Toxicity of an Organophosphate Pesticide (chloropyrifos) on a common Niger Delta Wetland Fish -*Tilapia guineensis* (Blecker 1862)

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ABSTRACT: The acute and sublethal toxicity of an organophosphate pesticide (Chloropyrifos) on the juvenile of Tilapia guineensis was evaluated to determine its effect on the survival, body functions and heamatological values. The fish was exposed to varying levels of the toxicant (0.0125mg/l; 0.025mg/l; 0.05mg/l and 0.1mg/l) using 96hrs static bioassay to determine the median lethal concentration and median lethal time for the different concentrations. The mortality was found to increase with exposure and increase in concentration. The 96hrs median lethal concentration was 0.002mg/l while the 96hr median lethal time of 56.2hrs; 42.7hrs; 31.1 hrs and 18.2hrs were recorded for 0.0125mg/l; 0..025mg/l, 0.05mg/l and 0.1mg/l respectively. The operculum beat frequency (F = Cal $6.89 > P = 3.49$)$_{0.05}$ and Tail beat frequency (F = Cal $4.46 > P (3.49)_{0.05}$ were significantly affected by the exposure. A sublethal exposure to various concentrations (0.0006mg/l; 0.00125mg/l, 0.0025mg\l, 0.005mg/l) were observed to cause a progressive reduction in the number of leucocyte (F – Cal $15.4 > P (3.01)_{0.05}$ and erythrocyte (F- Cal $14.39 > P(3.01)_{0.05}$ of the fish.The reduction in leucocye and erythrocyte number was significant indicating that the fish became anaemic.These conditions were more severe in the higher concentrations of the pesticide. @*JASEM*

Until recently, the adverse effects of pesticides and their residue on non-target organisms have not been seriously considered in Nigeria. Pesticides are often applied directly on agricultural land, primarily to control pest and improve crop yield to meet the high demand for food needed by the fast growing population. These pesticides applied in the field or those by accidentally spills of pesticides and careless discarding of pesticide containers; finally find their way into the aquatic environment either through storm water run off and or as aerosols carried by wind. Pesticides have been report to have negative ecological consequences on biota and the environment at large. On biota, the effect on the physiological processes (Singh *et al* 1992) reproductive failure (Bostveld *et al* 1993, Bostveld *et al* 1995), mortality (Dieter *et al* 1995); and instability in the ecosystem function (Bostveld *et al* 1993 and 1995, Grues, 198) have been reported. In Nigeria, similar studies have centered mainly on crude oil and petroleum products toxicity on aquatic organisms (Omoregie *et al* 1990; Dambo 1993, Omoregie 1995, Chindah 1998, Chindah *et al* 2001). Nonetheless, studies on pesticides toxicity are scanty despite several decades of its introduction, the wide use and magnitude (Chindah et *al* 2000). Therefore this study aims at determining the effect of a major organophosphate pesticide (chloropyrifos) used against soil insects of field and vegetable crops on fish mortality, body function and circulatory fluids on a common Tilapine fish species (*Tilapia guineensis*) prevalent in the Niger Delta waters.

MATERIALS AND METHODS

Tilapia guineensis of almost uniform length (5.5 $\pm$ 0 .8. cm) and weight (3.4 $\pm$ 0.3g) were collected with hand net from its natural habitat and held in holding tanks for 2 weeks prior to the experiment. The holding tanks were aerated, cleaned and water renewed daily. Fish were fed twice daily with NIOMR feed (pellets of 10% body weight).

Toxicity was determined by renewal static bioassay. All experiments were conducted in 15 rectangular glass aquaria (25 x 60 x 25cm) containing 20 litres of brackish water to which volumes of chlorphyfos was added to achieve different concentrations of the toxicant. Thirty minutes after the preparation of test solution, 20 juveniles fishes were carefully placed into each replicate tanks of 5 different concentrations (0.1mg/1,0.5mg/1, 0.025mg/ 1, 0.0125mg/1, and control 0.00mg/1). All experiments were conducted at room temperature and the tanks aerated. Fish were not fed during the experiment (Reish and Oshida 1986).

