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# TOXICITY OF GAMMA-BENZENE HYDROCHLORIDE (γ-BHC) TO *OREOCHROMIS NILOTICUS* (PISCES: CICHLIDAE)

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## ABSTRACT

The toxicity of gamma-benzene hydrochloride (γ-BHC) to *Oreochromis niloticus* was evaluated in a static bioassay system. At various concentrations and periods of exposure, changes in behavioural responses and mortalities were recorded in the test fish, while no such responses or any mortality was recorded in the control fish throughout the period of the experiment. Increasing concentrations and periods of exposure to γ-BHC caused uncoordinated movements, erratic swimming behaviour and extreme dullness prior to death of the test fish. There was progressive haemoconcentration, hyperproteinaemia, hypernatraemia, decreased plasma cation concentration, hypoglycaemia and increased plasma levels of ALP, AST and ALT with increasing concentration of γ-BHC and period of exposure of test fish. Death of test fish occurred earliest in the highest concentration of γ-BHC (200ppm) and with increasing periods of exposure. Postmortem lesions observed in the test fish include haemorrhagic and necrotic enteritis and myocarditis, hepatocellular degeneration and necrosis and non-suppurative meningoencephalitis. It is concluded that γ-BHC is very toxic to *O. niloticus*, causing severe acute dehydration, haemoconcentration, organ damage and death. Hence, it is suggested that the use of γ-BHC be properly controlled and regulated by appropriate legislation in order to prevent its bioaccumulation in the environment and imminent disastrous effect on the ecosystem.



## INTRODUCTION

Agrochemicals, 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 disease. However, their use is on the increase and has largely been uncoordinated, uncontrolled and discriminate, especially in the tropical countries (Bull, 1982; Goldberg, 1991; Tanabe, 1991). The use of these pesticides is strictly controlled in developed economies of the world because of their potential toxicological properties and ability to form residues in edible portions of crops and animal products (Akhtar, 1986). In addition, water bodies (sea, stream, rivers, dams, ponds and wells) receive the major amount of these chemicals (Bjerk and Brevik, 1980) when washed by rain from their points of application. Here they become readily available in the food chain and subsequent bioaccumulation in both aquatic and terrestrial flora and fauna (Melianby, 1967) with possible, accountable disastrous consequences on the ecosystem (Terry, 1987). For example, fish constitute two thirds of animal protein in Asia, and about a quarter of these fish is produced in fresh closed water ponds in rice plantations (Palmer, 1972). Severe toxicities and reduction in fish production has been recorded as a result of the use of chlorinated hydrocarbons in the control of

rice paddies (Palmer, 1972; Parrish, 1985). The study of the environmental dynamics of micropollutants, especially agrochemicals, is of importance when judging or predicting the potential short and long-term hazardous effects of these chemicals to man and wildlife. Hence, this study was carried out to determine the toxicity level of different concentrations and periods of exposure of γ-BHC to Nile tilapia, *Oreochromis niloticus*.

## MATERIALS AND METHODS

### *Experimental Fish*

Two hundred juvenile tilapia (*Oreochromis niloticus*) of average weight (5.01±0.05g) and length (8.30±0.12cm) were purchased from a commercial fish farm in Ibadan, Nigeria. The juveniles were randomly assigned into ten groups of 20 each in glass aquaria tanks of 45cm x 45cm x 60cm filled with air stones and electric power-driven aerators. They were fed with commercial fish pellets containing adequate nutrients (Reish and Oshida, 1986). Unconsumed feed and faecal wastes were removed, aquaria washed and water replenished regularly as recommended (Oyelese and Faturoti, 1995).

