INTRODUCTION

In the recent times in Africa, as a result of high population growth, accompanied by an intensive urbanization, an increase in industrial activities and a higher exploitation of cultivable land, there has been a steady increase in the quantity and diversity of discharges to aquatic environments (West, 1988). Aquatic pollution by pesticides results mainly from their widespread use in agriculture and in vector control campaigns. In Nigeria especially in the northern part of the country, there is an upsurge in the application of pesticides in agriculture. A major reason for the increased use of pesticide in the north is the development of dry-season irrigated farming (Mbagwu and Ita, 1994). These poisons are washed into water bodies through surface run-off during the rainy seasons. The American Chemical Society database indicates that there were some 13 million chemicals identified in 1993 with some 500,000 new compounds being added annually (FAO, 2004). The World Bank and other international organizations have warned that global environmental change may be reaching critical thresholds, and report increasing pressure on land and water resources in many parts of the world. Often these issues affect the poor and impoverished most, undermining the resource base upon which they depend and reducing their prospects for secure and sustainable livelihoods (EIA Training Manual, 2002).

Bretemite chlorpyrifos-Ethyl 40EC containing 400g/l chlorpyrifos-Ethyl active ingredient (0, O-diethyl O-3, 5, 6 -trichloro-2-pyridyl phosphorothioate) manufactured by Brethmont Ltd, London - is a broad-spectrum organophosphate insecticide. It is used for the control of termites, ants, mites, and a wide range of insect pests of soil and foliage in a variety of crops, e.g. cassava, maize, potato, beans, tomato, etc. It is also registered for direct use on sheep and turkeys, for horse site treatment, dog kennels, domestic dwellings, farm buildings and commercial establishments (EXTONNET, 1996). It is also most commonly used for stored grains and storage facilities. In this regard it is used for the control of granary weevil, rice weevil, red flour beetle etc. It is also sold mixed with Pyrethroid, such as Deltamethrin, to provide additional control of lesser grain borer, saw-toothed grain beetle and flat grain beetle (DowAgro, 2006). The application is usually by spraying a specific dose per hectare. It is directly toxic to the nervous system and in addition, it is transformed inside animals to Chlorpyrifos-oxon which is about 30-100 times as potent against the nervous system as chlorpyrifos itself (Cox, 1994). Like all Organophosphates, Chlorpyrifos and Chlorpyrifos-oxon kill insects and other animals including humans because of its toxicity to the nervous system. This is achieved by

ABSTRACT

Chlorpyrifos-ethyl with its mode of action was evaluated in laboratory studies to determine its 96-h acute toxicity, using juveniles of Clarias gariepinus. The fish was exposed in glass aquaria to 0.64mg/l, 0.80mg/l, 0.96mg/l, 1.12mg/l and 1.28mg/l. The test substance was found to have lethal effects on fish as they changed fish behaviour and caused death. The 96-h LC50 value for C. gariepinus was estimated as 0.92mg/l. Observations of behavioural and morphological responses were used as indicators of sublethal toxicity. The behavioural toxicology bioassay may be valuable in comparing and predicting the mode of action of new or unknown toxicants in this and other fish species.

Keywords: Chlorpyrifos-ethyl, acute toxicity, Behaviour, C. Gariepinus
inhibition of enzyme acetylcholinesterase (AChE) that breaks down acetylcholine, a chemical involved in transmitting nerve impulses across the junctions between nerves. Without functioning AChE, acetylcholine accumulates, producing rapid twitching of involuntary muscles, convulsions, paralysis, and untimely death (Cox, 1994). Drummond et al. (1986) evaluated the use of behavioral and morphological changes in fish as a diagnostic endpoint for screening and differentiating chemicals according to their mode of action. After exposing 30-d old fathead minnows (Pimephales promelas) to different chemicals, they observed unique morphological and behavioral signs of stress. The authors concluded that select abnormal responses are promising for predicting the mode of action of unknown xenobiotics. Behavioral responses are effective indicators of contamination and reflect acute and sublethal toxicity (Little, 1990).

The present study was conducted to determine the acute toxicity, behavioral, and morphological effects of Chlorpyrifos-ethyl on C. gariepinus, which is a popular tropical freshwater catfish indigenous to Africa and is common and in high demand in Nigeria.

