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ACUTE AND CHRONIC EFFECTS OF DICHLORVOS CONCENTRATIONS ON *CLARIAS GARIEPINUS*

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ABSTRACT
Environmental hazards pose to fish and other aquatic lives have been associated with 2, 2-dichlorovinyl-dimethyl-phosphate (dichlorvos) toxicity. The aim is to determine the acute and chronic effects of dichlorvos on *Clarias gariepinus*. Fish samples were inoculated with 4.42, 11.05, 22.10 and 44.20 mg/L for acute studies, also, 2.21 and 3.31 mg/L dichlorvos (DDVP) for chronic studies. Concentration was estimated as a total concentration in the liver, skin, fin and gill of catfish post-fingerlings treated with different concentrations of dichlorvos. Extraction of the pesticide residue was carried out and extracts were cleaned with dichloromethane. Identification of the pesticide was performed using gas chromatography-mass spectroscopy (GC-MS) in the selected ion monitoring mode. Dichlorvos residues were detected in all cultured fresh post-fingerlings exposed to the pesticide. The median lethal concentration (LC_{50}) for dichlorvos acute toxicity study were found to be 17.378 ± 2.737 and 8.913 ± 2.235 mg/L for the periods of 3 and 4 h respectively while median lethal concentration (LC_{50}) values for dichlorvos chronic toxicity study were found to be 85.114 ± 19.577, 50.119 ± 12.279, 39.811 ± 7.456 and 30.199 ± 4.541 mg/L for the periods of 6, 12, 24, and 36 h respectively. Dichlorvos was found to induce a cumulative sub-lethal and lethal effects in fresh and smoked *Clarias gariepinus* in aquatic environment and could be useful indicators of fish and aquatic environmental toxicity.

KEYWORDS: Dichlorvos, Pesticides, *Clarias gariepinus*, GC-MS, Toxicity.

INTRODUCTION
Dichlorvos (2,2-Dichlorovinyl dimethyl phosphate - DDVP), an acetylcholinesterase inhibitor[1] (Das, 2013), is classified by the World Health Organization as a Class 1B, 'highly hazardous' chemical.[1,2] The volatile and hydrophobic chemical is short lived and biodegradable and is acceptable for use by two third of the total population in India and China and Nigeria.[3]

It is used by farmers to control household and store product pests[4] and in public health to treat ectoparasites and *Pediculosis capitis* (head lice).[5] The route of degradation and pollution of DDVP in an environment range from discharge of municipal wastes from drainage to run-off that flows into the creeks.[6,7]

In fish, the long-term effect of water contamination by organophosphate compounds can cause histopathological alterations.[8,9] Besides, this also affect the essential polyunsaturated fatty acid profile, the nutritional value, the texture and the organoleptic properties (anti-inflammatory, antithrombotic, antiarrhythmic and vasodilatory properties) of fish.[10]

The overdose symptoms of dichlorvos in human range from weakness, headache, and tightness in chest, blurred vision, salivation, sweating, nausea, vomiting, diarrhea, and abdominal cramps, eye and skin irritation, miosis (pupil constriction), eye pain, runny nose, wheezing, laryngospasm, cyanosis, anorexia, muscle fasciculation, paralysis, dizziness, ataxia, convulsions, hypotension (low blood pressure), and cardiac arrhythmias among others.[1,2,3]

Studies on organometallic compounds, which are formulated as "pesticides" revealed that Sniper® (DDVP

1000 EC) contains 2, 2-dichlorovinyl dimethyl phosphate, tungsten, phosphorus, silicon and azafrin.[11] Result of previous studies by researchers and World Health Organization (WHO), recommends less toxic pesticides for use as "preservatives" such as actellic dust (pirimiphos-methyl) and synergized pyrethrins.[11]

The aim of the research work is to establish the presence of dichlorvos in fish and investigate the acute and chronic toxicity of the pesticide in harvested and preserved fish samples, and as well, ascertain their level of tolerance and suitability as bio-indicator in fresh water ecosystems.

