Original Research Article

RISK ASSESSMENT AND HEPATOTOXICITY OF ACUTE EXPOSURE TO CHLORVIEW (CHLORPYRIFOS 40% E.C.) VIA ORAL AND INHALATION ROUTES USING ANIMAL MODEL

ABSTRACT

Aims: This research aimed to determine the Hepatotoxicity via oral and inhalation routes using animal model and its Risk assessment when exposed to Chlorview pesticide (Chlorpyrifos 40% E.C.).

Study design: The animals were divided into two groups for oral and inhalation routes of exposure. They were divided into 4 groups of 5 animals each for oral and 4 groups of 4 animals each for inhalation.

Place and Duration of Study: This study was conducted in the Department of Biochemistry Laboratory University of Nigeria Nsukka.

Methodology: The hepatotoxicity and risk assessment studies were carried out using a colorimetric assay and standard methods respectively.

Results: Oral exposure to Chlorview led to a significant ($p<0.05$) increase in the activity of AST, ALT, and ALP when compared to the control. The risk assessment studies showed oral subchronic toxicity at the dose of 15.5 mg/kg b.w when exposed to Chlorview with a bioaccumulation factor (BAF) of 2.50. The oral exposure recorded a hazard quotient (HQ) of 3.4 and a risk quotient (RQ) of 0.1, a risk presumption of acute restricted use. There was no accumulation of the pesticide or its derivative via the inhalation route rather a macroscopic deposition of fat on the liver identified as cholesterol was seen.

Conclusion: This research suggests that exposure to Chlorview pesticide through oral and inhalation routes can pose a reasonable risk to human health.

Keywords: Risk Assessment, Hepatotoxicity, Chlorpyrifos, Organophosphate, Pesticide

INTRODUCTION

Farmers now use pesticides in their agricultural practices to reduce the loss of crops [1]. Pesticides are also heavily used in the household for the control of pests such as insects, arthropods and rodents. Potential adverse health impacts of organophosphate (OP) pesticide exposures are a serious public health concern for agricultural workers in low-middle income countries [2]. Chlorpyrifos (O, O-diethyl O-[3,5,6-trichloro-2-pyridinyl]-phosphorothioate, CPF) is a widely used organophosphorus insecticide utilized all over the world both for agricultural and nonagricultural purposes [3]. Lukaszewicz-Hussain [22] put forward the notification that chlorpyrifos disturbed the antioxidant status and its marker enzymes In the same line, a study conducted by Abel-Ghany*et al*., [23], it scrutinized the footprint of oral administration of an OP (fenitrothion) on rat organ. His results supported the hazards in the use of pesticides. Raina *et al*., [24] studied the impact of chlorpyrifos on wistar rats and as a consequence he's findings suggested hepatotoxicity and a restoration with ascorbic acid.

The risk of exposures to chlorpyrifos can occur in occupational and residential areas, during handling, mixing, loading, and application activities [4]. Risk assessment refers to the method deployed in the estimation of the possibility of increased human health problems as a result of exposure to a toxic pollutant [5]. Human risk assessments help to determine which potential hazards are the most significant. And these orders of significance help in proffering solutions to aid in curtailing hazards. These hazards are implicated as a probable causative agent or exacerbating agent in most complex diseases [6)] The four steps involved in Human risk assessment include hazard identification, exposure assessment, dose-response assessment and risk characterization [5; 7]. This work tried to evaluate the risk assessment and impact of chlorview on some liver marker enzymes exposed via oral and inhalation routes.

METHODOLOGY

The pesticide Chlorview® (Chlorpyrifos 40% E.C.) was purchased from commercial agrochemical vendors at Ogige market in Nsukka, Enugu State, Nigeria. Wistar rats were purchased from the animal house of the University of Nigeria Nsukka and fed with top finisher feed throughout the period of the experiment. The experiment was carried out in the Department of Biochemistry and other laboratories in the University of Nigeria Nsukka, as well as the Central Research Laboratory and Diagnostic Laboratory, Ilorin, Kwara State, Nigeria.

