ACUTE TOXICITY OF GAMMALIN 20 TO *CHRYSICHTHYS NIGRODIGITATUS* (LACEPEDE).

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ABSTRACT

The impact of acute exposure of Gammalin 20 (an Organochlorine pesticide) was investigated in a static bioassay test over a 96-(4-day) period on the fingerlings of *Chrysichthys nigrodigitatus* (lacepede). The 96-hLC50 of Gammalin 20 was determined as 2.31 Ugl^{-1} with lower and upper limits of toxicities as 2.10 and 4.44 Ugl^{-1} respectively. At higher concentrations, the colour of the exposed fish became darker, opercular movement slowed down while pigmentation pattern increased, respiratory distress, erratic swimming, tonic convulsion and no response to gentle prodding and finally death. The implications of these results were discussed with a suggestion of the total ban on the use of Gammalin 20 in capture fisheries due to its harmful and persistence nature in the aquatic environment.

Key words Toxicity, *Chrysichthys nigrodigitatus,* Gammalin 20.

INTRODUCTION

Gammalin 20 is a widely used organochlorine pesticide employed in veterinary and human medicine to treat ectoparasites and pediculosis (*Mario et al* 1998). It is also used in the control of a broad spectrum of phytophagous and soil-inhabiting insects, public health pests, and animal ectoparaties. It is used on a wide range of crops to control Aphidione larvae of coleoptera, diptera, etc in stored product warehouse and storerooms, public health treatments and seed treatments (Tomlin, 1997).

Pesticides and herbicides in drainage water and agricultural farm run-off are becoming a wide spread source of aquatic pollution, and enclosed water bodies such as lakes, reservoirs, small water bodies, and ponds are particularly at risk. Organochlorine compounds are of major concern because of their persistence in the aquatic environment (Lean et al 1990).

There is a considerable market for pesticides in developing countries because of the growing population and increased pressure on land for food production. For example, more than half of the $35 million spent in the control of the massive locust and grasshopper invasion of some West African countries in 1986 was spent on chemicals (African Farming 1988). A recent survey of the agrochemical industry in Nigeria concluded that the annual increase in sales of pesticides of 5-10% is likely to continue; this compare with an annual increase of less than 1% in industrialized nations (Ikemefuna, 1990). Use of agrochemical normally achieves the desired results but the environmental impact of intensive use of agrochemicals is not usually recognized.

In the United Kingdom the hazardous nature of agricultural wastes and pesticide residues on aquatic life led to the Local Government (Water Pollution) Act (Daly, 1984). Although there are considerable reports on the effects of pesticides in the tropics (eg. Omoregie et al 1990 and Avoajah and Oti 1997) very few farmers in developing countries are aware of the environmental consequences of the intensive use of chemical such as Gammalin 20 in killing fish (Ojike, 1983).

Mishra and Shukla (1994), reported the sublethal effects of an organochlorine pesticide on freshwater catfish and found that organochlorine pesticide exerts an inhibiting effect on electron transport and act directly on ATPS synthase complex which leads to an impairment in mitochondrial bioenergetic of the fish.

Clarke and Clarke (1974) reported that there are many situations in the field in which acutely deleterious concentrations of insecticides are present, exposing the fauna chronically to subacute concentrations of the toxicant. The problems associated with the presence of insecticides, mostly the organochlorines and some of the organophosphates arise primarily from their physical and chemical stability as reported by Clarke (1975). Because of this stability, they are extremely persistent and widely distributed in the aquatic environment (Risebrough, 1969). Fish species and birds which are top predators in the food chain may accumulate high levels of toxic compounds in their body tissues, especially in lipid content of their liver which may well present a health hazard to humans if consumed in sufficient quantity. (Gutierrez *et al* 1998) sediment bound polychlorinated aromatic hydrocarbons have been shown to have carcinogenic effects in fish species in lakes affected by their proximity to urban centres (Lean *et al* 1990).

The use of insecticides to control pest on farms and in granaries is a common practice in the tropics. Gammalin 20 is a broad-spectrum organochlorine pesticide with a strong contact and fumigant actions. It is commercially prepared as 25% liquid formulation of lindane and sprayed by farmers in protecting their cocoa against capsid bugs attacking mature cocoa trees, *Theobroma cacao.* Acute oral LC50 of Gamalin 20 for rats is 88-270mg/kg, mice 59-246mg/kg, Birds 120-130mg/kg, Guppies 0.16-0.3mg/L (Tomlin 1997). Therefore their exists the possibility of the existence of these chemicals in the aquatic environment.