MATERIALS AND METHODS

Equipment, Apparatus and Reagents

Aerator pressure pump (AC-9904, China), pH meter, thermometer, dissolved oxygen meter, analytical balance (Merck, Germany), white transparent plastic fish tanks (32 x 30 litres), 20-point and 12-point air circulating plastic connectors, connecting rubber tubules, air stones (China), 15 x 15 cm fish net, 1000 litre tarpaulin fish tank (Faculty of Agriculture, University of Uyo) were used for the study. All reagents were sourced from Sigma Aldrich Chemicals (USA), Merck (Germany) and Central Research Laboratory, Faculty of Pharmacy, University of Uyo.

Collection of Cultured *Clarias gariepinus* Post-fingerlings

Clarias gariepinus post-fingerlings (shooters) were obtained from Fish Culture in Vika farms Ltd, Mbiabong Etoi and Fadama Farms, Federal Housing Estate, Abak Road, all in Uyo Metropolis, Akwa Ibom State, Nigeria. The post-fingerlings were conveyed to the Nursery Fish Pond (Hatchery) at the Department of Fisheries and Aquatic Environmental Management, Faculty of Agriculture, University of Uyo, Uyo. The post-fingerlings were treated with 0.1% potassium permanganate as described by Joshi *et al.* 2002[12] and acclimatized for 14 days in the Hatchery.[13]

Acclimatization of Cultured Post-Fingerling (Shooter) in the Hatchery

A collection of post-fingerlings (shooters) brought into the hatchery, was acclimatized for 14 days in the Hatchery in a tarpaulin tank and kept unfed on the first day (24 h) before subjected to Weight and Length Analysis. The standard pond water quality was maintained and monitored in the course of acclimatization of the post-fingerlings in the hatchery.[12,13]

Grouping of Cultured Post-Fingerlings in the Hatchery

Selection of the post-fingerlings (shooter) based on the average weight (24.5 ± 6.5 g) and average length (12.5 ± 2.5 cm) according to Lawrence and Temiota, 2010 was carried out.[14]

Feeding and Inoculation of the Post-Fingerlings with Dichlorvos

The post-fingerlings were acclimatized to laboratory condition for 24 h before fed with 40% body mass of crude protein commercial feed (multi-feed) once daily.[13] Feeding was stopped 24 hours prior to the commencement of the experiment with a view to avoid any possible change *in situ* in the toxicity of dichlorvos.[15] On the 11th day, ten (10) healthy specimens were selected and kept in each of the aquaria containing 20 litres of pond water before the commencement of acute and chronic exposure studies. The acute and chronic toxicity of DDVP to cultured African catfish (*Clarias gariepinus*) post-fingerlings were assessed in a static renewal bioassay using transparent plastic fish aquaria for 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 12 h, 24 h, 36 h and 46 h.[15,16]

Experimental Design for Acute Toxicity: Based on the results of the range-finding test according to APHA 1998 and Abolagba *et al*, 2011[11,16] four (4) concentrations (4.42, 11.05, 22.10 and 44.20 mg/L) of the dichlorvos and four (4) control (0.0%) were prepared in duplicates. Ten (10) fish samples were introduced randomly into each of the 16 aquaria (of 30 litres capacity) and the tanks were filled up to 20 litres mark with standard pond water. The post-fingerlings were left to acclimatize to nursery temperature condition before the commencement of inoculation. Fish samples in four aquaria were inoculated in duplicate with 5 mL of 4.42, 11.05, 22.10 and 44.20 mg/L of the DDVP test solution respectively. Specimens selected as control were kept intact (without inoculation with pesticides) in each of the 8 aquaria containing standard pond water. The set up was aerated and pond water (in the control) was renewed daily. Fish samples showing no respiratory movement and response to tactile stimuli were considered as dead and removed immediately for storage at -20 °C until used for GC-MS analysis. Five (5) out of 10 fish samples from each of the aquaria were sacrificed and used for GC-MS analysis. Parameters such as weight loss, mortality and survival changes of each post-fingerling were measured *in situ* at 6 hours' intervals

