IMPACT OF LAMDA CYHALOTHRIN PYRETHROID INSECTICIDE ON THE UPTAKE OF CATIONS AND ANIONS BY THE GILLS OF FRESHWATER CATFISH HYBRID JUVENILE

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ABSTRACT

The impact of acute exposure of karate (Lambda cyhalothrin pyrethroid) insecticide was evaluated in a 4 – day exposure period at 20, 40, 60 and 80 ppm to Heterobranchus bidosalis(+) X Clarias gariepinus(♂) fingerlings showed the 96-hlc 50 as 25.11 ppm. The threshold value was 25. 11 ppm. The gills of the exposed fish analyzed showed a significant decrease in all major cations and anions ($C1^-$, Ca^{2+}, Na^+, K^+, Mg^{2+}) at $P < 0.05$). There was no inhibition of uptake of the cations and anions ($C1^-$, Ca^{2+} Na^+, K^+, Mg^{2+}). Their uptake increased rapidly during the 24 hr period and dropped at 48 hr and 72 hr and gradually increased at the end of 96 hr showing that it was time dependent. During the exposure period the fish stood in upright position with their snouts above the water surface gasping for air. Other behavioral characteristics of the exposed fish were peeling of the skin, initial increase in opercula movement, curvature of the body, loss of balance, erratic swimming and quietness. Based the outcome of this research and under similar experimental condition it is the recommendation of this research that this pyrethroid will affect the uptake of the major cations and anions. It further advises environmental officers, crop farmers and insecticides habitual users to be cautious on the use of this insecticide because of the resultant consequences of the misuse.

Keywords: Karate, Uptake, Cations, Anions, Gills, Catfish, Toxicity

INTRODUCTION

Aquatic organisms are bathed in solutions of trace metals at dissolved concentrations ranging from nanograms per litre in the open ocean, through level approximating micrograms per litre in coastal seas, to even higher concentrations approaching or exceeding milligrams per litre in estuaries and acid rich streams and salt lakes (Bryan and Gibbs, 1983). However the uptake of many trace metals from solution into aquatic organisms is facilitated by the use of gills, which is an appendage of fish body primarily concerned with the exchange of gases (Hoar 1975). Fish gills that can serve this purpose comprise a large part of fish body that contacts the external environment and they play an important role in the gas and ion exchange between the organism and environment.

They are important organs for the uptake of heavy metal compounds in fishes. Thus the gills are the very first site where metal-induced lesions may occur which may result in impaired gas and ion exchange (Witeska *et al.,* 2006). Subsequently, metal ions within the blood may affect the blood cells. (David and Philips, 1993).

The uptake of dissolved metals may take place all over the body surface of small and/or soft bodied organisms, as well as at particular site of high permeability such as the gills. Metal uptake from solution will also take place in the alimentary tract, when any of the medium is swallowed during "drinking" or the ingestion of food. For example, hypoosmoregulating crustaceans in littoral or salt lake environment will drink the medium to replace the water lost by osmosis, and all also excrete excess salts actively through the gills (Mantel and Farmer, 1983). Marine teleost fish are also hypo-osmotic regulator and drink seawater routinely, subsequently disposing of excess salts via gills and feaces.

Natural pyrethroids have proved of great value for use indoors for public hygiene, medicine and animal health. Uses include the control of lice and fleas in the homes and public buildings and the control of houseflies, mosquitoes and other insects that spread diseases of animals and humans importance (Hassall, 1990). Pyrethroids are ideal for home uses because they are of low toxicity to man and other warm-blooded animals. Moreover they are readily destroyed by heat during cooking or by digestive juices should trace get into food, onto fingers of children or onto the feet of domestic animals. Their outdoor use is severely restricted reason being that they are rapidly decomposed by light (Ruzo, 1982).

Although safe to higher animals, pyrethroids, both natural and synthetic are toxic to fish (Hassall, 1990). Pyrethroids are remarkable effective insecticides because of their ability to disrupt the insect nervous system at concentration that result in no mammalian toxicity.

Reports based on the work of Laufer *et al.* (1985) using the giant axon of the cockroach showed that pyrethroids interfered with the uptake and trans-membrane movement of sodium ions. Authors like Doherty *et al.* (1986) and Brooks and Clark (1987) observed that nonomolar concentration of pyrethroids reduce calcium ion uptake by 50 percent in housefly larvae. Hassall (1990) studied the inhibitory effect of Type 1 and type II pyrethroids on Y–aminobutyric acid (GABA) – dependent chloride ion influx into rat brain micro-sacs. Their findings showed that pyrethroids possess potent neurotoxic activity on several types of ion channels, such as chloride ion channels that are activated by GABA, voltage sensitive sodium channels and calcium channels. Furthermore, they reported that pyrethroids affects membrane pumps that involved Ca^{2+} dependent ATPase and Ca^{2+}/Mg^{2+} dependent ATPase.

