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Ezike CO
Department of Animal/Fisheries Science & Management, Enugu State University of Science & Technology (ESUT) Enugu, Nigeria

Correspondence
Ezike CO
Department of Animal/Fisheries Science & Management, Enugu State University of Science & Technology (ESUT) Enugu, Nigeria

Acute toxicity and heamatology of *Clarias gariepinus* (Burchell, 1822) Exposed to 2, 2-Dichlorovinyl Dimethyl Phosphate (Dichlorvos)

Ezike CO

Abstract
Static bioassay experiment was conducted to ascertain the acute effect of 2, 2 - dichlorovinyl dimethyl phosphate (DDVP) also known as Dichlorvos an organophosphate pesticide at 0.60, 0.80, 1.0, 1.2 1.4 and 0.00 mgL^{-1} on African catfish, *Clarias, gariepinus* juveniles (mean weight 13.86±0.46g) after 96 hours. The effects were assessed by examining the haematological profile against the control. Exposure to Dichlorvos caused a significant dose dependent ($P < 0.05$) reduction in haematocrit (PCV) and Haemoglobin (Hb) values. There was also a significant decrease ($P < 0.05$) of RBC in 1.4 mgL^{-1} concentration. The WBC elevation was significantly dose dependent ($P < 0.05$) in all concentrations except 0.60 mgL^{-1}. The inhibition of MCH and elevation MCHC were both non significant ($P > 0.05$) but the inhibition of MCV was significant between control and exposed group ($P<0.05$). The LC_{50} of dichlorvos for *Clarias gariepinus* was 0.93 mgL^{-1} while the upper and lower confidence limits gave 1.21 and 0.77 mgL^{-1} respectively. Safe concentration estimated gave 0.0093 mgL^{-1}. Behavioural responses in the fish included erratic and uncoordinated swimming which were observed to be more pronounced as the concentration increased. The results of this study highlight the stress of freshwater fish exposed to uncontrollable discharge of insecticides in the aquatic environment.

Keywords: Acute Toxicity, Heamatology, *Clarias gariepinus*, Dichlorvos

1. Introduction
Dichlorvos or 2, 2-dichlorovinyl dimethyl phosphate (DDVP) is an organophosphate pesticide widely used as an insecticide to control household, public health and stored products from insects. The compound had been commercially available since 1961 and has become controversial because of its prevalence in urban water ways and the fact that its toxicity extends well beyond insects [9]. Dichlorvos is used as an agricultural insecticide on crops, stored products, and animals. It is also used as an insecticide for slow release on pest-strips for pest control in homes as well as anthelmintic (worming agent) for dogs, swine, and horses, as a botacide. The United State Environmental Protection Agency requires cautionary warning labels on products containing dichlorvos [34, 35]. The 4-hour LC_{50} for dichlorvos in rat was $15mg/m^3$, and $13mg/m^3$ in mice [39]. It is estimated worldwide that 60% DDVP is used in plant protection, 30% for public health and vector control and 10% for protection of stored products [34]. It has been found effective in disinfection of aircrafts during flight. DDVP Vapour in the range of 0.15 to 0. 30 ug/litre of air produced 100% mortality of flies and mosquitoes after 30 minutes exposures [17]. No effect was noticed on the cholinesterase level of three individuals exposed 24 times to 30 minute of aircraft treatments, during which dichlorvos vapour concentration was 0.20 to 0.24 ug/litre of air [32]. The 96hour LC_{50} of fresh water and estuarine fish ranged from 0.2 to 12 mg/L [39] while marine fish was estimated to be more than 4mg/l for adults and pre-adult of Atlantic Salmon *Salmo salar* [31]. Due to its low persistence, repeated application is allowed for which reason large quantities find their way into the aquatic environment through careless handling, indiscriminate use, accidental spillage and discharge of untreated effluents into natural water ways.