Experimental Design for Chronic Toxicity: Two (2) chronic dichlorvos concentrations (2.21 and 3.31 mg/L) and two (2) control (0.0%) were selected in duplicate for chronic exposure studies.[11,17] Ten (10) healthy specimens were selected and kept in each of the aquaria containing 20 litres of standard pond water. Fish samples in 2 aquaria were inoculated in duplicate with 5 mL of 2.21 and 3.31 mg/L of the DDVP test solution respectively. Specimens selected as control were kept intact (without inoculation with pesticides) in each of the 2 aquaria containing standard pond water. The set up was aerated and pond water (in the control) was renewed daily. Fish samples showing no respiratory movement and response to tactile stimuli were considered as dead and removed immediately for storage at -20 °C until used for GC-MS analysis. Five (5) out of 10 fish samples from

each of the aquaria were sacrificed and used for GC-MS analysis. Parameters such as weight loss, mortality and survival changes of each post-fingerling were measured *in situ* at 6 hours' intervals.

Extraction of Pesticides in Fresh Fish samples

Pesticides extraction was carried out in fresh and smoked fish samples using dichloromethane. Eviscerated fresh fish sample (2 g) including gills, fins, skin and liver, was weighed, ground and transferred into 100 mL beaker. Extraction was done successively in duplicate using dichloromethane (3 x 10 mL). Each extract was filtered using a filter paper packed with anhydrous sodium sulphate and stirred for 15 minutes.[3,18] The same procedures for extraction were repeated for smoked fish from all the creeks.

Purification of Sample (Clean-up)

The sample clean-up was carried using a short column (15 x 1 cm) packed with 2 g silica gel (50 - 200 mesh) and 0.65 g of anhydrous sodium sulphate with dichloromethane as mobile phase. The eluates were concentrated in nitrogen gas and sent for GC-MS analysis.[18,19]

GC-MS Analysis

The screening and determination of dichlorvos in fish samples were established by the GC-Mass Spectroscopy (GC-MS QP2010 SE - Shimadzu, Japan). Separations were carried out on a Restek fused silica capillary column with injector port temperature of 250 ºC. The injection volume was 2.0 µL. The helium carrier gas flow rate was 1.0 mL min^{-1}. The electron-impact ionization was 70 eV and electron multiplier voltage 2000 V. Similarly, dried eluate of fish samples (control) were analyzed by GC-MS.

Statistical Analysis

Data were subjected to Probit analysis calculated as per Tainter and Miller method, 1944.[15] Probit logarithmic transformations of concentration were made. Slope function (S) and Confidence interval (upper and lower) of the Regression line chi-square test were calculated. The number of fish whose expected effects falls between 16% and 84% mortality were tested and the test results were considered during statistical analysis.

Four concentrations for acute test and two concentrations for chronic test of the dichlorvos were derived and used as the experimental concentrations. The median lethal concentration of 3 h, 4 h, 6 h, 12 h, 24 h and 36 h including LC_{16}, LC_{50} and LC_{84} values of dichlorvos on *Clarias gariepinus* were estimated and used for statistical analysis. LC_{50} ± SE levels of the pesticides in fish samples were evaluated and at p-values<0.05 were considered statistically significant.[15]

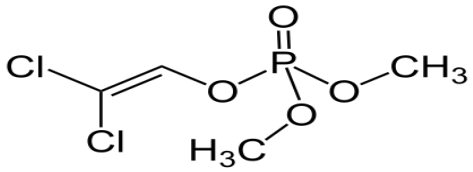

Figure 1: 2, 2-Dichlorovinyl dimethyl phosphate (DDVP).

RESULTS

Table 1: GC-MS result of fresh *Clarias gariepinus* inoculated with acute DDVP concentration.