### *Bioassay Procedure*

The γ-BHC - 200g/l EC lindane (Gammalin 20EC, I.C.I., England) - used in this study is liquid but insoluble in water. Hence, a 100ml stock solution of 1:20 of 200g/l E.C lindane was prepared in acetone, with serial dilutions into varying concentrations made from the stock solution. Preliminary tests were carried out to determine a suitable range of doses based on logarithm ratio, for example, 0.001, 0.01 *etc* according to Parrish (1985). The definite test concentrations were measured out in one control (zero ppm) and four treatments – 50ppm, 100ppm, 150ppm and 200ppm (i.e. 0.00001mg/l, 0.00002mg/l, 0.00003mg/l and 0.00004mg/l, respectively) of γ-BHC in 50 litres of dechlorinated water. Each control and test static bioassay was duplicated. Both control and test fish were starved for 24 hours prior to the commencement of the experiment in order to reduce contamination and further dilution due to faecal and uneaten feed material (Reisch and Oshida, 1986).

Observations were made on the behavioural patterns of both control and test fish at and between 1, 2, 4, 8, 16, 24 and 48 hours of the commencement assay. Haematology and serum biochemical studies were carried out on blood samples collected at just before commencement, 2, 8, 24 and 48 hours from fish per treatment as described by Fadina *et al.* (1999) and Oyelese *et al.* (1999) and. The parameters determined include packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) counts, total plasma protein (TPP), albumin (ALB), globulin (GLOB), plasma concentrations of $Na^+$, $K^+$, $Cl^-$, $HCO_3^-$, $Ca^{++}$, $PO_4^{--}$, urea, creatinine, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glucose. Mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and albumin:globulin ratio (AGR) were calculated (Jain, 1986).

Dead fish were promptly removed and examined for gross lesions after which they were slit open lengthwise ventrally and preserved in Bouins' fluid for 30 minutes and subsequently in 10%

phosphate-buffered formol saline. Samples collected from the gills, heart, intestines and brain were embedded in paraffin, sectioned at 5μ and stained with haematoxylin and eosin (H&E) for histopathology.

Data obtained from the duplicate assay groups were pooled for each treatment and subjected to analysis of variance (SAS, 1987), and treatment means were compared for significant differences at 95% confidence interval ($P<0.05$) as described by Duncan (1959).

## RESULTS AND DISCUSSION

No adverse behavioural changes or any mortality was recorded in the control fish throughout the period of the bioassay. However, a variety of erratic behavioural changes ranging from somersaulting, uncoordinated movements to opercular gasping, dullness and death were observed in the test fish with increasing concentrations of γ-BHC and periods of exposure. The skins of the test fish were also observed to be darker in colour than those of the control fish. At all the concentrations of γ-BHC, mortality was not recorded in the first two hours of exposure except in the 200ppm group, where 1(5%) fish died (Table 1). However, mortalities were recorded from 2 hours and above, especially with higher concentrations of γ-BHC, such that by the 8th, 16th and 24th hour, 40% mortality was recorded in each of tanks with 200ppm, 150ppm and 100ppm concentrations of γ-BHC, respectively. By the 8th hour, a total of 12(60%) of the test fish in 200ppm of γ-BHC have died compared to 6(30%), 2(10%) and 2(10%) in 150ppm, 100ppm and 50ppm, respectively. None of the fish in 200ppm γ-BHC survived beyond 24 hours (Table 1). All these findings show that γ-BHC is very toxic to *O. niloticus*, and this is in agreement with previous reports on the effects of chlorinated hydrocarbons on aquatic and terrestrial fauna (Cameroon, 1945; Clarke and Clarke, 1978; Bull, 1982; Terry, 1987).

Table 1: Mortality patterns of *Oreochromis niloticus* at different concentrations and exposure times to γ-BHC

| Concentration of γ-BHC (ppm) | Period of exposure (hours) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 8 | 16 | 24 | 48 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50 | 0 | 0 | 0 | (10)* | 4(20) | 6(30) | 8(40) |
| 100 | 0 | 0 | 0 | 2(10) | 5(25) | 8(40) | 5(25) |
| 150 | 0 | 0 | 2(10) | 4(20) | 8(40) | 4(20) | 2(20) |
| 200 | 0 | 1(5) | 3(15) | 8(40) | 6(30) | 2(10) | - |

*number of dead fish (% of total).