In Nigeria, agrochemicals that contain pesticides especially chlorinated hydrocarbons and the organophosphates, are routinely employed as part of the integrated farming practice to protect crops and animals from insects, weeds, and diseases [11]. Thus toxicity test become imperative to estimate potential hazards as part of risk assessment protocols in agriculture, especially in

fish farming. Toxicity tests are performed for the specific purpose of predicting what biological functions would be perturbed by the toxicant exposure or explicitly to quantify the effect of a toxicant on the health of an organism [28, 27]. A number of factors influence the responses of organism to toxicity test. These factors include age, disease, water quality, stage in life history/cycle, pollutant interaction, nutrient status, reproductive stages and species interactions. Different species of organism vary in their vulnerability to specific pollutant [36].

Haematological and acetycholinesterase changes in various fish species to different stressors has been used to determine acute and sub acute effect of pollutants [25, 22].

The exposure of fish to several types of chemical agents may induce changes in several haematological and physiological parameters, which are frequently used to evaluate fish health. *Clarias gariepinus* is hardy and can tolerate both well and poorly oxygenated waters. It is well cultivated and distributed in water bodies of Nigeria hence used as biological indicator in ecotoxicological studies [37]. The aim of this study was to investigate the lethal toxicity and haematological effect of Dichlorvos on a commercially important fish species, *Clarias gariepinus* under laboratory conditions.

2. Materials and methods

2.1 Collection and Acclimatization of Experimental Fish

One hundred and twenty (120) juveniles of *Clarias gariepinus* with a mean weight of 13.86±0.46g were conveyed to the experimental site and stocked 60 juveniles per tank into two (2) holding tanks (1.2x1.2x1m) for 14 days (Fawole *et al*., 2007) [13] and fed 3% body weigh with commercial feed at 9.00 and 16.00 hours daily.

2.2 Measurement of Water Quality Parameters

Temperature was measured using uniscope hand thermometer H19146-04, pH was determined using Hama pH meter model PHEP serial 1298, while alkalinity, ammonia and total hardness were determined with HACH analysis kit model FF-1A serial 2430-02. Dissolved oxygen was measure with oxygen metre (Asonye *et al*., 2007) [4].

2.3 Acute Toxicity Test.

Acute semi-static toxicity bioassay under laboratory condition was adopted for 96 hours (Ezike and Ugwu, 2015) [10]. Six graded concentrations of dichlorvos were used: 0.0mgL-1 (control) 0.6mgL^{-1}, 0.8mgL^{-1}, 1.0 mgL^{-1}, 1.2 mgL^{-1}, 1.4 mgL^{-1}. Control did not contain the toxicant. Ten (10) juveniles were stocked per replicate and estimated for mortality rate after 24, 48, 72 and 96 hours periods. Dead fish were removed immediately to prevent water pollution. Feeding was stopped 24 hours before commencement and during the experiment. Water in the tank was replenished daily with dechlorinated municipal tap water and fresh toxicant.

The 96hr lethal median concentration (LC_{50}) was determined as a probit analysis using the probit mortality versus the logarithm concentration. The LC_{50} was extrapolated from probit 5 to the log concentration. The antilog value gave the LC_{50} in ml^{-1}. Percentage mortality probit values were taken from Finneys Table (Finney, 1971) [14]. The safe concentration of Dichlorvos solution was estimated by multiplying LC_{50} by a factor of 0.01 (Koesoemadinate, 1980) [20, 21].

2.4 Haematological Analysis of the Experimental Fish

Blood samples were collected at the beginning of the experiment and every 24 hour, throughout the 96 hour (24, 48, 72 and 96 hours) respectively. The catfish blood was collected by means of heparinized plastic syringe after administration of clove oil to reduce stress. Blood samples were collected and immediately transferred into sterile ethylene diamine tetra-acetic acid (EDTA) container.

2.4.1 Packed cell volume (PCV)

The packed cell volume, also referred to as the haematocrit value, was determined from the blood sample earlier collected in heparinised tubes and stored at 4^0C for not more than 4 hours. The mixed blood was sucked into 75 x 1.5 mm capillary tubes and sealed at one end over a burner. The capillary tubes were placed into the micro haematocrit centrifuge with the sealed ends being at the outer end. The centrifuge lid was then closed. The tubes were centrifuged at 14,000 r.p.m. (revolutions per minute) for three minutes. Thereafter, the PCV percentage of total volume of whole blood was read from the haematocrit metre. (Svobodava *et al*., 2001) [33].

