ACUTE TOXICITY OF ATRAZINE TO ORECHROMIS NILOTICUS FINGERLINGS

By

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ABSTRACT

Acute Toxicity of Atrazine to *Oreachromis niloticus* was undertaken to find the lethal concentration (LC) 50 of atrazine using fingerlings. Different concentrations were prepared in mg/L. There were six different concentrations with a control and each treatment were replicated three times. A total number of twenty-one aquaria were used. The highest concentration was 30mg/L. Ten test organisms were used in cash aquarium.

At 24, 48, 72 and 96 hours there were LC 50 of 15.6mg/L 14mg/L, 11mg/L and 9.4L respectively. At 24, 48, 72 and 96 hours there were mean survival of 49.0%, 34.3% 28.6% and 28.1% respectively.

INTRODUCTION

The Tilapa (*Oreochromis niloticus*) is one of the important fish which provide animal protein source in the diet of most rural dwellers in Nigeria. Its importance hangs on the protific natural breeding ability and her hardy nature which enables her to inhabit and survive in most freshwater aquatic environment. Advancement in the agricultural practices and industrial activities has brought about degradation in most aquatic ecosystems. This has prompt the western world to become increasely concern about the excessive use of chemicals in agricultural practices. Nearly 200 years ago studies has beeen conducted to assesed the effect fo agricultural and industrial wastes on the environment and fish (Reish, 1987).

Several workers has reported the serious environmental problems pesticides have caused (Kemp *et al*, 1983; Jacknow, 1986; Agromisa *et al*, 1989; Ronald, 1989). Although most of these chemicals asre partially or completely banned in developed countries (Steward *et al.*, 1986), most continue to find their way to African countries for use in pests control. Some of these pesticides have not been screened neither have their toxicity effects on the animals or on aquatic organisms been assessd (Ita, 1991; Obiekezie and Okafor, 1995). Federal Environmental Agency (FEPA) in 1991 has set a uniform standard for all categories of pesticides allowed for discharge into inland waters in Nigeria at less than 0.01mg/l which seems impracticable because of lack of effective monitoring of the used of pesticides or effluents discharges from pesticides industries. Some attempts have been made to determine the levels of some pesticides and heavy metals in some inland waters in Nigeria (Adeniji *et al*, 1985). He recorded high concentration of DDT and other insecticides in the muscles of fish caught from Kainji and Jebba lakes areas of Nigeria. This was attributed to illegal use of Gamalin 20.

Acute toxicity tests provides basis for understanding the limiting effects of various chemicals on organisms. One of the early reports of the use of in toxicity studies was that of Peny

and Adams, 1963, when the effects of chemicals used in dye works onfish was checked. Chemicals such as pesticides are known to transfer their residual effects to the environment and the organisms that inhabit them (Alabaster, 1981; Gunkel, 1981; Adeniji *et al* 1985). Pesticides industries also get rid of their effluents through rivers and streams which ends up in larger aquatic environments. Inspite of the upsurge in the use of pesticides in agricultural practices in Nigeria, there has been little or no awareness of the dangers the environment is exposed to by the users. The increasing use of these pesticides has caused unwanted degradation of aquatic environments, fish fills and food and human poisoning (Alabaster, 1981; Rand and proccIli, 1985; Joseph, 1987; Igbedioh, 1991; Waiser and Robarts. 1997: Lanars, 1993). Apparently, information on their use, distribution and environmental impacts are scanty in Nigeria.

The objective of this work is to determine the toxicity of Atrazine (2-chloro-4-ernyl amino-o-iso ropyiaminol, 3, 5-triazine) to *Oreochromis niloticus* fingerlings. Atrazine is one of the commonent pesticides used in Nigeria to control weeds and pests in numerous crops. As reported by Van Schoubroeck, 1989; it is a white crystaline substance sold under a variety of trade name which include Primatol, Aatrex, Atranex, Actazin, Argeisin, Atrotol, Zeazin.