The values of haematological parameters and plasma proteins are within the normal range for healthy *Oreochromis niloticus* (Table 1) (Adedeji *et al.*, 2000). The test fish exhibited progressive polycythaemia (increased PCV and RBC values) and increased Hb concentrations and total and differential plasma protein levels with increasing concentrations of γ-BHC, suggesting progressive dehydration of the test fish which may be related to higher ionic strength of water containing γ-BHC than the internal milieu of the test fish. This in turn may lead to polydypsia in the test fish and eventual drinking of more water containing γ-BHC.

Plasma biochemical changes in test fish include hypernatraemia, decreased plasma cation ($Cl^-$ and $HCO_3$) concentration, hypoglycaemia and increased plasma levels of ALP, AST and ALT with increasing concentration of γ-BHC and period of exposure of test fish (Table 3). The increases in plasma $Na^+$ concentration and enzyme levels may be relative because of the dehydration (Kramer, 1989). However, haemoconcentration will promote sluggish vascular blood flow with resultant increase in blood cells to vascular wall contact, disseminated intravascular coagulation and ischaemia.

Apart from the fact that γ-BHC is potentially toxic in poultry and other animals causing haemorrhagic and necrotic lesions in the intestines and liver (Clarke and Clarke, 1978), loss of vital anions and cations from the test fish will cause derangements in homeostasis, loss or derangement of vital tissue functions and eventual death (Kaneko, 1989; Jubb *et al.*, 1995).

Death of test fish occurred earliest in the highest concentration of γ-BHC (200ppm) and mortalities increased with increasing concentration of γ-BHC and periods of exposure. Postmortem lesions observed include haemorrhagic and necrotic enteritis and myocarditis, hepatocellular degeneration and necrosis and non-suppurative meningoencephalitis. The manifestation of erratic behavioural patterns, especially those related to movements and reflexes may be attributable to body biochemical derangements as well as cardiac, hepatic and central nervous system lesions observed at postmortem (Robbins *et al.*, 1984; Ogunsanmi *et al.*, 1994a,b; Fadina *et al.*, 1999, Oyelese *et al.*, 1999). The enteric lesions may have been expressed as poor food consumption and growth rate if the fish had lived longer for these effects to be manifested. This implies that at very much lower concentrations of γ-BHC, but with continuous consumption, growth, reproductive performance and the general health condition of the exposed fish may be jeopardized. Because of the propensity for long term exposure at low doses, as may be the case in the environment, bioaccumulation of γ-BHC in the tissue of such fish will occur and this will serve as a source of low insidious poisoning in the food chain.

It is concluded from this study that γ-BHC is very toxic to *O. niloticus*, causing severe acute dehydration, haemoconcentration, organ damage and death. Hence, it is suggested that the use of γ-BHC, especially in the tropics be properly and strictly controlled and regulated by appropriate legislation in order to prevent its bioaccumulation in the environment and imminent disastrous effect on the food chain and the ecosystem.

Table 2: Haematology and plasma protein values of *Oreochromis niloticus* exposed to different concentrations of γ-BHC

| Concentration of γ-BHC (ppm) | PCV (%) | Hb conc (g/dl) | RBC counts (x10⁶/µl) | MCV (fl) | MCHC (%) | TP (g/dl) | ALB (g/dl) | GLOB (g/dl) | AGR |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 21.3±1.5[a] | 8.6±0.5[a] | 1.5±0.2[a] | 142.6±2.3[a] | 40.5±1.3[a] | 3.8±0.5[a] | 1.3±0.3[a] | 2.5±0.2[a] | 0.52±0.2[a] |
| 50 | 23.2±0.8[a] | 9.4±1.2[a] | 1.9±0.1[b] | 122.2±3.1[b] | 40.5±0.4[a] | 4.3±0.2[a] | 1.4±0.4[a] | 2.9±0.3[a] | 0.48±0.4[a] |
| 100 | 25.7±1.7[b] | 11.2±0.8[a] | 2.1±0.2[b] | 122.4±2.3[b] | 43.6±2.5[ab] | 4.9±0.8[a] | 1.7±0.3[ab] | 3.2±0.4[b] | 0.53±0.2[a] |
| 150 | 25.9±0.8[b] | 12.1±0.6[b] | 2.5±0.1[a] | 107.9±4.2[c] | 45.0±1.0[a] | 5.8±1.3[a] | 1.9±0.4[ab] | 3.9±0.2[a] | 0.49±0.4[a] |
| 200 | 27.5±0.3[a] | 13.4±0.2[a] | 2.8±0.5[a] | 98.2±3.1[c] | 48.6±3.8[a] | 6.3±1.1[a] | 2.2±0.3[a] | 4.1±0.5[a] | 0.54±0.6[a] |