2.4.2 Haemoglobin

The haemoglobin (Hb) was determined (Wickham *et al*., 1990) [38] using the cyano-haemoglobin method. To determine the haemoglobin from the blood, 5ml of the transformation solution was measured and poured into a test tube using a rinsing pipette, 20µl heparinised blood which has been collected and stored at 4^0C for not more than 24 hours was then collected and poured into the test tube containing the transformation solution and the content were stirred thoroughly. The transformation solution converted the haemoglobin into cyano haemoglobin rapidly and the value was read from the photocolorimeter after 3 minutes. The samples extinction measurement was performed in a 1cm cell at a wave length of 540-546nm against the transformation solution. The haemoglobin content was determined from the calibration curve which was drawn using the cyano-haemoglobin standard. Haemoglobin content was expressed in S.I unit of gdl-^{1}.

2.7.3 White Blood Cells

The WBC was determined following the method of Svodava *et al*., 2001 [33].

2.7.4 Haematological indices

The haematological indices of mean cell heamoglobin concentration (MCHC), mean cell Haemoglobin (MCH) and mean cell volume (MCV) were calculated using the total red blood cell count (RBC), haemoglobin concentration (Hb), and heamatocrit (HCT) according to the

Parameter were calculated as follows: Mean corpuscular volume (MCV) = HCT/RBC,

Mean corpuscular haemoglobin (MCH) = [Hb x 10]/RBC, Mean corpuscular haemoglobin concentration (MCHC) = [Hb x 10]/HCT x 100.

2.4.5 Red Blood cells

The red blood cells (RBC) count was determined within 6 hour blood sampling by diluting the heparinsed blood with the Prochazka-Skrobak solution at a ratio 1:200 Svobodava *et al*., 2001 [33].

2.5 Statistical Analysis of Data

Data obtained were expressed as standard mean ± standard error of mean and were analyzed with using Statistical Package (SPSS Inc. Chicago Illinois, USA). Differences in the test concentrations and control were subjected to one way analysis of variance (ANOVA) followed to by Turkey's Multiple Range Test was used to separate differences among means. Differences were considered significant at ($P < 0.05$).

3. Results

The physico-chemical parameters results obtained from the test solutions for the period of 96 hours showed that the values were close to the physio-chemical parameters of the control (Table 1).

Table 1: Mean physico-chemical parameters of water

Conc mgL^{-1}	DO mgL^{-1}	Temp^{0}C	Parameters pH	NH_3 mgL^{-1}	Total Hardness
0.00	6.24±0.04^a	26.38±0.5$^{a\ `}$	6.59±0.03^a	0.05±0.01^b	120±0.40^b
0.60	6.14±0.24^a	26.68±0.25^a	6.49±0.33^a	0.30±0.02^a	100 ±0.04^b
0.80	6.18±0.12^a	26.77±2.25^a	6.34±0.02^a	0.06±0.1^b	80.0±0.22^a
1.00	6.16±0.12^a	27.10±0.22^a	6.16±0.05^a	0.31±0.02^a	70.0±0.30^a
1.20	6.13±0.04^a	26.0±0.05^a	6.09±0.02^a	0.50±0.03^a	50.0±0.08^a
1.40	6.15±0.03^a	26.0±0.06^a	6.08±0.01^a	0.40±0.03^a	60.0±0.4^a

3.1 Mortality Responses

The 96 hour LC_{50} of Dichlorvos for *Clarias gariepinus* obtained from a plot of probit mortality versus log concentration gave a value of 0.93mgL^{-1} (Figure 1), while the upper and lower confidence limits gave 1.21 and 0.77mgL^{-1} respectively.

The behavioural responses of the test fish to the toxicant at different concentration (Table 2) indicated high secretion of slime (mucus) on the bodies and gills of exposed fish except control. Additionally, T_5 test fish were observed to be swimming with their backs about the 27th hour of exposure. Generally, observed behavioural changes include: fast opercula movements, air gulping, restlessness and loss of balance. The fish coughed in order to vomit the toxicant, swarm in a zig-zag direction and had their flesh peeled off. Mortalities were observed in all treatments except control.