MATERIALS AND METHODS

EXPERIMENTAL FISH

Healthy Clarias gariepinus juveniles (average weight 16.35±1.25 g and standard length 10.5±0.05 cm) were obtained from Kubweni Dam Ahmadu Bello University, Zaria. The fish were conveyed to fisheries laboratory in a portable well-aerated white polythene bag containing water from the Dam. They were held in large water baths of 160 L capacity at 24.5-25.5°C and acclimatized for two weeks in dechlorinated municipal water. A total of 180 specimens were randomly assigned to give a loading rate of 10 fish per tank to avoid overcrowding. During this period, the fish were fed with pelleted diet containing 35% crude protein twice per day at 5% body weight. Also, the water in the glass aquaria was changed once every two days. The fish were accepted as well as adapted to laboratory conditions when less than 5% death was recorded for the 14 days period and feeding was discontinued 24 hours before the start of the experimental run (Reish and Oshida, 1987).

ACUTE BIOASSAY

Acute 96-h static bioassays were conducted in the laboratory following the methods of Sprague (1975) and APHA (1985). The nominal concentrations for chlorpyrifos-ethyl were 0.64 mg/l, 0.80 mg/l, 0.96 mg/l, 1.12 mg/l, 1.28 mg/l, and a control with no toxicant. Each concentration was replicated three times. The desired stock solution was measured and introduced into 25 L of dechlorinated tap water in the glass aquaria. The mixture was allowed to stand for 30 minutes before introducing test fish. A total of 180 fish were stocked to give a loading rate of 10 fish per tank. Survival and mortality were recorded from 1 to 6, 8, 16, 24, 72, and 96 hours. Fishes were considered dead when the opercular movement ceased and there was no response to gentle probing.

BEHAVIORAL AND MORPHOLOGICAL ASSAYS

Observations of behavioral and morphological response of C. gariepinus juveniles exposed to chlorpyrifos-ethyl were conducted at 1 through 6, 8, 12, 24, 48, 72 and 96-h during the acute toxicity tests. The methods developed by Drummond et al. (1986) were used for this study. Controls without toxicant were monitored, along with the nominal concentrations, to provide a reference for assessing any behavioral and morphological changes. Responses were recorded if they differed from the controls and occurred in 10% of the fish within each test chamber. Five behavioral and morphological indicators were observed in this study: loss of equilibrium, general activity, startle response, hemorrhage, and deformity (including postural indicators). Each test chamber was observed for 5 to 10 minutes. Startle responses were monitored by lightly touching the fish with a wooden applicator stick (tactile stimulus).

STATISTICAL ANALYSIS

The percentage mortality and probit kill were determined. Graph of probit kill against log concentration, was used to determine the 96-h LC50 (Finley, 1971)
## RESULTS

Table 1: Diagnostic Behavioural Effects of Chlorpyrifos-ethyl on juveniles of *C. gariepinus*

<table>
<thead>
<tr>
<th>Behavioural and Morphological Symptoms</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of equilibrium</td>
<td>Yes</td>
</tr>
</tbody>
</table>
| General activity                       | Hyperactive to Hypoactive<sup>d</sup>  
Backward movement<sup>d</sup>  
Change in skin pigmentation<sup>d</sup> |
| Startle response                       | Underreactive<sup>b</sup>              |
| Hemorrhage                             | None      |
| Deformities                            | Occasional breakage (Fracture) of vertebral column at caudal regions<sup>ad</sup>  
Lordosis/Scoliosis<sup>ad</sup>  
Pectoral fins (forward)<sup>cd</sup>  
Swollen abdomen<sup>de</sup> |

<sup>a</sup>In caudal area  
<sup>b</sup>Severely intoxicated fish ceased to respond  
<sup>c</sup>Pectoral and pelvic fins postured at 45° to 90° angle from the ventral surface of the head and the lower abdomen, with little or no movement  
<sup>d</sup>Distinguishing behaviour, morphological change or signs of stress  
<sup>e</sup>About 24h after exposure and only in two highest nominal concentrations.
Plate 1: (BV) Breakage (Fracture) of vertebral column at the caudal region in the chlorpyrifos exposed fish.
Fish mortality occurred in all the test tanks except control, and the mortality was dose-dependent. By 2-3hrs, there was 50% mortality in the highest nominal concentrations of 1.28mg/l while 1.12mg/l concentration recorded an average of 25% mortality within the same time period. The 96-h median lethal concentration (LC50) of chlorpyrifos-ethyl to Clarias gariepinus was estimated to be 0.92mg/l. (Fig. 2).