Ethical clearance for animal use and care

Ethical clearance was obtained from the committee constituted by the faculty of biological sciences, University of Nigeria Nsukkafor the purpose of animal use and care for an intending experimental research

EXPERIMENTAL DESIGN

A total of 64 male Wistar rats were used for the experiment. The animals were divided into two groups (32 in each) for oral and inhalation routes of exposure. The experimental groups were composed of 20 rats divided into 4 groups of 5 animals each for both the oral and inhalation route. The four experimental groups consist of 3 treatment groups and a control group. The treatment doses were obtained by dividing the value of the median lethal dose (LD_{50}) that will be established from the acute studies by a factor of 40, 25 and 10 to get 3 treatment doses representing groups 1, 2 and 3 respectively for both forms of exposure. The treatment doses were as follows:

Oral
Group 1 - 3.8mg/kg b.w
Group 2 - 6.2mg/kg b.w
Group 3 - 15.5mg/kg b.w
Group 4 - Control

Inhalation
Group 1 - 35.36 mg/kg b.w
Group 2 - 56.57 mg/kg b.w
Group 3 - 141.421 mg/kg b.w
Group 4 - Control

2.2 ACUTE TOXICITY STUDIES

Acute toxicity studies were carried out using a modified Lorke's [8] method. Oral acute toxicity study was done using 16 wistar rats of weight range 140g to 200g divided into 4 groups of 4 animals each. Adjusted doses of 50, 120, 200 and 270 mg/kg b.w. of the pesticide were administered orally via olive oil to each group representing groups 1, 2, 3 and 4 respectively. Inhalation acute toxicity was carried out using

16wistar rats of weight range 133g to 223g divided into 4 groups of 4 animals each, adjusted doses of 1000, 2000, 3000 and 6000 mg/kg b.w. of the pesticide was administered to each group representing groups 1, 2, 3 and 4 respectively. This reflects the range of LD_{50} values already reported in various literatures.

Experimental animals were observed for signs of sub-acute and acute toxicity for and the median lethal dose (LD_{50}) determined after 24 hours. Blood samples (in EDTA coated tubes) of the experimental animals were evaluated for hematological and biochemical (dry tubes) parameters. The animals were decapitated and their liver harvested for further studies.

2.3 Determination of median lethal dose (LD_{50})

The median lethal dose was determined using the following formula[9]

$LD_{50} = \sqrt{(D_0 \times D_{100})}$

D_0 = Highest dose that gave no mortality

D_{100} = lowest dose that gave mortality

Converting Emulsifiable Concentration (E.C.) to mg/ml

$(g/L) \div 10 = E.C.$

$(g/L) = E.C.x\,10$

For 40% E.C.

$(g/L) = 40\,x\,10 = 400g/L$

- To convert g/L to mg/m

  1g = 1000mg

  1L = 1000ml

  $400g/L = (400 \times 1000)/1000 = 400mg/ml$

There is 400mg of chlorpyrifos in 1ml of the pesticide.

2.4 Assays for Aspartate and Alanine Aminotransferases Activities

Aspartate Aminotransferase Activity using Reitman and Frankel [10] method. Pipette 0.5ml of R1 (buffer) to the blank test tube. Mix 0.1ml of sample (serum) and 0.5ml of R1 to the sample test tube. Incubate for exactly 30mins at 37°C. Add 0.5ml of R2 (2.4-dinitrophenylhydrazine) and 0.1ml of sample (serum) to the blank, add 0.5ml of R2 (2.4-dinitrophenylhydrazine) to the sample test tube. Mix and allowed to stand for exactly 20mins at 20 - 25°C. Add 5.0ml of sodium hydroxide to both test tubes. Absorbance of the sample mixture (A_{sample}) was read against the blank after 5mins. This was done thrice to obtain a triplicate value.