Studies on Atrazine

Agricultural uses of atrazine has been reported in some African countries, Australia, New zealand, Venezueia and in most European countries. The major producer is Ciba Geigy corporation. The products are usually used as water spray or liquified fertilizers which could applied as pre-mergence or post-mergence fertilizer. Atrazine is leached into the soil by ram or irrigation water and its also taken away by runoff water (Ronald, 1989). Kemp *et al*, (1983) have associated the application of herbicides, atrazine inclusive to the over all decline in the abundance of fish and wildlife in many aquatic systems as a consequence to serious disturbance in the ecological balance which cummulate in the decline of sea grasses and freshwater submerged vascular plants which provides food for the entire habitat. Denoyelles *et al.*, (1982) also observed that atrazine concentrations between 1 and 5ug/l adversely affected higher levels of the food chain beginning with zooplankton. At concentrations of 1 to 5ug/l and exposure period of 5 minutes to 7 weeks, Mayasich *et al.*, (1986) also reported adverse effects in sensitive species. Meck *et al.*, (1976) reported that adverse effect level to selected species of aquatic invertebrates and fishes ranged from 120ug/l to 500ug/l based on life time exposure. However, Deway (1986) as cited by Ronald (1989) observed that ambient concentrations of as low as 20ug/l have beeen associated with adverse effects on freshwater aquatic fauna, including benthic insects. In the studies of Kettle *et al.*, (1987) cited by Ronald (1989) it was noted that the production of channel catfish (*Lctalurus punctatus*) and blucgills as measured by number of young per pond was reduced by more than 95%. It was also reported that the number of prey items in stomach were significantly higher in control ponds than in the treated ponds. He thus concluded that the effects of atrazine on channel catfish and bluegills were probably indirect, and that the reduction of macrophytcs that had provided habitat for fodd items led to improvished diets and more canibalism by adult blue gills.

Bioaccumulation of atrazine from freshwater into aquatic organism muscles is limited and food chain biomagnification is negligible. Studies have shown that in a farm pond treated once with 300ug atrazine/l, residues at 120 days post treatment ranged between 204 and 264ug/kg in mud and water (Kadoum, 1979; cited by Ronald, 1989). Gunket (1981) in his studies with freshwater snail (*Achylus fluviatilis*) and fry of white fish (*Coregonus fera*) found that atrazine was rapidy accumulated from the medium by both species and saturation was reached within 12 - 24 hours.

Materials and Methods

The was obtained through induced breeding from the hatchery facilities of National Institute for Freshwater Fisheries (NIFFR), Kainji New Busaa, Nigeria. The fishes were of the same approximate size, each weighing about 1.3gms and measuring 3-4cm. They were kept in

glass aquaria measuring 60 x 30 x 30 cm in the diseases and mortality on daily basis. The fishes were maintained on dried commercial fish feed produced by NIFFR, but the fishes were starved for twenty-four hours prior to the test. They were also exposed to sixteen hours of light and eight hours of darkness as described by APHA Standard methods (1980) during acclimatization and experiment. The wide spectrum fourescent tubes were used as a light source similar to day light.

During acclimatization and the experiment, aerators pumps were used to aerate each aquarium tank so as to maintained dissolved oxygen level close to saturation. Municipal water was used for the test and it was analysed for some physico-chemical parameters (Temperature, Hydrogen ion (pH), Dissolved oxygen and Hardness).

Preparation of the Stock Solution

Atrazine powder was dissolved in water with the aid of ethanol as an organic solvent which serves as the ispersing agent. The powder contains 80% pure atrazine. A correction factor for obtaining the exact quanties of pure atrazine was then calculated as follows: % contents of pure atrazine in the atrazine powder X weight of atrazine powder in grams/100. The correction factor being in grams was further reduced to milligrams by multiplying it by 1.000. The correction factor was then used to determine the amount of powder needed to prepare a known concentration of stock solution.

Preparation of the test Solution

The test solution was prepared on litre basis, so as to get a test solution containing the required concentration per litre. This was done by pipeting the required quantity of the stock solution and introducing it into a known volume of water in each aquaria to get the test solution of a required concentration.

Test Procedures

Six different atrazine test solutions were prepared. Each concentration was replicated twice. A control was set up which was replicated twice also. Ten specimens of test organisms (*O. niloicus*) were kept in each aquaria tank. Each tank was observed daily for a period of 96 hours. Observations were made at 15 and 30 minutes, 1,2,4,8,12,16, 20 and 24 hours on the fish day of the experiment. Subsequent observations were ast intervals of 6 hours on daily basis. Temperature, pH, dissolved oxygen and hardness concentration of each tank were taken on daily basis. Test organisms were not feed during the test period so as to minimized the accumulation of fecal materials. Each experimental units were observed closely for any abnormal swiming behavior, hyperventilation or mortality. Death was the adverse effect used to reflect acute toxicity of the test solution. This was indicated by lack of movement of the operculum and response to stimulus.