Plate 1: (BV) Breakage (Fracture) of vertebral column at the caudal region. (SA) Swollen Abdomen in Chlorpyrifos exposed fish.

MORTALITY
DISCUSSION

Acute toxicity of chlorpyrifos-ethyl on *C. gariepinus* was also carried out by static bioassay method, and the result revealed that the insecticide was highly toxic to *C. gariepinus* with 96-h LC₅₀ value of 0.92 mg/L and NOEC estimated as 0.0092 mg/L. This result is consistent with the findings of several other workers. EXTOXNET (1996) reported the 96-hour LC₅₀ for chlorpyrifos as 0.009 mg/L in mature rainbow trout, 0.098 mg/L in lake trout, 0.806 mg/L in goldfish, 0.01 mg/L in blugill and 0.331 mg/L in fathead minnow. Behavioral monitoring is a promising diagnostic tool for screening and differentiating chemicals according to their mode of action (Drummond et al., 1986). They proposed that chemicals with different modes of action will evoke a distinct behavior pattern. In this study, effects of chlorpyrifos-ethyl clearly showed loss of equilibrium in the exposed fish. This finding is similar to that of Drummond et al., (1986), Patricia et al. (1997) and Ault (2001). However, Patricia et al. (1997) concluded that this effect alone is not diagnostic for any chemical or mode of action. Also, other distinguishing symptoms included lordosis/scoliosis in the caudal and mid regions with characteristic backward movement initiated with the tail.

One to two hours after exposure is marked by hyperactivity and increased aggression, this progressively decreased as fish became severely intoxicated. Occasionally there was breakage of bone at the caudal and mid region. Others include continuous forward posturing of the pectoral and pelvic fins. The time of initial onset of morphological responses and mortality was dose-dependent. Fish exposed to nominal concentrations held their pectoral and pelvic fins at 45° to 90° angles from their body with little or no movement. The dorsal and ventral fins were also fully stretched even when fish was in resting position. The sign of intoxication occurred at 1 and 2.5 hour for concentrations of 1.28 mg/L and 1.12 mg/L respectively. This may be due to the inhibition of AChE by Chlorpyrifos-ethyl. In guppies, very low concentrations of 1 parts per billion (ppb) significantly inhibited AChE activity, which did not return to normal after two weeks of keeping the test fish in uncontaminated water (van der Wel et al., 1989). Abnormal growth (spinal deformities) occurred at very low concentrations of 5 ppb in juvenile rainbow trout and at 5 ppb in fathead minnows (Holecombe et al., 1982). Exposure periods of 5 hours were sufficient to cause these deformities (Jarvinen et al., 1988). Carbofuran and atrazine produce adverse behavioral changes in goldfish after a short-term exposure to sublethal concentrations (Saglio and Trijasse, 1998). According to Bretaud et al. (2000), disturbances in AChE activity can affect locomotion and equilibrium in exposed organisms and may
impair feeding, escape, and reproductive behaviour. Cholinesterase inhibition in brain and muscle produces adverse effects in movement because AChE participates in neuronal and neuromuscular transmissions (Fernandez-Vega et al., 2002).

Occurrence of swollen abdomen was also dose-dependent, although it was restricted to the high treatment groups of 1.28mg/l and 1.12mg/l. This may be associated with inhibition of the enzymes carbonic anhydrase and Na-K-ATPase activity in the gill and the intestine of the fish. Both enzymes play a pivotal role in teleost intestine and gill physiological functions like gas exchange, acid-base balance, osmoregulation and clearance of waste products from nitrogenous metabolism (Lionetto et al., 2000). Carbonic anhydrase activities in gill and intestinal homogenates were significantly inhibited by CdCl₂ (Lionetto et al., 2000). It is also opined that the Chlorpyrifos-ethyl or its breakdown products (Chlorpyrifos-oxon) generated excessive reactive oxygen species (ROS), which induced lipid peroxidation in the lining of the intestine leading to oxidative stress. This led to loss of membrane integrity and subsequent leakage of fluid into the stomach. Nestel and Joan (1975) reported swelling as a result of PCB lesion in the kidney of rainbow trout, and opined that glomerular filtration may have exerted a deleterious effect on cell membranes resulting in increased permeability and thus allowing fluid to enter the cells. Gail et al. (2000) also observed gastric lesions which included chronic inflammation of the serosa with lymphocytes, mononuclear cells and occasional plasma cells infiltration, in the stomach of adult female rainbow trout (Oncorhynchus mykiss) treated with TCDD. Recent evidence suggests that waterborne contaminants which generate reactive oxygen species (ROS) may be a significant source of toxicity for aquatic organisms living in polluted environments and may be partially responsible for disruptions in physiological function (Livingstone, 2001). In addition, it has been reported that organophosphates are reactive and may cause direct oxidative damage to membranes (Galloway and Handy, 2003). Oakes et al. (2004) argued that excessive ROS production in response to xenobiotics inducing compounds can overwhelm endogenous detoxifying mechanisms producing the cumulative damage to cellular constituent's termed oxidative stress.