Concentration of DDVP Solution (mg/L)		GC-MS Result	
Test	**Control**	**Test**	**Control**
4.42	0.00	+	ND
11.05	0.00	+ +	ND
22.10	0.00	+ + +	ND
44.20	0.00	+ + + +	ND

+ = Moderately available; ++ = Available; +++ = Readily available; ++++ = Most readily available. ND = Not detected.

Table 2: GC-MS result of fresh *Clarias gariepinus* inoculated with chronic concentration of DDVP.

Concentration of DDVP Solution (mg/L)		GC-MS Result	
Test	**Control**	**Test**	**Control**
2.21	0.00	+	ND
3.31	0.00	+ +	ND

+ = Moderately available; ++ = Available; +++ = Readily available; ++++ = Most readily available. ND = Not detected.

Table 3: Post-inoculation mortality variations of fresh cultured *Clarias gariepinus* exposed to acute concentration of DDVP.

Concentration of DDVP Solution (mg/L)	Mortality Changes (%)				
	1 h	**2 h**	**3 h**	**4 h**	**5 h**
4.42			10	60	100
11.05		10	60	100	
22.10		10	80	100	
44.19	10	100			

h = hour

Table 4: Post-inoculation mortality variations of fresh cultured *Clarias gariepinus* exposed to chronic concentration of DDVP.

Concentration of DDVP Solution (mg/L)	Mortality Changes (%)				
	6 h	**12 h**	**24 h**	**36 h**	**48 h**
2.21	10	40	60	90	100
3.31	30	60	80	100	

h = hour

Table 5: Acute and chronic toxicity test in tolerance of fresh *Clarias gariepinus* exposed to DDVP concentrations.

Period of Exposure (Hours)	LC_{50} (mg/L)	Standard Error of LC_{50} (SE)
DDVP Acute Toxicity Test		
3	17.378	± 2.737
4	8.913	± 2.235
DDVP Chronic Toxicity Test		
6	85.114	± 19.577
12	50.119	± 12.279
24	39.811	± 7.456
36	30.199	± 4.541

Median lethal concentrations are presented as LC_{50} ± SE.

DISCUSSION

The results of the acute and chronic exposure studies of *Clarias gariepinus* post-fingerlings treated with dichlorvos (Figure 1) pesticide concentrations revealed significant different variations ($p<0.05$) and positive correlations in mortality rate (Table 3 and 4 and Figure 2 and 3), median lethal concentration (LC_{50}) values of DDVP toxicity (Table 5), GC-MS results (Test and Control) of fresh (Table 1 and 2) *Clarias gariepinus* exposed to different lethal and sub-lethal DDVP concentrations. However, in Figure 2 and 3, there were inverse relationships (inverse post-inoculation variations) in the periods of exposure between cultured post-fingerlings exposed to acute (1, 2, 3, 4 and 5 h) and chronic (6, 12, 24, 36 and 48 h) concentrations of dichlorvos test solution. These results revealed positive correlations in acute and chronic toxicity, the accumulation of the hazardous chemical in fish and its concentration in water and other marine food chain.[2,6]

The pattern of variations according to the results of acute toxicity studies, revealed the occurrence of mortality after shorter periods of exposure of post-fingerlings to DDVP test solutions. The trend of occurrence of 1st mortality (Table 3) with regards to *Clarias gariepinus* post-fingerlings exposed to four different acute concentrations (4.42 mg/L; 11.05 mg/L; 22.10 mg/L; 44.20 mg/L) of DDVP test solution were 10% after 3 h, 10% after 2 h, 10% after 2 h and 10% after 1 h respectively. Similarly, at chronic levels of exposure of post-fingerlings to dichlorvos (2.20 mg/L; 3.31 mg/L) test solutions, their corresponding mortality indices and periods of exposure (Table 4) gave statistically significant (p-value <0.05) variations of 10% after 6 h and 30% after 6 h respectively.

