the fish to lindane, there was a marked congestion in peripherally placed hepatocytes in the test fish (plate f). All the lessons observed indicates impending liver damaged prior to death of the test fish.

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*. [18] as optimal requirement for *C. gariepinus*. Thus, suggesting that the parameters did not seem to alter the toxicity of the insecticide to the test fish. However, temperature, hardness, pH, alkalinity, sex, age and other physiological status of the test animals was reported to have profound effects on the toxicity of agro-chemical [19, 20].

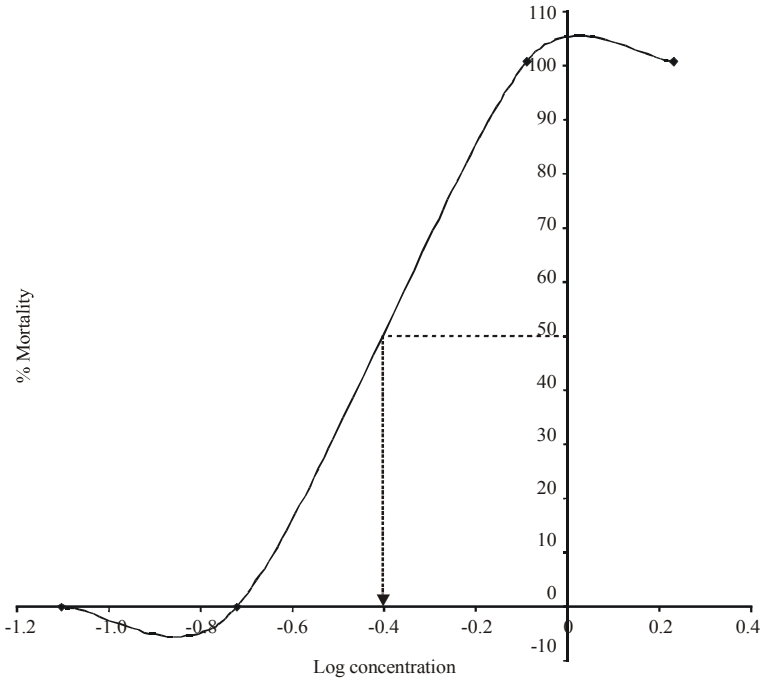

Fig. 1: The 96 h LC50 of Lindane to *Clarias gariepinus* juveniles

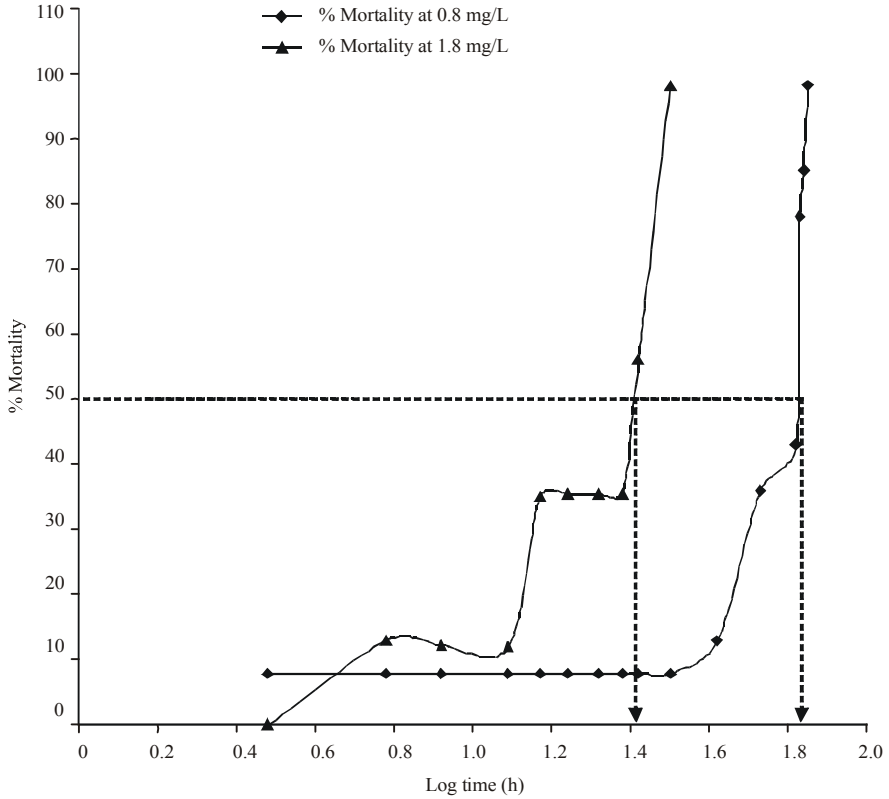

Fig. 2: Time-mortality graph to determine LT50 at different concentrations of Lindane to *Clarias gariepinus* juveniles