Pyrethroids are extremely toxic to aquatic animals due to impaired metabolism, resulting in LC_{50} values 10 -1000 times higher for fish than for mammals. Fish toxicity is greater in the presence of α-cyano moiety and is inversely related to temperature (Hassall, 1990). Field exposure studies have shown that pyrethroids are not as toxic to fish populations in their native environment as in bioassay studies conducted in the laboratory. This observation is explained in part by the hydrophobic nature of pyrethriods, which results in high levels of nonspecific binding to apolar humic substances and organic particulate matter, thereby decreasing the effective concentration of bioavailable pyrethroids (Hassall, 1990).

"Heteroclarias" hybrid juveniles were chosen for this study because of their economic viability and strong genetic potential for aquaculture programmes. They are known for their breed easy technology. They are ubiquitous in Africa and are now very popular among fish farmers and have become African most cherished and adaptable aquaculture candidate. They constitute one of the main fish families of economic value as food fish. Several authorities have advocated using them for restocking programmes but there is strong opposition to that because they might pollute the genetic strains of the catfish family (Fagbenro, 1982).

The Karate (Lambda cyhalothrin pyrethroid) insecticide was purchased from "Clemagro" Agrochemical Nigeria Limited, Umuahia, Abia State, Nigeria. This insecticide cyhalothrin (pyrethroid) was introduced by ICI Australia and ICI Agrochemicals (now Zeneca Agrochemicals). The common name is cyhalothrin (BSI, draft E – ISO BAN); cyhalothrin (f) draft F – ISO). The IUPAC name (RS) - - cyano –3 – phenoxybenzyl (Z) – (IRS, 3 RS, 3RS) – (2 – chloro = 3, 3, 3 – frifluropropenyl) – 2, 2 – dimethylcy clopropanecar boxylate. Roth: (RS) - - cyano – 3 – phenoxy benzyl (Z) – (IRS) – cis – 3 – (2 – chloro – 3, 3, 3, - trifluoro = propenyl) – 2, 2 – dimethyl – cyclopropane carboxylate (Tomlin 1997).

The objective of this study was to examine the impact of acute exposure of karate (Lambda cyhalothrin pyrethroid) on the uptake of some major cations and anions (Na^+, CI^-, K^+, Mg^{2+}, Ca^{2+}) in the gills of the freshwater catfish hybrid (*Heterobranchus bidosalis* X *Clarias gariepinus*) Juvenile.

MATERIALS AND METHODS

Catfish Hybrid Procurement and Management: Freshwater catfish (hybrid of *Heterobranchus bidorsalis* (male) X *Clarias gariepinus* (female) were obtained from African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt, River State Nigeria. The hybrid juveniles averaging 9.26 ± 0.05 cm (Total length) and 6.23 ± 0.02g body weight were used for this study. The fish were held in the laboratory in large water baths of 160 L capacity at 24.3 – 26.0^{0}C and acclimated for two weeks prior to experiment. The fish were fed 5% body weight with Pfizer pelleted diet during acclimation. A daily photoperiod of 16: 8h light: dark was maintained during acclimation and experimental phases.

LC_{50} Screening Test: LC_{50} screening tests (Range finding tests) were carried out in one litre conical flasks containing 30 juvenile catfish. Concentrations of pyrethroid insecticide which caused 50% fish death within 30 minutes were omitted from the test. Acute concentrations of insecticide used were 80, 60, 40, 20, and 0.00 ppm (control). Ten catfish hybrid juvenile were exposed to acute concentrations of 20, 40, 60, 80 ppm of insecticide while the 0.00 ppm served as the control. The experimental set up consisted of 15- circular plastic water tanks (30L capacity) filled to 20-litre mark with well-aerated dechlorinated tap water. Insecticide concentrations were obtained by diluting stock solution prepared weekly. The already prepared concentrations were introduced into the first four tanks serially, the fifth tank served as the control (devoid of the toxicant Karate); while the remaining ten tanks served as replicates. The test media were changed every 24 h, replacing the old media with fresh solution.