Table 2: Mortality of *Clarias gariepinus* exposed to different concentration of Dichlorvos

Concentration mgL^{-1}	Log concentration	%mortality	probit
0.00	0	0	0
0.2	-0.699	10	3.12
0.4	-0.398	30	4.48
0.8	-0.097	60	5.26
1.6	0.204	90	6.28

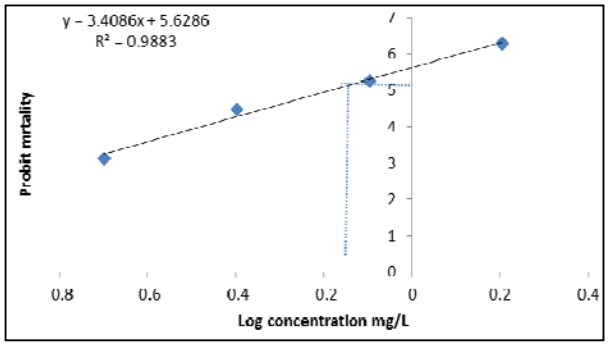

Fig 1: Logarithimic probit line for determination of 96-h LC_{50} DDVP to *C. gariepinus*

3.1.2 Haematological Responses

Exposure of Juveniles of *Clarias gariepinus* to Dichlorvos for 96 hours significantly ($P < 0.05$) inhibited packed cell volume and haemoglobin in the fish blood. The percentage reduction from control values of packed cell volume and haemoglobin were 31.00 and 29% respectively (Table 3). The inhibition of these parameters in the exposed fish was significant ($P < 0.05$) and dose dependent (Table 3). The RBC values of 0.60 – 0.80 increased from that of the control. Also there was significant reduction ($P < 0.05$) of RBC in the nominal concentration 0.60- 1.40 mgL^{-1}. The WBC showed a dose dependent percentage elevation from the least to the highest concentration (Table 3).

Table 3: Haematological parameters of the exposed fish to DDVP.

Blood parameters	Control 0.0	Concentrations (*mgL-1*) 0.6	0.8	1.0	1.2	1.4
PCV 24 (%)	31.00±0.02^a	29. ±0.12^a	24.01±0.4^b	25±0.01^c	21±0.01^a	22. ±0.01^b
PCV 48	25±0.03^e	20±0.35^a	19±0.07^b	17±0.01^a	24±0.02^d	24±0.05^d
PCV 72	22.10±0.10^d	17±0.01^c	19±0.3^b	20±0.09^c	17±0.03^a	23±0.09^e
PCV 96	18.01± 0.13^b	16±0.02^a	19±0.03^a	20±0.09^c	18±0.08^b	18±0.09^b
Hb 24(g/dl)	10.20±0.10^d	9.7±0.03^b	8.0±0.05^c	7.0±0.03^a	8.0±0.08^e	9.0122^a
Hb 48	10.10±0.01^e	8.8±0.03^c	7.1±0.03^b	6.6±0.03^a	10.1±0.5^e	10.1±0.04^e
Hb 72	8.01±0.01^d	6.9±0.06^a	6.8±0.05^c	7.0±0.03^c	8.0±0.08^b	9.0122^c
Hb 96	6.14±0.03^b	5.3±0.08^a	6.2±0.05^c	5.60.08^c	5.4±0.06^d	5.7±0.01^e
WBC 24(10^3/mm^3)	6000.01±0.009^c	64000±0.01^b	6600±0.00c	6800±0.00^d	6900±0.00^e	7000±0.0^e
WBC 48	5400.01±0.00^a	60000±0.01^b	6200±0.00^c	6400±0.00^d	6500±000^d	6700±000^e
WBC 72	5500.01±0.00^b	5300±0.03^a	5700±002^c	6000±0.08^d	5900±003^e	6000±0.05^e
WBC 96	5300.01±0.01^b	5100±0.01^a	5500±0.05d	5600±0.08^c	5400±0.06^d	5700±0.01^e
RBC 24(10^6/mm^3)	68100±0.00^d	70000±0.03^b	75000±0.03^c	42000±0.03^a	65000±0.03^a	66000±0.02^e
RBC 48	53100±0.00^d	65000±0.03^c	72000±0.03^b	40000±0.03^c	39000±0.06^a	40000±0.04^a
RBC 72	47000±0.00^d	50000±0.03^a	60000±0.3^c	45000±0.03^c	40000±0.05^b	38000±0.01^b
RBC 96	37200±0.02^b	55000±0.03^b	60000±0.04^b	40100±0.02^c	39000±0.02^b	37000±0.01^b

