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Acute toxicity of dichlorvos on tropical freshwater snail (*Pila ovata*)

C.N. Ariole[1*], O. Ezevununwo[2]

[1]*Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria*

[2]*School of Science Laboratory Technology, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria*

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Abstract

The acute toxicity of dichlorvos (dimethyl 2, 2-dichlorovinyl phosphate) to freshwater snail (*Pila ovata*) was studied in toxicity bioassay. The test organisms were exposed to dichlorvos in a static renewal bioassay for 96hours. There was an initial range finding test to determine the concentrations of dichlorvos to be administered on the test organisms in the definite test. Five concentrations of the dichlorvos were prepared in the definitive test as 0.5, 1.0, 1.5, 2.0 and 2.5 ppm and a control experiment (0.0 ppm). The median lethal concentration (LC_{50}) at 24hr, 48hr, 72hr and 96hr was 2.91, 1.74, 1.01 and 0.54ppm respectively. The median lethal time (LT_{50}) at dichlorvos concentrations of 0.5ppm, 1.0ppm, 1.5ppm, 2.0ppm, 2.5ppm were 65.68hrs, 57.91hrs, 50.91hrs, 41.54hrs and 35.56hrs respectively. Mortality increased with increase in dichlorvos concentration and the number of survivors in each concentration differ significantly ($p<0.05$) from others. The present findings indicate that dichlorvos has mortality effect on *Pila ovata* and may adversely affect other aquatic organisms.

* **Corresponding Author:** C.N. Ariole ✉ cnariole@yahoo.com

Introduction

Pila ovata, a freshwater snail is an essential source of protein among Nigerians especially people living in the Niger Delta. The organism is widely distributed in streams, lakes and rivers across the southern rain forests. Effluents from both natural and anthropogenic sources are discharged into water bodies in the Niger Delta either directly or indirectly through run-off, leaching or seepage especially during the rainy season. In Nigeria, many pesticides such as insecticides are available to enhance agricultural productivity and to improve public health.

Pesticide is defined by United Nations Environment Programme (UNEP, 2005) as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides are widely used in the world in the control of pests on crops and on animals for vector control. One of the adverse effects of their use is the contamination of the environment which often results from direct application of pesticides into crops, animals, soil and water. It has been estimated that only about one percent of applied pesticides land on the target and that the rest contaminates the environments (Lawson *et al.*, 2011).

Dichlorvos also known as DDVP (Dimethyl 2, 2-dichlorovinyl phosphate) (USEPA, 2007) is an organophosphate insecticide and has been applied in Nigeria as insecticide over the decades since its commercial manufacture started in 1961 (BCERF, 1999). Detailed risk characterization of dichlorvos has been well documented in CEPA (1996), its toxicological profile in ATSDR (1997) and environmental assessment in APVMA (2008). Most standard industrial products indicate safety information which is not adhered to (Musa *et al.*, 2010).

The toxicity of dichlorvos to aquatic organisms such as ciliate of protozoa (*Tetrahymena pyriformis*) (Mojzis *et al.*, 1993), freshwater green algae (*Selenastrum capricornutum*) (MOE/Japan, 2002), water flea (*Daphnia magna*) (MOE/Japan, 2002), snail (*Lymnaea accuminata*) (Tripathi and Agarwal, 1998) and fish (*Oryzias latipes*) (MOE/Japan, 2002) have been reported. Snails have been used for a purpose of bio-indicator for contamination by industrial waste dump (Pihan *et al.*, 2000). However, there is dearth of information on the toxicity of dichlorvos on the mollusc, *Pila ovata*.

Bioassays are used to determine the toxicity of chemical substances and to indicate which organisms are the most sensitive to such chemicals (Lawson *et al.*, 2011). These data are used to rank chemicals, determine their water quality criteria and set standards for effluent discharges (Finney, 1971). Therefore, the present study aimed to determine the potential toxicity of dichlorvos (Dimethyl 2, 2-dichlorovinyl phosphate) used routinely as agricultural and household insecticide in Nigeria on freshwater snail (*Pila ovata*) so as to ascertain its level of tolerance and its suitability as bio-indicator in freshwater environment.