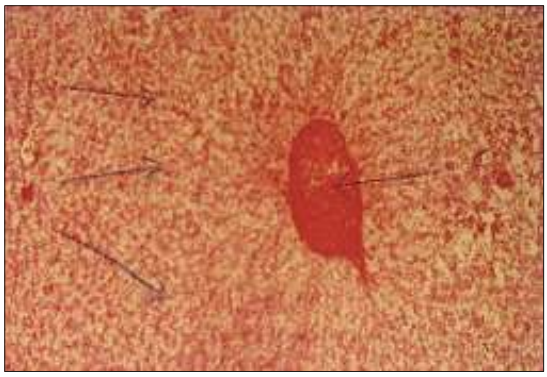

Plate a: Photomicrograph of liver of *C. gariepinus* not exposed to Lindane. No significant lesion was seen

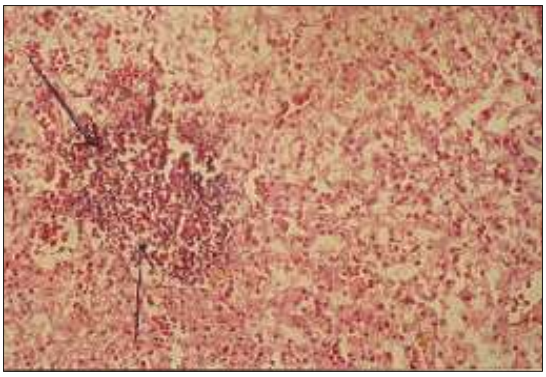

Plate d: Photomicrograph of liver of *C. gariepinus* after 96 h exposure to 0.4 mg/L Lindane Moderate faty degeneration (F) in hepatocytes with focal lymphocytic (L) infiltration (I) arrow seen in the portal area

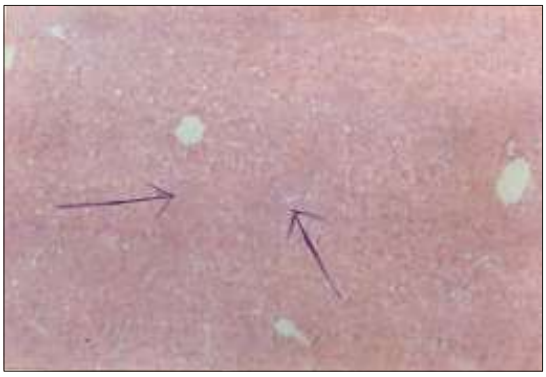

Plate b: Photomicrography of liver of *C. gariepinus* after 96 h exposure 0.08 mg/L Lindane. Moderate fatty change (F) and focal lymphocytic (L) infiltration (I) arrow seen in the portal area

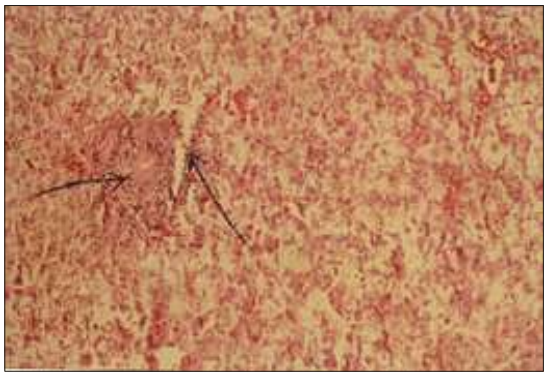

Plate e: Photomicrography of liver of *C. gariepinus* after 96 h exposure to 0.8 mg/L Lindane. Moderate diffuse fatty degeneration (F) in heapatocytes with focal lymphocytic (L) infiltration (I) arrow seen in the liver

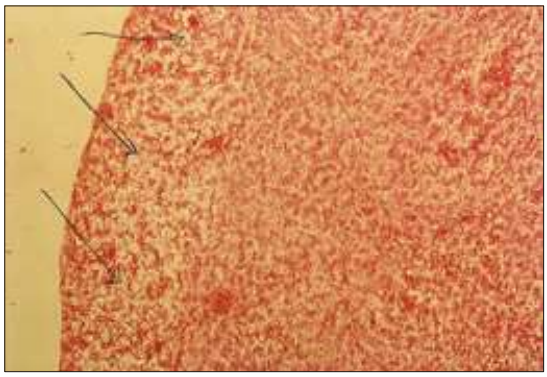

Plate c: Photomicrography of liver of *C. gariepinus* after 96 h exposure to 0.18 mg/L Lindane. Moderate fatty degeneration (F) in hepatocytes with focal lymphocytic (L) infiltration (I) in the liver, arrow seen in the portal areas

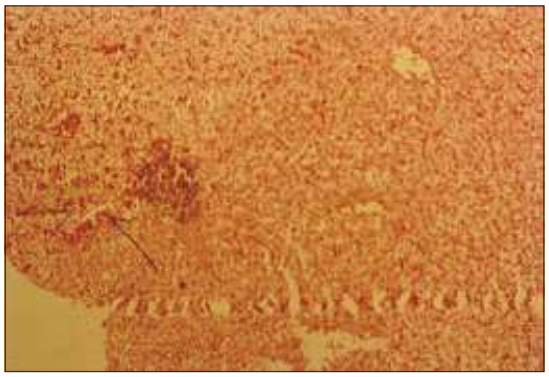

Plate f: Photomicrography of liver of *C. gariepinus* after 96 h exposure to 1.8 mg/L Lindane. Moderate diffuse fatty degeneration (F) in heapatocytes with focal lymphocytic (L) infiltration (I), arrow seen in peripherally placed hepatocytes