The 3 h and 4 h median acute concentration (LC_{50}) of DDVP to *Clarias gariepinus* (Table 5) gave 17.378 ± 2.737 and 8.913 ± 2.235 mg/L respectively. The 6 h, 12 h, 24 h and 36 h median chronic concentration (LC_{50}) of DDVP to *Clarias gariepinus* (Table 5) were found to be 85.114 ± 19.577, 50.119 ± 12.279, 39.811 ± 7.456 and 30.199 ± 4.541 mg/L respectively. Thus, DDVP induced wide range of toxicity to fish, even at a low dose, considering its median lethal.

The minimum LC_{50} of DDVP after 6 h, 12 h, 24 h and 36 h of exposure (Table 5) were 65.114, 37.840, 32.355 and 25.658 mg/L respectively while the maximum LC_{50} of DDVP after 6 h, 12 h, 24 h and 36 h of exposure ranged from 104.691, 62.398, 47.267 to 34.740 mg/L. At 95% confidence interval, the range between minimum and maximum LC_{50} of dichlorvos after 3 h and 4 h exposure (Table 6) were found to be 14.641 - 20.1641 mg/L and 6.678 - 11.148 mg/L respectively. The 6 h, 12 h, 24 h and 36 h LC_{50} values for the same fish (*Clarias gariepinus*) were found to be much lower in another study carried out by Das, 2013.[2] Secondly, there were some similarities between the results of this study and the studies executed which recounted some health implications of pesticide residues in smoked catfish (*Clarias gariepinus*) in Nigeria, acute toxicity of organophosphate insecticide, dichlorvos in relation to selected water hardness for the freshwater zooplankters and effects of 2,2-dichlorovinyl dimethyl phosphate (DDVP) on the sodium, potassium and calcium content in the kidney and liver of *Clarias gariepinus*.[6,11,20]

The LC_{50} of DDVP is the lethal concentration of DDVP given all at once, which causes the death of 50% (one half) of cultured fresh *Clarias gariepinus* post-fingerlings.[2,5] The LC_{16} (lethal concentration of DDVP that triggers 16% mortality) and LC_{84} (lethal concentration of DDVP that triggers 84% mortality) are determining indices of maximum and minimum median lethal DDVP concentrations. The LC_{50} of DDVP is also a determining factor of the median lethal dose (LD_{50}) which measures the short-term or long-term of DDVP poisoning potential (lethal toxicity) of the post-fingerlings.[2,5]

The distributions of different acute and chronic levels of exposure of cultured *Clarias gariepinus* post-fingerlings to DDVP test solutions and their corresponding mortality rates after 1, 2, 3, 4, 5 h (acute) and 6, 12, 24, 48 h (chronic) periods of exposure are illustrated in Figure 2 and 3 respectively. DDVP, according to the results of analysis, though it is short live and non-bio-accumulative in fish, is very potent thereby reducing the weight of fish and rendering fish unsafe for human consumption. This research work clarified the reason why wide use of DDVP in fish harvesting and preservation reports toxicity not only to fish but also to aqua cultures and non-target animals.[2]

CONCLUSION

The result of analysis revealed that the median lethal concentration (LC_{50}) values of DDVP in cultured fresh and smoked *Clarias gariepinus* showed wide range of pesticides toxicity. The LC_{50} values were indicators of potential health risk to fish and human who depend on fish as food, and could as well be useful potential diagnostic tools for comparative purposes since the

effects of regular exposure to pesticides in food are hard to detect and quantify.

The compound sampled as dichlorvos was present in both aquatic and terrestrial environments. Dichlorvos induced accumulative lethal and sub lethal effects in fresh and smoked *Clarias gariepinus*.

The LC_{50} values for DDVP lethal toxicity could serve as useful indicators of food (fish) and environmental toxicity. The levels of dichlorvos in captured fresh and smoked fish were statistically significant (p-value <0.05) and that there were positive correlations in its application in local fish industries to reduce post-harvest losses, boost fish yield and preservation.

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