Observations were recorded every 12hrs; number of dead fish were removed. The tail beat and opercular beat frequencies were taken every 24hrs. Experiment lasted for 96hrs for the different concentrations of chloropyrifos. The susceptibility of fish to insecticide

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was determined using probit method for LC_{50} at 96hrs.

From the result of acute toxicity, sublethal concentrations (0.005mg/1, 0025mg/1, 0.00125mg/1, and 0.0006mg/1) were prepared. Ten glass aquaria were used with 2 replicates per treatment and with the same conditions as in acute toxicity. Group of 10 fish (9 ± 1.2g, 7.4 ± 1.3cm TL) were exposed to different concentration for 8 weeks. During which freshly prepared test solution were added on regular basis to maintain the concentration level after the waste were siphoned out. The water was changed once weekly and the tanks cleaned daily.. Fish and water quality parameters (Temperature, pH, salinity, conductivity, alkalinity, and Dissolved Oxygen) of the test solution were monitored through the duration of the experiment.

Every forth night, 2 fishes were sacrificed and blood collected using insulin syringe and needle *(size)* that were rinsed with EDTA to determine the various hematological parameters. The significant differences among means were tested with 2-way analysis of variance (ANOVA, 0.05).

RESULTS

The water quality parameters (Temperature, dissolved oxygen, salinity, alkalinity, pH conductivity) monitored during the exposure period did not differ within the various concentrations of pesticides as well as with the control. pH and alkalinity values tended to increase with increasing concentrations of the test chemical, however, values between treatments were not significantly different (ANOVA, $P < 0.05$). Temperature and dissolved oxygen values were almost uniform all through the study irrespective of the treatment. Similarly, salinity and conductivity values exhibited minimal variation in values, which were statistically not significant within and between treatments (ANOVA, $P < 0.05$). The limited variation in the physicochemical variables is similar to the trend observed in the wild, which is tolerated by the test organism even in the wild (Table 1).

Table 1: Mean concentration (ppm), range and standard deviation of physiochemical parameters for chloropyrifos treated tanks.

Conc.	pH	Temp (^{O}C)	DO (mg/l)	Salinity ($^o/_{oo}$)	Conductivity (µS/cm)	Alkalinity (mg/l)
0	6.93± 0.15 (6.7 – 7.0)	27.7± 0.30 (27.3 – 27.9)	3.98± 0.32 (3.5– 4.1)	15.9± 0.08 (5.8-16.0)	25850± 129.1 (25700-26000)	76.4± 7.3 (69.8-86.8)
0.0125	6.98±0.15 (6.9-7.1)	27.6± 0.36 (27.1-27.9)	3.95± 0.32 (3.75-4.42)	17.1± 0.87 (15.8-17.6)	27650± 1369.9 (25000-28400)	78.8± 5.9 (70.0-84.2)
0.025	6.98± 0.15 (6.8-7.1)	27.5± 0.36 (27.1-27.8)	3.97± 0.45 (3.59-4.62)	17.1± 0.89 (15.8-17.8)	27725± 1381.7 (25700-28800)	80.4± 9.2 (70-91.6)
0.05	7.03± 0.05 (7.0-7.1)	27.5± 0.50 (27.0-28)	3.94± 0.40 (3.62-4.61)	16.83± .68 (15.9-17.5)	27250± 1066.1 (25800-28300)	81.1± 8.7 (69.6-90.2)
0.1	7.05± 0.05 (7.0-7.1)	27.5± 0.48 (26.9-27.9)	3.91± 0.50 (3.52-4.61)	16.68± 0.39 (16.1-16.9)	27125± 485.6 (26400-27400)	86.1± 7.94 (85.2-92.0)