Methods for acute toxicity tests as described by Sprague (1973) were employed for this investigation. The fish were not fed 24h prior to and during the exposure period which lasted for 96h. The 96–h Lc_{50} confidence limit was calculated as a summary of the percentage mortality data using formula as described by Lichfied and Wilcoxon (1949) thus: $S = (LC_{84}/LC_{50}) + (LC_{50}/LC_{16})$, where LC_{84} is the probit 84, LC_{50} is probit 50 and LC_{16} is probit 16 from graph.

Experiment 1: Mortality, Opercular Ventilation Rate and Behavioural Studies: Mortality was recorded every 24 h, though the tanks were inspected every 3h for dead fish which were immediately removed. The opercular ventilation rates per minute, were read at the start of and every 24h thereafter. Behavioural responses of the exposed fish were observed on a 3 – hourly basis.

Experiment 2: Ions and Water Chemistry: In the second experiment four fish was removed from each tank containing different concentrations of toxicant including the control which was devoid of the toxicant with the aid of scalpel.

The gills were dissected out and analysed to determine the effects of the toxicant (Karate) on the uptake of Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+} by the gills. This was done daily and it lasted for 4 days. The cations and anions were determined by Atomic Absorption spectrophotometer model (AA – 670, Shimadu, Kyoto) with background compensation capability for dissolved oxygen, free carbondioxide, total hardness, alkalinity and pH. These parameters were monitored every 24h using methods described by APHA (1989).

Data Analysis: Results obtained from these investigations were analysed using the analysis of variance (ANOVA) methods to test for level of significance at the 0.05 probability level.

RESULTS

The mean of water quality parameters obtained during the period of exposure of the *H. bidosalis* X *C. gariepinus* hybrid to the different concentrations of pyrethroid insecticide showed that the temperature was 24.3 $\pm$ 0.6^{0}C, pH, 7 ± 0.04, Dissolved Oxygen, 5.79 ± 0.5 mg/l; Carbon (IV) Oxide 1.17 ± 0.1 mg/L and Total Alkalinity was 8.04 $\pm$ 0.2 mg/l. The water quality parameters in the treatment tanks did not very significantly ($P < 0.05$) from those of the control tanks (Table 1).

Behavioural patterns observed during the exposure period include; the fish stood upright with their snouts above the water surface gasping for air, curvature of body, peeling of the skin, initial increase in opercula movement, loss of balance, erratic swimming and quietness. The fish finally died.

Mortality increased with increase in pesticide concentrations. Thus mortality was highest in 80 ppm and lowest in 20 ppm. There was no mortality in the control experiment (Table 2). The 96-h LC_{50} was 25.12 ppm while the threshold value was 25.11 ppm.

The uptakes of the major cations and anions by the gills during the exposure have been presented in (Table 3). There was a significant difference between the uptake of the cations and anions among the treatment tanks (P < o.05). Uptake of Mg^{2+} and Ca^{2+} were significantly different at ($P < 0.05$), while the uptake of K^+, Na^+ an Cl^- were not significantly different at ($P < 0.05$).

There was no total inhibition of uptake of the cations and anions (Mg^{2+}, Ca^{2+}, Na^+, K^+, and Cl^-) but their uptake increased rapidly during the 24 h period and then dropped at 48 h and 72 h, and gradually increased at the end of the 96 h indicating that its uptake was time dependent (Table 3).

DISCUSSION

In the present study, there was no significant difference in the physicochemical parameters of the treatment medium (water). The water quality parameters of the treatment tanks during the exposure did not vary significantly from those of the control tanks. All were within suggested tolerance levels (Mackereth, 1963). The value of 96-h LC_{50} of 25.12 ppm reported in this experiment was within the range earlier reported by Oti (1999) for several tropical freshwater catfish species exposed to insecticides. Amadi (2004) and Nmerole (2004) reported a 96-h LC_{50} of 25.00, and 26.08 pp for fish *Heterobranchus bidorsalis and Clarias gariepinus* exposed to Dichlorvos insecticide and round up herbicide respectively. Several other workers have reported 96-h LC_{50} for several species of the African catfishes exposed to different pesticides in contrast to what was observed in this experiment. Aguigwo (2002) reported 96-h LC_{50} of 4.17 $mg1^{-1}$ for *Clarias gariepinus* exposed to cymbush pesticide. Rahman et al (2002) reported 96-h LC_{50} of 6.55, 3.09 and 2.72 ppm for *A. testudineus*, *C. punctatus* and *B. gonionotuse* exposed to Diazinon. Perschbacher and Sarkar (1989) reported 2-h LC_{100} values of 15 ppm for snakehead exposed to rotenone. We however noted that the differences, LC_{50} values of in the present study from those of cited workers may be attributed to difference in fish species, age, size, sex, strain, fish condition and experimental techniques adopted (Brown, 1990).