2.5 Assay for Alkaline phosphatase Activity

Colorimetric method of Klein *et al*., [11] was used. Pipette 0.01ml of sample (serum) and 0.5ml of reagent into a test tube. Mix and aspirate into the RX Monza.Pipette 0.05ml of sample and 3.0ml of reagent into macro cuvette, 0.02ml of sample and 1.0ml of reagent into semi-micro cuvette and 0.01ml of sample and 0.50ml of reagent into micro cuvette. This was done thrice to obtain a triplicate value.

2.6 Identification of volatile compounds

Gas chromatography (Agilent USA) hyphenated to a mass spectrometer with triple axis detector equipped with an auto injector (10µl syringe) was used and the carrier gas was Helium gas.Chromatographic separation was carried out on a capillary column (specification: length; 30m, internal diameter; 0.2µm, thickness; 250µm), treated with phenyl methyl silox. Other conditions for GC-MS include ion source temperature (EI), 250°C, interface temperature; 300°C, pressure; 16.2 psia, out time; 1.8min, 1µl injector

in split mode with split ratio 50:1 with injector temperature of 300°C. The column temperature started at 35°C for 5mins and changed to 150°C at the rate of 4°C/min. the temperature was raised to 250°C at the rate of 20°C/min and held for 5mins. The total elution was 47.5mins; system control and data acquisition was achieved using MS solution software. NIST library (NISTII) was used to carry out compound identification by comparing the mass spectra obtained with those of the standard mass spectra in NIST library.

2.7 Determination of bioaccumulation factor (BAF)

The transfer coefficient was calculated by dividing the concentration of chlorview in animal tissue by the total chlorpyrifos concentration applied. [12].

$$BAF = \frac{C\,liver}{C\,admin}$$

Where; BAF represent the transfer factor of liver
C_{LIVER} = pesticide concentration in liver tissue,
C_{ADMIN} = pesticide concentration in soil,
BAF > 1 indicates that the liver accumulated pesticide residues (Bio-accumulation)
BAF < 1 means that the liver excluded the pesticide residues from the body.

2.8 Determination of Average daily dose (ADD)

The Daily intake of chlorview was calculated using the following formula used by Olowoyo and Lion [13].
$ADD = DI \times MF_{LIVER}/WB$
Where; ADD = represents the average daily dose (mg/kg/d) of the pesticide.
DI = is the daily intake of chlorpyrifos.
MF_{LIVER} = is the trace pesticide concentration in the tissues (mg/kg)
WB = represent the body weight of investigated animal

2.9 Determination of Hazard quotient (HQ)

The Hazard Quotient (HQ) was used to calculate the possible human health risks associated with the exposure of chlorview to farmers, applicators and household. The following equation for calculating human health risk of exposure of chlorview used to calculate the Hazard Quotient of animal tissue [14].

HQ is the ratio between exposure and the reference oral dose (RFD)
If the ratio is lower than one 1, there will be no obvious risk.
HQ = ADD/RFD
Where; ADD = represents the average daily dose (mg,kg/d) of the chlorview.
RFDc = is the reference dose (mg,kg/d) [15]

2.10 Statistical Analysis

IBM SPSS software version 23 was used to carry out the statistical analysis. A one way analysis of variance (ANOVA) was carried out at p = 0.05, and Duncan's multiple range test was used to show the source of the observed differences.

3 RESULTS AND DISCUSSION

3.1 Acute Toxicity

The toxicity signs looked out for in these animals were there activity, feeding and drinking patterns and eventually death

3.1.1 Oral Acute Toxicity

Median lethal dose (LD_{50}) for oral toxicity of chlorview was determined within 24hours. Table 1 result shows that group 1 to group 4 with dose of 50, 120, 200, and 270 mg/kg respectively were observe and there were mortality rate at group 3 (1 death) and 4 (2 death) respectively. No toxicity signs were observed in group 1 treated with 50 mg/kg dose however, group 2 was not fully active as usual which indicates a sign of toxicity. The acute toxicity test shows that the higher the concentration of Chlorview the more the toxicity as shown in table 1.