Mortality: Data on survival of *T. guineensis* to chlorphyfos is shown in Fig. 1. No mortality was observed in the control, however mortality increased as concentration and time increased. Ten (10) percent mortality was recorded in 0.0125mg/L concentration tank at 24hrs, this value increased to 32% in 36hrs, 38% in 48hrs, 52% in 60hrs, 62% in 72hrs, 78% in 84hrs and 85% in 96hrs respectively, thus indicating increased mortality over time. At 0.025mg/L concentration, 22% mortality was observed in 24hrs followed by 45%, 50%, 67%, 77%, 82%, and 92% for 36hrs, 48hrs, 60hrs, 72hrs, 84hrs, and 96hrs respectively. The fish exposed to 0.05mg/L concentration had mortality sequence of 38% (24hrs)< 63% (36hrs)< 77% (48hrs) < 88% (60hrs) < 95% (72hrs) <100% (84hrs) = 100% (96hrs). Similarly, at 0.1mg/L concentration, high mortality values of 68% and 88% were recorded for 24hrs and 36hrs respectively. Slight increase was observed in 48hrs (95%) and 60hrs (97%) until 100% mortality was observed in 72hrs. The 96hrs LC_{50} was 0.002mg/l and the LT_{50} for 0.0125mg /l; 0.025mg/l; 0.05mg/l and 0.1mg/l were 56.2hrs 42.7hrs, 31.1hrs and 18.2hrs respectively (figure 2). The LT_{50} decrease with increase in concentration while the LC_{50} decreases as time increase (Fig.3). The influence of chlorphyfos concentration on mortality was significant with F cal (23.85) $> P =3.26_{0.05}$.
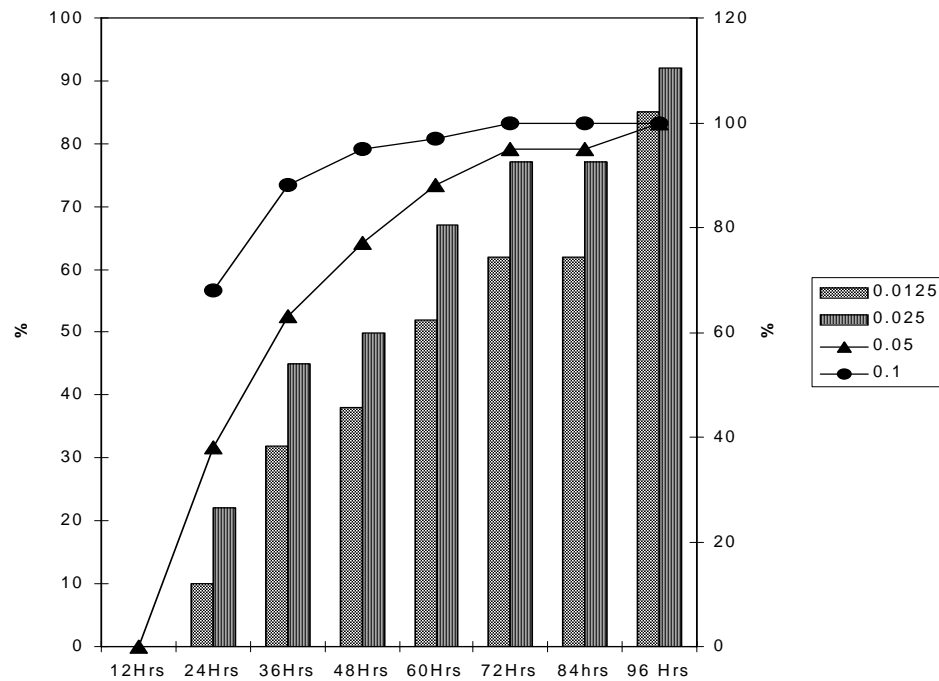
Fig 1: Percentage mortality of *Tilapia guineensis* expose for several hours of exposure at different concentrations of chloropyrifos
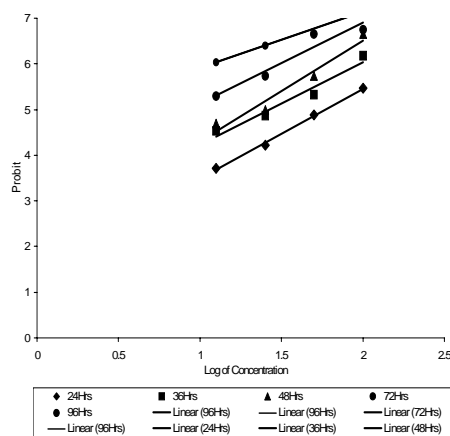