During the exposure period, the test fish showed abnormal pathological sign of peeling of the skin and several behavioural changes such as increased opercula and respiratory activities, curvature of the body, erratic swimming, loss of balance, strong spasm, paralysis and finally death. These behavioural movements are in agreement with earlier reports of Avoaja and Oti (1997), Oti (1999), Witeska *et al.* (2006) and Rahman *et al.* (2002). These behavioural responses were indications of stress and nervous disorder.

The initial increase in opercular activities of fish exposed to pyrethroid insecticide as reported in this findings suggests that fish exposed to karate insecticide tended to exhibit avoidance syndrome during the first few hours of exposure. However, as the exposure period was prolonged the fish became fatigued hence subsequent drop in opercula activity. Indices of opercula activities have earlier been reported by Saroj and Gupta (1987) as a strong indicator of stress when fish were exposed to toxicants. Basha *et al.* (1984) have shown initial increases in the respiratory rate of fish exposed to malathion, this was soon followed by a decreased in respiratory rate. The combined effect of fatigue and toxicity of the karate to the exposed fish often led to death.

According to Gill *et al.* (1988) respiratory distress in *P. conchonius* exposed to dimethoate was due to degeneration of secondary gill lamellae, edematous separation of respiratory epithelium and degenerated chloride cells in the interlamellar crypts of gill tissue. It may be assumed that as a result of reduced efficiency of damaged gills to respiratory activity other metabolically active; tissues like liver and muscle receive less oxygen leading to severe tissue hypoxia. Development of such internal hypoxic conditions may be responsible for the overall stressful conditions of exposed fish observed in the present study.

Table 1: Water quality parameters during exposure to Karate

Parameter	Toxicant Concentration (ppm)				
	20	**40**	**60**	**80**	**Control**
Temperature (^oC)	24.3± 0.8	24.2 ±0.	23.8 ±0.4	24.3 ± 0.01	24.9 ± 0.5
pH	10 ± 0.2	9.5 ± 0.07	7.03 ± 0.21	7.02 ± 0.3	7.15 ± 0.4
Dissolved Oxygen (Do) (mg/l)	6.20 ± 0.4	5.88 ± 0.004	5.3 ± 0.6	5.10 ± 0.5	6.43 ± 0.2
Total alkalinity (mgl)	8.2 ± 0.2	8.0 ±0.4	8.0 ± 0.6	5.0 ± 0.1	11 ± 0.2

Table 2: Mortality rates of experimental *Heterobranchus bidosalis X Clarias gariepinus* exposed to different concentration of Karate

Conc. (ppm)	Log. Conc. (ppm)	Mortality (hrs) 24	48	72	96	Total mortality	Survival %	Mortality	Probit kill
0	0	0	0	0	0	0	10	0	0
20	1.3010	0	1	1	2	4	6	40	4.75
40	1.6021	0	1	1	2	5	5	50	5.00
60	1.7782	1	2	2	3	8	2	80	5.85
80	1.9031	1	2	3	3	9	1	90	6.30

Table 3: Cations and anions uptake by gills of exposed fish

Conc. ppm	Exposure period 24h			48 h			72 h			96 h		
	Na+	**CL^-**	**K**	**Na^+**	**CL^-**	**K^+**	**Na^+**	**CL^+**	**K^+**	**Na^+**	**CL**	**K^+**
0	92.0	0.14	75.0	94	0.15	96	97	0.14	75	105	0.15	88
20	70.5	0.13	66.0	86	0.14	57	86	0.15	68	86.5	0.15	72.5
40	81.5	0.15	62.5	94	0.15	77	95	0.15	66	96.5	0.15	77
60	74.5	0.15	60.0	83	0.15	76.5	77.5	0.14	65	83.5	0.14	70.5
80	89.5	0.15	63.0	90	0.15	72.5	76.5	0.14	64	77.5	0.15	67.5
	Mg^{2+}	**Ca^{2+}**		**Mg^{2+}**	**Ca^{2+}**		**Mg^{2+}**	**Ca^{2+}**		**Mg^{2+}**	**Ca^{2+}**	
0	1945	4408.8		243.1	451		291.7	802		246	762	
20	972.5	4408.8		143.6	512.05		121.6	561		505.5	559.5	
40	972.5	5210.4		145.9	401		145.9	381		173.45	441	
60	1094.05	5210.4		133.75	721.5		461.9	41.5		27.1	514	
80	850.95	6613.2		121.6	501		158.05	541		139	520.5	