Table 1: 24 hour oral acute toxicity study

Groups	Dose in mg/kg body weight	Observation	Mortality
Group 1	50	No sign of toxicity	0/4
Group 2	120	Signs of toxicity	0/4
Group 3	200	Signs of toxicity	1/4
Group 4	270	Signs of toxicity	2/4

3.1.2 Inhalation Acute Toxicity

A 24 hour inhalation toxicity study was carried out in order to establish a median lethal concentration for Chorview®. Table 2 result shows that group 1 to group 4 with dose of 1000, 2000, 3000, and 6000 mg/kg respectively were observed and there were mortality rate at group 2, 3 and 4 with 1 , 3 and 3 deaths respectively. No toxicity sign was observed in group 1 with 1000 mg/kg dose. The acute toxicity test shows that the higher the concentration of Chlorview the more the toxicity shown in table 2.

Table 2: 24 hour inhalation acute toxicity study

Groups	Dose in mg/kg body weight	Observation	Mortality
Group 1	1000	No sign of toxicity	0/3
Group 2	2000	Signs of toxicity	1/3
Group 3	3000	Signs of toxicity	3/3
Group 4	6000	Signs of toxicity	3/3

The results from acute toxicity studies revealed that oral exposure to Chlorview® (Chlorpyrifos 40% EC) gave a median lethal dose (LD_{50}) of 155 mg/kg b.w. while inhalation exposure gave a median lethal concentration (LC_{50}) of 1414 mg/kg b.w. for 60 minutes. This result agrees with the findings of Clegg and Van Gemert (2010) who reported an oral LD_{50} of 118-245 mg/kg b.w. The result varies with the report of Smegal[4], who reported an oral LD_{50} of 223 mg/kg and an inhalation LC_{50} of 200mg/m^3. This might be due to the difference in the formulation of the pesticide used for the experiment. Some components of the formulation may have aggravated the toxicity of the pesticide as opposed to the used of pure chlorpyrifos.

3.2 Hepatotoxicity

3.2.1 Oral Hepatotoxicity

Table 3 shows the effects of oral exposure to Chorview® (Chlorpyrifos 40% E. C.) on the Liver marker enzymes of Wistar rats. The results from hepatotoxicity studies showed that oral exposure to Chlorview® led to a significant ($p=0.05$) increase in the activity of Aspartate amino transferase (AST) in group 3 (46.00±4.00) when compared to group 4 (25.67±3.21 U/L). A significant ($p=0.05$) increase in Alanine amino transferase (ALT) activity was recorded in group 3 (11.67±2.08) when compared to group 4 (4.33±0.58 U/L). Alkaline phosphatase (ALP) activity in group 3 (32.92±1.89 U/L) significantly ($p<0.05$) increased when compared to group 4 (29.53±0.42).However, the activities of Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) in group 1(3.8mg/kg b.w) and group 2 (6.2mg/kg b.w) increased non-significantly ($p=0.05$) when compared to the group 4 (Control). This implies that oral exposure to Chlorview® caused a significant liver damage on Wistar rats at 15.5mg/kg b.w. This result is *in tandem* with the report of Uzun and Kalender [16], who report a statistically significant increase in ALP, ALT and AST activities (50%, 41%, 38% respectively) on exposure to chlorpyrifos.

Table 3: The effect of oral exposure to Chlorview® on Liver marker enzymes

GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L)
Group 4 (Control)	25.67±3.21[a]	4.33±0.58[a]	29.53±0.42[a]
Group 1 (3.8mg/kg b.w)	27.00±1.73[a]	5.00±1.00[a,b]	30.63±1.12[a,b]
Group 2 (6.2mg/kg b.w)	29.33±3.79[a]	7.33±1.53[b]	31.86±2.07[a,b]
Group 3 (15.5mg/kg b.w)	46.00±4.00[b]	11.67±2.08[c]	32.92±1.89[b]

Means with the same superscript across the groups are non-significantly (p=0.05) different. n = 3