Chrysichthys nigrodigitatus is a common member of the African tropical freshwater ichthyofounna and is currently listed as one of the endangered fish fauna especially in the Cross River Southeastern Nigerian where they are commonly caught with Gammalin 20 and other obnoxious fishing methods (Oti; 1999). Although this species is ubiquitous in Africa (Fagbenro 1982) they are seriously threatened by aquatic pollution. They constitute one of the main fish families of economic value as food fish. They are very popular with fish farmers and consumers.

Because Gammalin 20 is a popular insecticide commonly used on farm in the tropics and subtropical Africa a good proportion may be washed into streams and ponds with deleterious adverse effects on aquatic biota. The objective of this study was to determine the effects of acute toxicity of Chrysichthys nigrodigitatus exposed to Gammalin 20.

MATERIALS AND METHODS

Lindane (gamma HHC or BCH) insecticide with the trade name "Gammalin 20" is an organochlorine insecticide commercially prepared as 20% liquid formulation (Tomlin 1997) was supplied as liquid emulsible concentrate (Gammalin 20 EC).

The experimental fish, *Chrysichthys nigrodigitatus* with mean weight of 0.50 ± 0.12g were collected from Cross River in Afikpo, Nigeria. The fish were transported to the laboratory where they were acclimated to laboratory conditions for 2 weeks in glass aquaria containing dechlorinated tap water. During the period of acclimation, the experimental fish were fed 4% of their body weight with artificially prepared diet daily throughout the period of the acclimation. It is from the acclimated fish that the test fish were selected for the bioassay studies. Mortality did not exceed 5% during acclimation period. Acute concentrations of insecticide used were 5.00, 2.50, 1.25, 0.625, 0.315 and 0.00NgL^{-1}. During the exposure period, 10 fish each was introduced into 12 glass aquaria (measuring 39x29x18cm) containing 20L of well aerated dechlorinated tap water. The already prepared concentrations were introduced into the first five aquaria serially, the sixth served as the control (devoid of the solution Gammalin 20), while the remaining six aquaria served as replicates.

Methods for acute toxicity tests as described by Sprague (1973) were employed in this investigation. The fish were not fed 24h prior to and during the exposure period, which lasted for 96h. The 96-hLC50 confidence limit was calculated as a cumulative of the percentage mortality using formula as described by Litchfield and Wilcoxon (1949) simplified method of evaluating dose-effect experiments. Mortality was recorded every 24h, though were read at the start and every 24h thereafter. Behavioural responses of the exposed fish were observed on a 3-hourly basis. During the exposure, the temperature, dissolved oxygen, free carbon dioxide, total hardness alkalinity and pH in each aquarium were monitored every 24h using methods described by APHA, AWWA and WPCF (1989). Results obtained from this investigation were subjected to statistical analysis using the analysis of variance methods to test for level of significance at the 0.05 probability level.

RESULTS AND DISCUSSION

The water quality parameters in the treatment tanks did not vary significantly ($P>0.05$) from those of the control tank (Table 1). All were within the suggested tolerance ranges (Mackereth 1963). The mortality rates of the test fish exposed to the various concentrations of Gammalin 20 are presented in Table 2. Percentage mortality increased significantly ($P<0.05$) with increase in the Gammalin 20 concentrations. No mortality was recorded in the control groups of fish exposed to 0.312 and 0.00 Ugl^{-1} of Gammalin 20, while 90% mortality was recorded in the group exposed to 5.00 NgL^{-1} The 25% and 10% mortality recorded in the groups exposed to 1.25 and 0.625 NgL^{-1} respectively were observed during the first 48h of the exposure period. At 1.25 Ugl^{-1} concentration and above the colour of the exposed fish became darker and respiratory distress, erratic swimming loss of balance and no response to gentle prodding were observed before death finally occurred. At lower concentrations, such changes were minimal, while the control fish showed no such behavioural responses. The value of the 96-hLC50 of 2.31 $\pm$0.12 Ugl^{-1} reported here is within the range earlier reported by Oti (1999) for several Tropical freshwater catfish species. Mishra and Shukla (1994) reported a lower 96-hLC50 in freshwater catfish. It is however important to note that the difference in the present study from those of these earlier workers could be attributed to difference in fish species, age and experimental techniques and conditions.

During the exposure period, the test fish exhibited serious behavioural changes before death occurred. These include erratic swimming, respiratory distress, tonic convulsion, darkening of the skin and no response to gentle prodding. These behavioural responses are in agreement with earlier reports of Okwuosa and Omoregie (1995) Avoajah and Oti (1997) when fish were exposed to various concentrations of toxicants. These behavioural responses are indications of death due to nervous disorder and insufficient oxygen supply.

The darkening and increased pigmentation observed in exposed fish in this investigation has also been reported by Novales (1959), Mishra and Shukla (1994) on several other catfish species. The result stated that the dark colour of fish is due to the dispersion of melanin pigments in the chromatophores when stimulated by melanocyte stimulating hormones (M.S.H) in the presence of Na+ ions in the medium.