There was varying percentage inhibition of mean corpuscular volume (MCV) in the exposed fish (Table 4). The percentage inhibition was dose dependent from 0.80 – 1.40mgL^{-1} exposed fish. There was significant difference between control and exposed fishes for MCV but no significant difference ($P > 0.05$) among exposed concentrations except for 0.60mgL^{-1}. There was no significant difference ($P > 0.05$) between control and exposed fishes and among treatment concentrations for MCH and MCHC (Table 4).

Table 4: Haematological indices

Conc.mgL^{-1}	MCV (ft/cell)	MCH (pg)	MCHC (gdL^{-1})
0.00	2.16±0.04^{a}	0.73±0.01^{a}	33.30±0.01^{a}
0.60	1.51±0.01^{b}	0.50±0.00^{a}	33.10±2.19^{a}
0.80	0.91±0.03^{c}	0.46±0.02^{a}	49±2.19^{a}
1.00	0.93±0.07^{c}	0.50±0.4^{a}	54.20±6.87^{a}
1.20	0.96±0.03^{c}	0.53±0.14^{a}	50.20±64^{c}
1.40	1.03±0.02^{c}	0.50±0.00^{a}	48.30±6.1^{c}

Tabe 5: Behavioral changes of *C. garipinus* exposed to different concentrations of dichlorvos for 96 hours

Behavioural changes	0.0	0.6	DDVP (mgL^{-1}) 0.8	1	1.2	1.4
Mucus secretion	-	-	-	x	xxx	xxxx
Uncoordinated movement	-	-	-	x	xx	xxx
Air gulping	-	-	-	x	xx	xxx
Opercular ventilation	-	X	xx	xxx	xxx	xxxx
coughing	-	-	-	x	xx	xxx
restlessless	-	-	-	x	xx	xxxx
Swimming with back	-	-	-	-	-	xx
Tailfin movement	-	X	x	xx	xxx	xxxx
Lossof balance	-	-	-	x	xx	xxx

-none, x mild, xx moderate, xxx strong, xxxx very strong

4. Discussion

The lethal toxicity test showed that fish in the control tank survived whereas mortalities were recorded in all treatment concentrations. Mortality increased with increasing concentration of the toxicant in water. Many authors made similar observation on different fish species exposed to varying levels of toxicants (Agbon *et al*., 2002 [1]; Omoregie *et al*., 2009 [29]; Umar *et al.*, 2010) [36]. Mucus observed on the gills of dead fish might be responsible for mortality recorded in this study. Omoniyi *et al*. (2002) [28] reported accumulation of mucus on gills which reduced respiratory activity due to inability of the gill surface to actively carry out gaseous exchange and to mortalities. The results of the water quality of the test media was within the optimal range recorded by FAO (2000) [12] and Omoregie *et al*. (2009) [29]. The fish exhibited stressful behaviour which increased with toxicant concentration. Similar behaviour was reported by Fafioye *et al*. (2001) [11] on *Clarias gariepinus* and Rahman *et al*. (2002) [30] on *Clarias punctatus.* The observed restlessness and uncoordinated swimming might be due to its stimulant effect which Umar *et al*. ((2010) [36] specifically identified to inhibit cholinesterase enzymes to acetylcholine receptors in the nervous system of exposed fish which resulted to excitation and restlessness. The 96-h LC_{50} value of 0.93mgL^{-1} in this investigation is in agreement with the value of 0.93mg/L reported for rainbow trout (Bayer, 1980) [7] but higher than 0.48 and 0.55mg/L reported for blue gill and sports (Lukaszewicz-Hussain, 2010 [23]; Kenaga, 1979) [19]. The 96-h LC_{50} value of 1.9mg/L obtained in tilapia mossambica and 2.7mg/L in carp exposed to DDVP are higher than the value of this investigation (Koesoemadinata, 1983) [21]