3.2.2 Inhalation Hepatotoxicity

Table 4 shows the effects of Inhalation exposure to Chorview® on the Liver marker enzymes of Wistar rats. Hepatotoxicity studies on Wistar rats exposed to Chlorview® via inhalation follow the same trend as those exposed via oral route. There was a significant (p=0.05) increase in the activity of Aspartate amino transferase (AST) in group 3 (94.47±6.87 U/L) when compared to group 4 (49.81±2.99 U/L). A significant (p=0.05) increase in Alanine amino transferase (ALT) activity was recorded in group 3 (64.50±0.71) when compared to group 4 (21.04±1.96 U/L). Alkaline phosphatase (ALP) activity in group 3 (269.56±57.25 U/L) significantly (p=0.05) increased when compared to group 4 (170.27±9.00 U/L). This may be due to death of the hepatocytes and subsequent release of these marker enzymes caused by administration of higher doses. However, the activities of Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) in group 1(35.36 mg/kg b.w) increased non-significantly (p=0.05) when compared to the group 4 (Control). There was also a non-significant (p=0.05) difference between ALT and ALP activities of group 2 and group 3. Across treatment groups in both oral and inhalation studies, there is a significant increase in the activities of liver marker enzymes (AST, ALT and ALP) of group 1 (least dose) when compared to group 3 (highest dose). This implies that the toxicity of chlorpyrifos is dose dependent and increases with increase in dose. This finding corresponds with the report of Karami-Mohajeri*et al*. [17], who reported a dose-dependent liver damage in rats exposed to sub-lethal doses of organophosphate pesticide.

Table 4: The effect of inhalation exposure to Chlorview®on Liver marker enzymes

GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L)
Group 4 (Control)	49.81±2.99[a]	21.04±1.96[a]	170.27±9.00[a]
Group 1 (35.36 mg/kg b.w)	53.12±2.09[a,b]	41.33±8.73[b]	238.74±26.67[a,b]
Group 2 (56.57 mg/kg b.w)	69.62±9.79[b]	60.80±5.73[c]	235.47±3.84[a,b]
Group 3 (141.421 mg/kg b.w)	94.47±6.87[c]	64.50±0.71[c]	269.56±57.25[b]

Means with the same superscript across the groups are non-significantly (p>0.05) different. n = 2

3.3 Risk studies

3.3.1 Oral bioaccumulation studies

Table 5 shows the bioaccumulation of the total biotransformed derivatives of chlorpyrifos (3,5,6-trichloro-2-pyridinol (TCP) and diethylphosphate) in the liver of wistar rats exposed to the pesticide via oral route. In oral route of exposure, GS-MS analysis of the liver homogenate detected TCP and diethylphosphate in group 2 (79.43 g/l) and only TCP group 3 (998.21 g/l) whereas group 1 and group 4 (control) showed total absence of chlorpyrifos derivatives. This is supported by the report of Clegg and VanGemert [18], who stated that TCP is the major metabolite of chlorpyrifos following oral dosing.

Bioaccumulation factor (BAF) value of group 2 (0.19) and group 3 (2.50) shows that the pesticide accumulated more in group 3 treated with 15.5mg/kg b.w of chlorpyrifos and in group 2 treated with 6.2 mg/kg b.w of chlorpyrifos. This may be due to the sequestration of the pesticide derivatives in the Liver. This result is supported by the report of Tanvir*et al*.[19] who reported accumulation of chlorpyrifos in the liver. In contrast, Tanvir*et al*. [20] reported that nearly 90% of the administered dose was absorbed and deposition of chlorpyrifos was detected only in adipose tissue. Almost 80% CPF was absorbed with dose dependent effects seen in rat's plasma and brain [21]. Chlorpyrifosbioaccumulates over time and exerts toxic effects on animals [20]. Bioaccmulation factor greater than one (BAF>1) is estimated to be significant while bioaccmulation factor less than one (BAF<1) is estimated to be non-significant. Hence,

groups 3 (BAF = 2.50) showed a significant bioaccumulation, this implies a high risk of subchronic toxicity at the dose of 15.5mg/kg b.w of chlorpyrifos.

Table 5: Bioaccumulation of derivatives chlorpyrifos in wistar rat exposed to Chorview® (Chlorpyrifos 40% E. C.) via oral route.