Fig 2 . Probit plot of *Tilapia guineensis* mortality against log of chloropyrifos concentrations
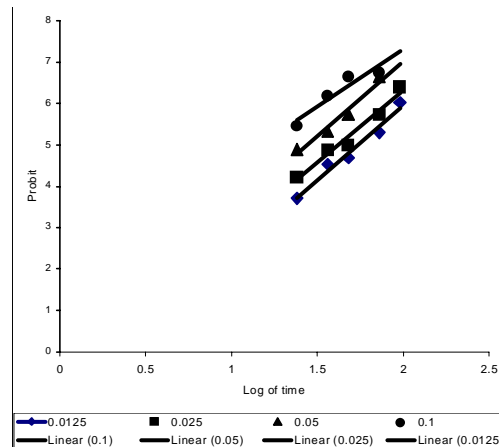

Figure 3 Probit plot of mortality against log of time of *Tilapia guineensis* expose to various concentrations of chloropyrifos.

Opercular Beat Frequency (OBF): The effect of chlorphyfos on OBF of *T. guineensis* In all cases, except the control fish that shows relatively uniform value (91-92 counts/minutes) Table 2. A progressive and significance (F- cal $(6.89) > P = 3.49_{0.05}$) decline in OBF values was observed with exposure time.

Tail Beat Frequency (TBF): The fishes in the control experiment showed limited variation in the TBF whereas other treatments had an initial increases values at the 24hrs, which declined after 48hrs (Table 3) statistical analysis revealed that there was

significant difference in the TBF between the treatments (F cal $(4.46) > P = 3.49_{0.05}$).

*Leucocyte and Erythrocyte***:** In addition, significant (F cal $(15.4) > P = 3.01_{0.05}$) reduction in leucocytes and erythrocytes values was observed at chlorphyfos sublethal concentrations. The erythrocyte (RBC) and Leucocytes (WBC) values for treated fish (Table 4) indicated significant decline for both the leucocyte (F cal $(15.4_{0.05}) > P = 3.01$) and erythrocyte (F cal $(14.39) > P = 3.01_{0.05}$) when compared with the reference fish.

Table 2: Summary of OBF values of *Tilapia guineensis* exposed to various concentrations
of chloropyrifos in 96 hours.

Exposure Time (hr)	Concentration.(mg/l)				
	control 0.0	0.0125	0.025	0.05	0.1
24	90.7± 0.6	106.3 ± 5.5	122 ± 1.7	130 ± 0	145 ± 5
48	92.3 ± 2.5	93.7 ± 5.5	113.3 ± 2.9	45.3 ± 5.5	30.3 ± 0.6
72	90 ± 0	58.3 ± 2.9	102.7 ± 3.8	30 ± 0	20.7 ± 1.2
96	91.3 ± 1.5	42 ± 2.6	48 ± 2.6	19.5 ± 0.7	-

Table 3: Tail Beat Frequency of *T.guineensis* exposed to various concentrations of
chloropyrifos in 96 hours.

Exposure Time (hr)	Concentration (mg/l)				
	Control 0.0	0.0125	0.025	0.05	0.1
24	7.3± 0.6	11 ± 1.7	15.3 ± 2.5	18.7 ± 0.6	25 ± 5
48	7.7 ± 0.6	9.7 ± 0.6	11.7 ± 2.9	4.3 ± 0.6	2.7 ± 0.6
72	7.3 ± 0.6	4.7 ± 0.6	5 ± 2	2 ± 0	1.7 ± 0.6
96	7 ± 0	3 ± 1	2.3 ± 1.5	1 ± 0	-