[a]Data presented as mean ± standard error of mean.
n=15 for samples at 0, 50 and 100ppm; while n=12 and 9 for samples at 150 and 200ppm, respectively.
Means along the same column with different superscripts differ significantly (P<0.05).

Table 3: Plasma biochemistry of *Oreochromis niloticus* exposed to different concentrations of γ-BHC

| Concentration of γ-BHC ppm | Na+ (mMol/l) | K+ (mMol/l) | Cl- (mMol/l) | HCO3 (mMol/l) | Ca++ (mMol/l) | PO4 (mMol/l) | Urea (mg/dl) | Creat (mg/dl) | ALP (IU/l) | ALT (IU/l) | AST (IU/l) | Glucose Mg/dl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 124.5 ±1.9[a] | 4.2 ±0.5[a] | 151.5 ±5.2[a] | 30.5 ±3.1[a] | 8.5 ±0.3[a] | 4.6 ±0.2 [a] | 10.5 ±0.5[a] | 0.7 ±0.1[a] | 76.0 ±2.1[a] | 35.3 ±3.1[a] | 19.3 ±0.3[a] | 36.3 ±4.1[a] |
| 50 | 126.2 ±0.8[a] | 4.4 ±1.2[a] | 128.2 ±2.9[b] | 25.2 ±1.1[b] | 9.1 ±1.4[a] | 4.1 ±0.4 [a] | 10.4 ±0.1[a] | 0.6± 0.1[a] | 89.8 ±5.6[b] | 43.7 ±5.2[a] | 30.0 ±2.2[a] | 52.2 ±3.1[b] |
| [illegible] | [illegible] 7[b] | 4.1 ±0.3[a] | [illegible] ±1.2[c] | 23.4 ±0.3[b] | 9.4 ±0.1[a] | 4.9 ±0.4 [a] | 10.7 ±0.5[a] | 0.7 ±0.2[a] | 105.3 ±10.9[ab] | 59.3 ±2.9[b] | 47.0 ±2.5[b] | 56.0 ±1.8[b] |
| [illegible] | [illegible] ±2.7[b] | 5.4 ±1.5[a] | 92.5 ±5.4[c] | 21.9 ±0.5[c] | 8.5 ±0.3[a] | 4.7 ±0.4 [a] | 11.2 ±0.1[a] | 0.8 ±0.2[a] | 112.9 ±12.4[a] | 68.4 ±3.4[b] | 51.9 ±1.4[b] | 44.3 ±2.7[b] |
| 200 | 162.1 ±3.5[a] | 4.4± 0.7[a] | 88.0 ±1.5[c] | 19.2 ±1.2[c] | 8.6 ±0.3[a] | 4.3 ±0.5 [a] | 10.8 ±1.3[a] | 0.7 ±0.2[a] | 120.4 ±15.5[a] | 74.8 ±3.7[a] | 58.1 ±5.2[a] | 42.5 ±3.8[b] |

*Data presented as mean ± standard error of mean.
n=15 for samples at 0, 50 and 100ppm, while n=12 and 9 for samples at 150 and 200ppm respectively.
Means along the same column with different superscripts differ significantly ($P<0.05$).

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