Toxicity of lindane to *C. gariepinus* is relatively lower when compared with other species of fishes. The 96 h LC50 value (0.38 mg/L) obtained in the present study is lower than the values reported in literature for other species of fish.Vittozzi and De angelis [21] summarized the 96 h LC50 values of other organophosphate pesticides like parathion (0.056-1.99 mg/L), methyl parathion (1.95-8.91 mg/L) and malathion (0.091-22.9 mg/L) for different species of fishes. These values indicate that these compounds are more toxic to fish than lindane. 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. Differences in metabolic pathways among species may result in different patterns of biotransformation, leading to more or less toxic metabolites [22, 23]. The magnitude of toxic effects of pesticide also depends on length and weight, corporal surface/body weight ratio and breathing rate [23-25]. Metabolic differences between different animal classes may also be responsible for differential toxicity of chemicals.

Increase physical activity, convulsion, excess secretions of mucus, incessant gulping of air, erratic swimming, respiratory distress, strong spasm, paralysis, sudden quick movement, increase in opercula ventilation and prior to death darkening of fish were associated with lindane toxicity in this study. This agreed with the findings of Alkahem *et al*. [23] on *Oreochromis niloticus exposed to trichloroform*. Omitoyin *et al*. [14] reported similar observation in *Sarotherodon galilaeus* (Tilapia) fingerlings exposed to piscicidal plant extracts of *Tetrapleura tetraptera*. Fafioye [16] also reported similar changes in fish exposed to *Parkia bioglobosa* and *Raffia vinifera*. Rate of gill ventilation and oxygen consumption increases in fishes treated with sub lethal concentration of insecticides [23, 26-28]. Moreover, disrupted structural integrity of fishes gill by pesticide [29-31] and deposition on them would reduce gaseous exchange. Meletev *et al*. [32] reported that pesticides affect the gas exchange of fish and other aquatic organisms. Thus, a hypoxic condition may be reduced at tissue level due to highly demand and reduced supply of oxygen. Previous investigation by Fernando and Moliner [33] and Gopal *et al*. [34] clearly indicated that a hypoxic condition developed in fish after pesticides exposure. This in turn may prompt a stress mediated physical activities such as convulsion, opercula gasping, uncoordinated movement, dullness and death. Fish under stress (physical, chemical or starvation) mobilizes triglyceride and protein to fulfill and increased demand for energy by the fish to cope with detrimental conditions imposed by the toxicant/xenobiotic and to meet energy required to sustain increased physical activity bio-transformation and excretion of xenobiotic [23, 35-38].

The manifestation of erratic behavioural or increased physical activity especially those related to movement and reflexes may be attributed to body biochemical derangement as well as hepatic lesion observed at postmortem [12, 39-42]. This implies that at very much lower concentration of lindane to fish with continuous exposure, growth, reproductive performance and general health condition of the exposed fish may be endangered because of the propensity for long term exposure at low doses as may be the case in the aquatic ecosystem. Bioaccumulation of lindane in the tissues of such fish will occur and this will serve as a source of low insidious poisoning in the food chain.

At the agricultural dose of 0.08 and 0.18 mg/L, mild moderate fatty changes and focal lymphocytic infiltration were observed in the portal areas and in few cases vacuolation were recorded for the fish species. At the highest dose of 1.80 mg/L, severe fatty degenerative changes of hepatocytes and in some samples, severe degenerative changes of hepatocytes like necrosis, vacuolation, rupture of blood vessel causing haemorrhage were observed in the test fish. Kabir and Begum [43] reported cytoplasmic degeneration, pyknotic nuclei in liver tissues; vacuolation in hepatic cells and ruptured of blood vessels. Shastry and Sharma [44] exposed *Channa punctactus* to a sub-lethal concentration 10.01 mg/L) of eldrin and observed hypertrophy of hepatic cells and liver corddisarray, vocuolation of cytoplasm and necrosis, rupture of hepatic cells membrane and necrotic centrolobular area.

The results of the present study indicate that lindane exacts toxic effects on fish. The 96 h LC50 value (0.38 mg/L) for *C gariepinus* juveniles suggest that the fish show a quick response to the toxicant. This is in agreement with the findings of Joshi *et al*. [45], who reported that lindane creates haematological disturbances and causes metabolic disorders to fishes which may ultimately lead to the deterioration of general health of fish.

Thus, the use of lindane should be properly and strictly controlled and regulated by appropriate legislation in order to prevent it's bioaccumulation in the environment and imminent disastrous consequences on the aquatic ecosystem.

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