There was no total inhibition in the uptake of the cations and anions (Na^+, $C1^-$, K^+, Mg^{2+} studied but there was slight reduction in the uptake. Similar observation was recorded by Brooks and Clark (1987) when they exposed the rat brain to nanomolar concentrations of pyrethroid with an alpha - cyano groups. They observed the calcium ion uptake was reduced by 50 percent. Laufer et al. (1985) reported similar findings in the giant axon of the cockroach exposed to pyrethroid. They observed that pyrethroids interfere with the uptake of sodium and chloride ions as well as transmembrane movement of these ions. It has been suggested that pyrethroids appear to affect membrane pumps that involve Ca^{2+} dependent ATPase and Ca^{2+}/ Mg^{2+} dependent ATPase (Hassall 1990).

The uptake of many trace elements concentrations in the tissues of aquatic organisms at their permeable surfaces is generally considered to be a passive process not requiring the expenditure of energy (Simkiss and Taylor, 1989). This situation contrasts markedly with that pertaining to the major ions of alkali metals (e.g. sodium, potassium, calcium), which are taken up through active transport pumps. The key to this difference lies in different chemistries of the two groups of metals (Nieboer and Richarson, 1980). The uptake of dissolved metal may take place all over the body surface of small and/or soft bodied organism, as well as particular sites of high permeability such as the gills (David and Philips, 1993).

Uptake of potassium, sodium and chloride ions did not vary significantly between the concentrations at ($P < 0.05$) while the uptake of magnesium and calcium ions varied significantly at ($P < 0.05$). The relationship between metal uptake and loss dictates the particular metal accumulation strategy of on organism (David and Philips, 1993).

Whether the usual uptake of this ions from solution is by passive facilitated diffusion or by incorporation into active transport pumps the rate of uptake of an element will be directly proportional to the external concentration of dissolved metal over typical environmental range of uptake of most circumstances, however, the rate of uptake of trace elements respond proportionally to increases in external dissolved concentrations (Rainbow and White, 1990), Nugegoda and Rainbow (1989) have shown that changes in salinity and osmolatity had different effects in the uptake of zinc by *Palaemen elegans*. Campbell and Jones (1990) have shown the physiological response of prawn to the uptake of these ions. In contrast to the case of Heteroclarias" hybrid" catfish it appears that progressive physiological reduction in the uptake of cations and anions in the initial hour 24 and 72 hours of exposure observed in this research may have been the result of external concentration of pyrethroid bound linkages which exerts strong osmotic pressure with concomitant reduction in the available ions of the sample.

The gradual rise in metal ions during the 96 hours period indicated a recovery phase in the experimental animals. At the end of the recovery period the cation and anion values was near to control value. Similar trends of event have been documented by Begum (2004) for *Clarias batrachus* exposed to carnofuran insecticide.

Similarly the significance different at (P<0.05) in the uptake of potassium and chloride ions may be as a result of acid – base balance and osmotic regulation in the test fish. Sodium and potassium are associated with chloride in acid/base balance and osmo-regulation (McDonald *et al.,* 1995)

The increase in the uptake of these cations and anions at the 24 hour and decrease at 48 hour and 72 hour, and finally gradual increase at the end of 96 hours may be linked to initial phase of acclimation and or adjustment process to acute concentrations of xenobiotic compound. The high metal uptake of ions in the control fish may be because the control tank was devoid of the toxicant. The above result also show that the uptake of these cations and anions (Na^+, Cl^-, Ca^{2+}, Mg^{2+}, K^+) is time dependent.

The uptake of these cations and anions varied significantly between the treatment tanks and the control groups (P > 0.05).

It may be concluded that in general that acute exposure of *"Heteroclarias"* to karate (Lambda cyhalothrin) pyrethroid insecticide under similar environmental conditions and experimental techniques will affect the uptake of the major cations, and anions, although it may not totally inhibit, the uptake of these metal ions. As a follow up to this, it is advisable for environmental officers, crop farmers and insecticide habitual users to be cautious on the use and application of this insecticide as its misuse may affect the hydrodynamics and stability of ambient water quality standard and the aquatic life therein.

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