The opercular ventilation rate of fish exposed to the Garmmalin 20 initially increased sharply, the increase being directly proportional ($P<0.05$) to the Gammalin 20 concentration (Table 3). Gammalin 20 concentrations of 0.315 Ugl^{-1} did not significantly alter the opercular ventilation rate when compared with the control groups ($P<0.05$). The Opercular ventilation of fish exposed to 0.625 Ugl^{-1} and above dropped below that of control by the 72 hour of exposure.

The initial hyperventilation of opercular reported in this research work suggest that fish exposed to Gammalin 20 tended to exhibit avoidance syndrome during the first few hours of exposure. However, as the exposure period is prolonged, the fish became fatigued, hence the subsequent drop in ventilation rate. The combined effect of this fatigue and direct toxic effect of the Gammalin 20 on the exposed fish led to their death. Even if the action of a toxicant is associated with the nervous system the role played by fatigue in the exposed fish is also shown to be important. Indices of opercular ventilation have earlier been reported by Saroj Gupta (1987) to be a strong indication of stress when fish are exposed to toxicants.

Gammalin 20 is widely used in the developing nations and most essentially in the tropics to control farm insect pests of cocoa. Though the use of these compounds is legal due to their low mammalian toxicity (Eto 1977) the resultant harmful effects on aquatic life subjected to nominal acute exposure in ponds and streams contaminated with runoff from treated farms calls for immediate evaluation of the legal status of these chemicals.

The use of Gammalin 20 and other similar pesticides by local fishermen in rivers, streams and lakes is seriously ill advised, as the resultant deleterious effects on fish subjected to acute exposure will subsequently lead to death. If the present rate at which this pesticides are being used is not checked the continuous existence of the aquatic fauna including some biologically important fish species, will be in serious jeopardy. Environmental managers, especially within the tropical zones of the world, need to set new water quality standard on the use of these recalcitrant and xenobiotic ompounds in the aquatic ecosystems.

Table 1: Mean (+SD) Water quality values during acute bioassays with *Chrysichthys nigrodigistatus* exposed to Gammalin 20.

Variable	Toxicant concentration (Ng/L)					
	5.00	2.50	1.25	0.625	0.313	0(Control)
Temperature (°C)	23.01	23.0	23.01	23.04	23.01	23.02
	±0.03	±0.02	±0.02	±0.11	±0.02	±0.04
Dissolved oxygen (mg/L)	7.76	7.75	7.72	7.70	7.72	7.68
	±0.04	±0.04	±0.07	±0.03	±0.04	±0.03
00_2 (mg/L)	3.54	3.55	3.52	3.50	3.51	3.51
	±0.02	±0.04	±0.02	±0.04	±0.02	±0.01
Alkalinity (mg/L)	34.10	34.08	34.07	34.04	34.00	34.20
	±0.02	±0.01	±0.04	±0.02	±0.01	±0.011
pH	6.71	6.70	6.69	6.66	6.71	6.00
	±0.01	±0.04	±0.02	±0.01	±0.01	±0.02

Table 2: Mortality rate of *Chrysichthys nigrodigitatus* exposed to various concentrations of Gammalin 20 for 96h.

Toxicant Conc. Conc. (NgL⁻¹)	Log (Ugl⁻¹)	Mortality rate in 2 replicates 24h	48h	72h	Mean Mort. 96h	%	Mean Probit Mort.
5.00	0.69	2 3	3 3	3 2	2 1	90	6.27
2.50	0.39	2 3	2 1	2 1	0 1	50	5.00
1.25	0.09	2 1	0 1	0 0	0 0	25	4.30
0.625	-0.20	2 1	0 1	0 0	0 0	25	3.70
0.315	-0.50	0 0	0 0	0 0	0 0	0 0	0.00
0.00	0.00	0 0	0 0	0 0	0 0	0 0	0

Table 3: Opercular ventilation (Mean of six reading S.E) rates per minute of *Chrysichthys nigrodigitatus* (Lacepede) exposed to various concentrations of Gammalin 20.

Conc. Ugl⁻¹	Time Start	24h	48h	72h	96h
5.00	139	120	112	100	108
	±0.02	±0.04	±0.01	±0.01	±0.03
2.50	130	128	125	99	101
	±0.04	±0.02	±0.04	±0.04	±0.04
1.25	118	116	115	98	103
	±0.06	±0.04	±0.08	±0.02	±0.02
0.625	110	109	106	98	105
	±0.04	±0.04	±0.04	±0.04	±0.08
0.315	108	104	106	97	105
	±0.02	±0.01	±0.02	±0.01	±0.02
0.00	86	85	84	80	84
	±0.04	±0.02	±0.02	±0.04	±0.04

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