Matsumura (1975) reported that hyperactivity is a primary and principal sign of nervous system failure due to pesticide poisoning, which affects physiological and biochemical activities. Pal and Konar (1987) also reported that disruption of the functioning of nervous system of fish might be the cause of slow and lethargic swimming, erratic movement and loss of equilibrium. Similar observations were made by Ogueji (1997) and Rukiye et al. (2003). Patricia et al. (1997) also reported distinguishing symptoms of Chlorpyrifos to 30-day-old Latipes during sublethal exposure as continuous forward posturing of the pectoral fins, hemorrhage (vertebral area), and scoliosis in the caudal region. They also reported that the time of initial onset of morphological responses and mortality was dose-dependent. Chlorpyrifos is an organophosphorus insecticide that causes the inhibition of AChE. Excess acetylcholine causes continuous firing of muscle fibers (Fukuto, 1990). Drummond et al. (1986) reported that fish exposed to one of several AChE inhibitors showed common tetany, lordosis, and scoliosis. Jarvinen et al. (1988) reported 50% deformities, which consisted of a lateral bend in the spine, in fathead minnows after 15 h of 0.122mg/l chlorpyrifos. Chlorpyrifos is transformed inside animals and chlorinated water to chlorpyrifos-oxon which is about 3000 times as potent against the nervous system as Chlorpyrifos itself (Cox, 1995). The behavioural and morphological changes observed in juveniles of C. gariepinus were consistent with the findings of Patricia et al. (1997).

CONCLUSION

In conclusion, the mode of action of chlorpyrifos and their metabolites is inhibition of enzyme acetyl cholinesterase (AChE), although this was not studied, it is opined that this was responsible for most of the observed morphological and behavioural abnormalities. Africa's inland fisheries play a critical role in supporting the livelihoods and food security of millions of people across the continent. It generates employment and income for households and provides a major source of animal protein and essential micronutrient. Yet catches from most inland fisheries have reached their maximum capacity and many of them are considered to be over fished (Ye, 1999). Nigeria has abundant freshwater resources, with fish constituting about 40% of animal protein intake (Olutunde, 1989; Ila, 1993). Inland fisheries sub-sector contributes 86% of the domestic fish production, and about 0.3mm Nigerians engage in artisanal fish production. This means that any short fall in fish availability will reduce employment and animal protein intake of Nigerians especially those in the low income bracket. Aquaculture production has increased from 100 000 tonnes in 1994 to 520 000 tonnes in 2003 (Ye, 1999). The largest share of the output continues to be generated by Egypt (86 percent), followed by Nigeria (6 percent) and Madagascar (2 percent) (Ye, 1999). Average yields in most countries remain low, commercial operators have yet to develop in many areas, and producers are few in number, with very little organization or capacity (Ye, 1999). Pollution arising from
indiscriminate use of pesticides has continued to endanger freshwater fish stocks. According to Dickson et al. (1980), there are two roles that biomonitoring must play if we are to protect our aquatic resources from damage. (1) We must be able to provide a real-time (i.e., continuous) assessment of present conditions. (2) We must use our biomonitoring technology to predict the effects of new chemical substances likely to reach aquatic ecosystems. In addition, we must use our biomonitoring tools to predict the future ecological effects of chemical substances and utilize this knowledge to prevent these substances from reaching hazardous concentrations in the ecosystem. In the current study, a distinguishing behaviour or set of behavioural and morphological symptoms was observed for Chlorpyrifos-ethyl against control. Changes in behaviour and morphology were proven to be more sensitive diagnostic endpoints than was mortality. 

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