Materials and methods

Collection and acclimatization of test organisms

The specimens of *Pila ovata* were collected from Okpuhur Creek in Odhieke Community in Ahoada West Local Government Area, of Rivers State, Nigeria. The specimens were brought to the laboratory in plastic container filled with oxygenated and cool habitat water to reduce their activity and stress before reaching the laboratory. Active and healthy organisms were selected for acclimatization. Acclimatization was for 10days at room temperature according to the static test procedure (APHA, 1998) in dilution water obtained from organism's habitat.

Bioassay

The dichlorvos (DDVP 1000EC), organophosphate group, used for this experiment was purchased from a reputable shop (UZO-Best Chemical Nigeria Limited, Port Harcourt). A range finding test was carried out as described by Rahman *et al.*, (2002) to determine the concentrations of dichlorvos used in the definitive test. The following concentrations of

dichlorvos were used for the definitive test- control (0.0ppm), 0.5ppm, 1.0ppm, 1.5ppm, 2.0ppm and 2.5ppm. Sixty (60) specimens of juvenile *Pila ovata* of fairly equal size were randomly assigned in equal number (10) into six test tanks (29cm by 29cm by 30cm) separately containing the definitive concentrations of dichlorvos. Each of these test tanks was replicated thrice to give a total of eighteen (18) experimental units (test tanks) containing 180 specimens of *Pila ovata*. The control (0.0ppm) contained only 10 individuals of *Pila ovata* without dichlorvos. During the bioassay, the test solution in each tank was renewed every 24hours. Dead snails were promptly removed and mortality was specifically recorded at 24, 48, 72 and 96 hours of exposure time as described by Odiete (1999).

Statistical analysis

Each test concentration and the corresponding percentage mortality were transformed into probit (Sprague, 1973). The median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were determined according to the method described by Finney (1971). Analysis of variance (ANOVA) was used to test for significant differences in the number of survivors in different concentrations of the toxicants (dichlorvos).

Results and discussion

Results obtained showed that generally, percentage mortality increased with increasing concentration of dichlorvos (Fig. 1) and with increase in exposure time (Fig. 2). The LC_{50} and LT_{50} were obtained from the probit graph (Finney, 1971). The 24, 48, 72 and 96hr median lethal concentration (LC_{50}) of dichlorvos to *Pila ovata* were 2.91, 1.74, 1.01 and 0.54 respectively (Table 1). The result showed that the LC_{50} value of dichlorvos to *Pila ovata* decreased as the exposure time increased (Table 1). The correlation coefficient (r^2) between concentration of the dichlorvos and probit mortality showed that there were strong and positive correlations between concentration and mortality values for 24, 48, 72 and 96hr (Fig. 1). The median lethal time (LT_{50}) of dichlorvos to *Pila ovata* at dichlorvos concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5ppm were 65.68hr, 57.91hr, 50.91hr, 41.54hr and 35.56hr respectively (Fig. 2 and Table 2). The LC_{50} of dichlorvos vary considerably when previous reports on invertebrates are compared and also with LC_{50} values obtained in this study.

Table 1. Median lethal concentration (LC50) of dichlorvos to *Pila ovate*.

Time (hr)	LC_{50} (PPM)
24	2.91
48	1.74
72	1.01
96	0.54

Table 2. Median lethal time (LT50) of dichlorvos to *Pila ovate*.

Concentration (ppm)	Time (hr)
0.5	65.68
1.0	57.91
1.5	50.91
2.0	41.54
2.5	35.56

Table 3. Survivors of *Pila ovata* exposed to different concentrations of dichlorvos.

Concentration (ppm)	Survival (%) (mean ± S.D.)
Control (0)	$100^{a} \pm 0.00$
0.5	$23.33^{b} \pm 0.77$
1.0	$20^{c} \pm 0.77$
1.5	$15.33^{d} \pm 0.77$
2.0	$9.67^{c} \pm 0.77$
2.5	$3.33^{f} \pm 0.77$

Mean values which do not have the same superscript letter are significantly different ($p<0.05$).