The significant elevation of RBC in 0.60 and 0.80mgL^{-1} nominal concentrations may be due to the release of red blood cells into the blood stream. This agreed with observations of Al-khem *et al*. (1998) [2] when Trichlorofon (organophosphate pesticide) was exposed to fish for 96 hours which they attributed to stress mediated condition that triggered release of new erythrocytes from the erythropoietic tissue in order to improve oxygen carrying capacity of exposed fish blood, with resultant higher values of erythrocyte count. Increase at lower concentration may suggest a compensatory response of *Clarias gariepinus* to increase, their oxygen demand and energy to meet up with the challenge of insecticide detoxicification. Similar observation and explanation had been reported by Atamanalp and Yanik (2002) [5].

Haemoglobin concentration and packed cell volume inhibition suggest that the insecticide exerted effect similar to the production of acute anaemia in exposed fish. This is consistent with the report of Mgbenka *et al.* (2005) [24] in *Clarias albopunctatus* exposed to sub lethal actellic concentrations. Anaemia associated with erythropenia has also been reported for several freshwater fish species (Auta, 2001[6]; Svoboda *et al*., 2001 [33]; Gbem *et al*., 2003) [15]. The significant dose dependent inhibition in the Haemoglobin concentration observed in the present investigation may be due to increase in the rate at which Hb was destroyed, or decrease of Hb synthesis. The implication of these findings indicates that acute Dichlorvos exposure to *Clarias gariepinus* may impair its oxygen supply to various tissues. Thus reductions in Haematocrit and Haemoglobin in *Clarias gariepinus* suggest that Dichlorvos may have impaired erythropoietic activity in the fish especially in the highest nominal concentrations (Nussey *et al*, 1995) [26].

The MCV has been reported to provide information on the size and status of erythrocytes (Nussey *et al*., 1995) [26] thus the inhibition in PCV and MCV as observed in the current investigation indicate that Dichlorvos may have interfered with the normal physiology of RBC. Anees (1978) [3] earlier reported that methyl parathion and Dimethoate caused consistent decrease in cellular and nuclear diameter of erythrocytes. The significant ($P < 0.05$) elevation of RBC in 0.60 and 0.80mgL^{-1} nominal concentration and concomitant decrease in PCV may show the extent of shrunk cell size.

In the current investigation, no significant differences were observed in the levels of MCHC and MCH. Giron-Perez *et al*. (2006) [16] also reported that chlorpyrifos had no effect on MCH and MCHC of Nile tilapia (*Oreochromis niloticus*). Similarly, Svobodava *et al*. (2001) [33] observed that values of MCH and MCHC in common carp (*Cyprinus capio*) registered after 96 hours exposure to Diazinon were comparable with control group. The increase in the WBC can be correlated with an increase in the antibody production which helps in survival and recovery of fish exposed to pesticides (Joshi *et al*., 2000) [18].

5. Conclusion

Acute toxicity level at 0.93 mgL^{-1} of DDVP could cause 50% mortality and adverse effects on blood physiology of exposed fish. Levels below 0.0093mgL^{-1} may be safe for juvenile freshwater catfish but cautionary measures must be employed

in its application as agro-chemical and other insecticidal uses.

6. Recommendations

Government through the Ministry of Agriculture should conduct periodic awareness campaigns on impacts of pesticides (insecticides) on environment and their threshold concentration to ensure appropriate use. Monitoring and patrol team by the Ministry of Environment through the Environmental Protection Agency (EPA) should access levels of insecticide contamination in Nigerian inland waters to guarantee safety of aquatic life and tertiary consumers. There should be strong restriction by government and non-governmental organizations in both urban and rural areas on excessive level of application of pesticide (insecticide) on agricultural fields to avoid their drift into water ways by runoff.

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