DISCUSSION

The observed increase in OBF and TBF within 24 hours of exposure to chloropyrifos and decline thereafter had been reported earlier by Omoregie (1995). The initial increases in OBF and TBF may be associated with the sudden response to shock. In addition, the behavioural response to chloropyrifos with marked deviation in the rate of OBF and TBF from reference sample (control) imputes an adjustment in physical fitness as a result of the stress condition (Edwards and Fusher 1991, Leight and Van Dolah 1999). Grillitsch *et al*. (1999) reported that organisms exhibit behavioural responses to chemical stress both at acute and sublethal toxicity. This elicits the potency and sensitivity of the fish - *Tilapia guineensis* to the test chemical. The ecological importance of this is that the damage to non-target species in the environment and such attribute of the organism could be effectively used as toxicity biosensor of chemical stress. Other studies on different toxicants especially petroleum related hydrocarbon compounds indicated damage to epithelial cells of the gill chamber (Omoregie 1995), destruction of liver (Omoregie 1995), possible nervous breakdown, failing organs and retarded physiological processes in fish body functions (Omoregie 1995 Meyer et al. 1992 and Fryday *et al*

1996). Chindah *et al* (2001) noted that aquatic organisms (shell and fin fishes) which is indirect continuous contact with the medium in addition to breathing and feeding will be vulnerable to respiratory tract damage and other organs of the body.

The result of haemotological parameters showed marked reduction between control fish and the fish exposed to different concentrations of chloropyrifos, which is an indication of the deleterious effects of the chemical pollutant to the body fluid. The most adverse effects were observed in the fish exposed to 0.1mg/l of the toxicant. In consistence with our data, other research has shown a reduction in these parameters in fish exposed to various toxic environments (Omeregie *et. al* 1990; Sikoki & Enajekpo 1991; Wedemayer and Yasutake 1977 and Singh et al 1992). Reduction in erythrocyte and leucocyte reported in this investigation indicated that *Tilapia guineensis* exposed to sublethal concentration of chloropyrifos became anaemic and leukopenia (Kerting and van Wijngaarden1992). This according to Hontela *et al*. (1992) is due to hemodilution resulting from impaired osmoregulation across the gill epithelium. The change in osmoregulation such as plasma osmolarity which is associated with rise in blood volume and tissue water content suggesting increased influx of water through the gills from

changes in gills permeability to water, a failure of electrolyte regulatory mechanism (MCLeese *et al* 1980, Scheck 1990, Weber and Spieler 1994, Hontela *et al*. 1992,, Habig and Guillo 1991). In view of the multifarious uses of agrochemical (pesticides) by man, their ecological effects must always be kept in mind. Our results confirm the views of other researchers in this field, that the pesticide chloropyrifos have deleterious effects on the survival, body and haematological processes in *T. guineensis* (Singh and Narain1982, Singh *et al* 1992). The sublethal dose in the wild may in addition cause growth lag, reproductive failure and/or death (Stein *et al* 1992, USEPA 1995, Pratt and Cairns 1996).

Table 4: The average leucocytes and erythrocyte of *T. guineensis* after 8 weeks of exposure to various concentrations of Chloropyrifos

Variable /Concentration of Chloropyrifos(mg/l)	Duration of exposure (weeks) (cells/mm3)				
Leucocytes					
Conc.	0	2	4	6	8
0.000	9995	9989	9998	9998	9995
0.0006	9998	9789	9783.5	9761	9781
0.00125	9998	7534.5)	7241)	7091	6544
0.0025	9998	76021	5992.5	5588	5203.5
0.005	9995	5667	5215	5196	5100
Erythrocytes					
Conc. (Mg/l)	0	2	4	6	8
0.000	47150	47150	47150	47150	47100
1.0006	47150	42500	40100	35550	35150
0.00125	47150	37100	31000	27500	25000
0.0025	47150	22550	22000	20950	18500
0.005	47100	21450	20950	18500	15950

It is therefore our view that effort should be made to study the levels of pesticides in our natural water bodies and adjoining environment in order to provide baseline data, which could be used as a strategy for assessing the distribution, impact of contaminants on specific areas and the ecological risk on native organisms in the Niger Delta water sheds. This shall provide critical tools for formulating regulations and appropriate management options.

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