Many studies have been conducted to assess the hazardous effects of dichlorvos on organisms in the environment using indices such as mortality, immobilization and growth inhibition (CERI, 2007). The acute toxicity of dichlorvos to invertebrates has

been reported in freshwater crustacea, water fleas, scud, mosquito, midge, shell (snail) and oligochaete. Results showed that the 48hr EC_{50} values of immobilization in water flea were 0.000144 and 0.000266mg/L (Brooke, 1991; MOE/Japan, 2002), the 96hr LC_{50} in minaminuma shrimp was 0.00719mg/L (MOE/Japan, 2002) and 96hr LC_{50} in one of freshwater pulmonate (*Physella virgata*) was 0.17mg/L (Brooke, 1991). In addition, the LC_{50} values ranged from 0.0158 to 0.0176mg/L in insects and 0.0075 to 0.31mg/L in shell (snail), which indicates strong effects of dichlorvos on these organisms similar to those on crustacean (CERI, 2007) and the result obtained in this study.

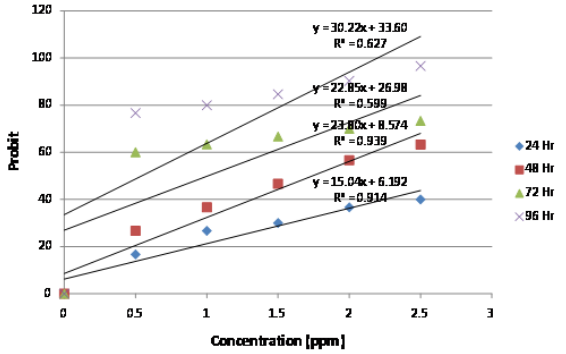

Fig. 1. Median lethal concentration (LC50) of dichlorvos to *Pila ovate*.

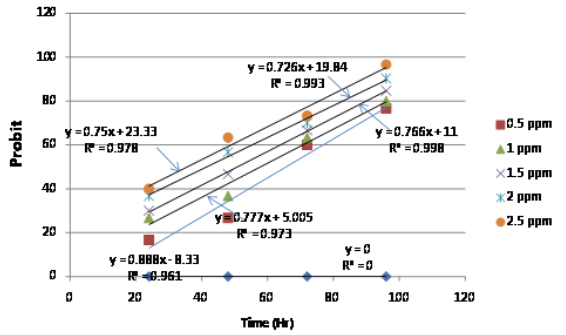

Fig. 2. Median lethal time (LT50) of dichlorvos to *Pila ovate*.

The differential toxicity of dichlorvos to freshwater snail and the other invertebrates can be attributed to the differences in susceptibility and tolerance related to its accumulation, biotransformation and excretion (CERI, 2007). At this stage we did not investigate the mode of accumulation of the toxicant. It is possible that accumulation occurred by absorption through the gastrointestinal and respiratory tracts and skin. Differences in metabolic pathways among species may result in different patterns of biotransformation, leading to more or less toxic metabolites (Johnson and Toledo, 1993). The magnitude of toxic effects of pesticide also depends on length and weight, corporal surface/body weight ratio and breathing rate (Murty, 1986). Metabolic differences between different animal classes may also be responsible for differential toxicity of chemicals.

The number of survivors of *Pila ovata* exposed to different concentrations of dichlorvos differ significantly ($p<0.05$) from others (Table 3). The dead organisms were exposed to dilution water (no toxicant added) and observed for 1hour. None of the dead organisms revived. This is evidence that death was irreversible. The mode of toxicity was not evaluated at this stage. It is likely that the absorption of the toxicant molecules on the respiratory tract reduced the oxygen uptake rate which invariably affected metabolic activity.

The levels of this toxicant in the environment may be lower than the concentrations tested in this study. However, the results show the potential toxicity of this toxicant to the organisms which will result in reduced catch and economic loss to the local baiters. Consumption of the dead snails is an indirect route of accumulation of this toxicant by humans this poses health risk because dichlorvos is considered to have carcinogenicity to experimental animals and has been categorized as group 2B (the agent is possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC). The study showed that dichlorvos is toxic to *Pila ovata* and its use as pesticide in agriculture should be strictly regulated to prevent its adverse consequences on aquatic ecosystem and man. Currently, work is continuing in our laboratory on the potential toxicity of other pesticides on the organism and also the tolerance of the various life stages of this organism to pesticides.

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