Results

The calculation of the LC_{50} at 24 - 96 hours by the arithematic graph method are shown in figures 1 - 6. Percentage survival, mortality data and the 24h LC_{50}, 48h LC_{50}, 72h LC_{50}, and 26h LC_{50} for the various atrazine concentrations are presented in tables 1 and 2 respectively. No death was recorded in the controls and fishes in the lower concentrations of test solution did not show any abnormal behavior, however, at lethal concentrations fishes showed signs of stress, loss of equillibrium therefore shows cratic swiming behavior, gulping for air near water surface, over turning when swiming, slogishness and finally death results.

The pH and the dissolved oxygen values ranges between 6.09 and 7.03 and 2.13mg/l and 2.94gm/l respectively. Hardness decreases with increase in the exposure period. Its value ranges between 9 to 17.3mg/l. The mean variations in these parameters are shown in table 3.
(insert 2 graph here).

Table 1: **Records on percentage survival and percentage mortality rates of *O. niloticus* fingerlings in different concentrations of atrazine solution.**

{PRIVATE}	EXPERIMENTAL DURATION							
	24 HOURS		48 HOURS		72 HOURS		96 HOURS	
conc.	x%surv.	x% mort	x%surv.	x% mort	x%surv.	x% mort	x%surv.	x% mort
	100	0	100	0	100	0	100	0
	100	0	100	0	83.3	16.7	83.3	16.7
	76.7	23.3	20	80	1.33	86.7	13.3	86.7
15mg/i	56.7	43.3	167	833	33	90	0	100
20mg/i	10	90	33	96.7	0	100	0	100
25mg/1	0	100	0	100	0	100	0	100
30mg/f	0	100	0	100	0	100	0	100

conc. = concentration x%suurv. = mean percentage survival; x% mort = mean percentage mortality.

Table 2: **The experimental duration and LC_{50} of atrazine toxicity to the fingerlings of *O. nilolucus*.**

{PRIVATE }**Period of exposure in hours**	LC_{50}
24	15.6mg/l
48	14mg/l
72	11mg/l
96	9.4mg/l

Table 3: Mean variations in some physico-chemical parameters measured in the experimental units.

{PRIVATE}												
	24 hours			48 hours			72 hours			96 hours		
Conc	DO	pH	Hard	DO	pH	Hard	DO	pH	Hard	DO	pH	Hard
0mg/l	2.73	7.06	16.3	2.80	7.09	16.6	2.50	6.98	17.3	3.00	6.99	17.3
5mg/l	2.13	6.95	15.6	2.40	6.09	16.0	2.66	6.92	15.6	2.53	6.93	16.3
10mg/l	2.73	6.95	13.3	3.20	6.98	12.6	2.73	6.96	12.3	2.80	6.94	12.6
15mg/l	2.60	6.89	13.3	2.80	6.95	13.0	2.66	6.92	12.3	2.60	6.92	12.0
20mg/l	2.66	6.95	11.3	2.40	6.00	11.0	2.60	6.91	10.3	2.73	6.92	10.0
25mg/l	2.80	7.09	10.3	2.86	7.02	10.6	3.00	6.99	9.6	2.80	6.99	9.3
30mg/l	2.93	7.03	10.0	2.86	7.02	9.6	3.00	6.97	9.0	3.00	6.96	9.0

Conc. - concentration in mg/l, DO - Dissolved oxygen, pH - Hydrogen ion; Hard - Hardness.

Discussion

There were great significant differences at different concentrations. Swimming in an airmiess and erratic manner, gulping for air near water surface, overturning whilke swimming, dullness and finally death were the common responses of the test organisms to the test solution. These behaviours were also observed when Liong *et al* (1988) studied toxic effects of twenty-seven pesticides on Tilapia fish. There was 100% mortality at 96 hours at a concentration of 15mg/l and at less than 5mg/l the fish did not seem to be affected in any way.