Groups	Dose administered (mg/kg b.w.)	Concentration administered (mg/l)	Concentration in Liver (mg/l)	Bioaccumulation factor (BAF)
Group 1	35.36	400	ND	ND
Group 2	56.57	400	ND	ND
Group 3	141.421	400	ND	ND
Group 4	-	-	ND	ND

ND – Not detected

3.3.2 Inhalation bioaccumulation studies.

Table 6 shows the bioaccumulation of the biotransformed derivative of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCP) in the Liver of wistar rats exposed to the pesticide via inhalation route. TCP was not detected in group 1, group 2, group 3 and group 4 (control). Bioaccumulation factor greater than one (BAF>1) is estimated to be significant. However, Bioaccmulation factor less than one (BAF<1) is estimated to be non-significant.

Accumulation of chlorpyrifos residues and derivatives were not detected in groups exposed via inhalation route, however there was macroscopic (high) deposition of fats in the liver of the wistar rats with majorly was identified as cholesterol.

Table 6: Bioaccumulation of derivatives chlorpyrifos in wistar rat exposed to Chorview® (Chlorpyrifos 40% E. C.) via Inhalation.

Groups	Dose administered (mg/kg b.w.)	Concentration administered (g/l)	Concentration in Liver (g/l)	Bioaccumulation factor (BAF)
Group 1	3.8	400	ND	-
Group 2	6.2	400	79.43	0.19
Group 3	15.5	400	998.21	2.50
Group 4	-	-	ND	-

ND – Not detected

3.8.3 Oral average Daily Dose (ADD) and Hazard Quotient (HQ)

Table 7 shows the average daily dose and hazard quotient of wistar rats exposed to Chorview® via oral route. ADD and HQ were calculated for group 2 and group 3, who recorded accumulation of the pesticide in the liver of the wistar rats.

The calculated Average daily dose (ADD) for groups exposed via oral route was given as group 2 (0.0012 mg/kg b.w.) and group 3 (0.017mg/kg b.w.), while the Hazard quotient (HQ) was given as group 2 (0.24) and group 3 (3.4).

Table 7: Oral average Daily Dose and Hazard Quotient

Groups	Dose administered	Concentration in Liver	Average daily dose (ADD)	Hazard quotient (HQ)

	(mg/kg b.w.)	(g/l)	(mg/kg b.w.)	
Group 1	3.8	ND	-	-
Group 2	6.2	79.43	0.0012	0.24
Group 3	15.5	998.21	0.017	3.4
Group 4	-	ND	-	-

ND – Not detected

3.8.4 Inhalation Average Daily Dose (ADD) and Hazard Quotient (HQ)

Table 8 shows the average daily dose and hazard quotient of wistar rats exposed to Chorview® (Chlorpyrifos 40% E. C.) via inhalation route. ADD and HQ were not calculated for group 1, group 2 and group 3, who recorded no accumulation of the pesticide in the liver of the wistar rats.

Table 8: Inhalation Average Daily Dose and Hazard Quotient

Groups	Dose administered (mg/kg b.w.)	Concentration in Liver (g/l)	Average daily dose (ADD)	Hazard Quotient (HQ)
Group 1	35.36	ND	-	-
Group 2	56.57	ND	-	-
Group 3	141.421	ND	-	-
Group 4	-	ND	-	-

ND – Not detected

4.1 CONCLUSION

This research suggests that exposure to Chlorview® (Chlorpyrifos 40% EC) poses a reasonable risk to human health both through oral and inhalation routes of exposure affecting many biochemical processes. Chlorpyrifos accumulates in the liver at high oral doses of exposure (≥ 6.2 mg/kg b.w). The observations suggest that oral exposure affects risk assessment indices significantly whereas inhalation exposure affects biochemical indices significantly. The mechanism of toxicity of chlorpyrifos at low doses involves pathways other than the classical acetylcholinesterase inhibition. The liver, the testis and the brain represent a highly susceptible organ for the subchronic toxicity of chlorpyrifos at low dose.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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