Toxicological Effects of Chronic Inhalation Exposure of 2-2 dichlorovinyl dimethyl phosphate on Haematological indices of New Zealand white Rabbits.

Abstracts

Aim: To assess the Toxicological Effects of Chronic Inhalation Exposure of 2-2 dichlorovinyl dimethyl phosphate on Haematological indices of New Zealand white Rabbits.

Study design: This is an experimental study.

Methodology: A total of twenty four male New Zealand white rabbits, two months old weighing between 1.0 and 1.2 kg, were used for the study. They were divided into three (3) groups, each consisting of four (4) rabbits and a corresponding number of matched controls, for long-term toxicological effects of dichlorvos on the rabbits (30 days, 60 days and 90 days).The rabbits received ten (10%) of the LD50 dose . The LD50 dose was 0.5 mg/m^3, while 10% of the median lethal dose of dichlorvos which was 0.05 mg/m^3 was diluted with 1.0 milliliter of distilled water. It was administered by spraying in a closed cage containing the rabbits every day for thirty, sixty, and ninety days. At the end of each month, a set of rabbits in the experimental group with their matched control were sacrificed using chloroform. Five milliliters (5 mls) of blood was collected from each rabbit at the stipulated period for haematological investigations which included the following indices: Total White Blood Cells, Red Blood Cells Count, Hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Platelets Count, Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophil. They were analyzed using an automated haematology analyzer (Sysmex XP-300 automated CBC Hematology Analyzer) Coulter). Data generated were expressed as mean $\pm$SD. ANOVA and Tukeys multiple comparison test were used to compare the results between means of groups. Variation in mean of parameters were considered statistically significant at $P<0.05$.

Results: The results of the haematological indices at 30, 60 and 90 days exposure showed significant decreases in the following parameters: PCV (32.75±1.71%, 32.50±1.29%, 27.75±1.26%) WBC: (3.03±0.43 x 10^9 /L, 2.10±0.14 x 10^9 /L, 1.68±0.30 x 10^9 /L), PLT 48.58±2.42%, 36.28±5.03%, 24.98±29.31%) count, MCV: (70.00±0.82fl, 63.00±2.16fl, 58.50±2.65fl ,MPV: (5.59±0.36fl,63.00±2.16fl, 58.50±2.65fl) and neutrophils. Lymphocytes, Basophils, Monocytes and Eosinophils were significantly increased as the duration of exposure increased from 30 to 90 days.

Conclusion: Dichlorvos caused severe alteration in the haematological parameters of rabbits and the severity of the changes were dependent on the duration of exposure.

Keywords: Chronic toxicological effects, 2, 2-dichlorovinyl dimethyl phosphate (Sniper), haematological parameters, Rabbits.

1. INTRODUCTION

Despite being hazardous to both humans and animals, dichlorvous (sniper) is widely used as a pesticide in agriculture and homes. If the Occupational Safety and Health Administration's (OSHA) daily exposure limit of 1 mg/m^3 is exceeded, it may have detrimental consequences on one's health. Dichlorvous is classified as a very dangerous substance by the WHO [1]. An estimated 3 million cases of dichlorvos poisoning are reported globally each year, with over 250,000 fatalities [1]. There are indices that can be used in dichlorvous poisoning to determine the degree of poisoning and death. One indicator of acute dichlorvous poisoning's prognosis is blood cholinesterase activity. For medicinal and medicolegal objectives, poisoning diagnosis is crucial for both live and deceased patients. Between 2010 and 2015, the annual discharge of dichlorvos into the environment for agricultural purposes was around 11,45 tons for African nations, 4342 tons for Caribbean countries, and 10,013 tons for Central American countries. Numerous health risks have been brought about by the widespread use of organophosphate pesticides in agriculture, as well as in residences, parks, schools, hospitals, airplanes, and other public areas [2)

The integrity of the haematological parameters needs to be examined to determine the toxic effects of any exogenous compound, as these parameters are crucial in determining the toxicity of any compound. Any compound that accumulates excessively in erythrocytes is typically a sign of a pathological condition [3]. A drop in blood parameters during the assessment of haematotoxicity may be a sign that the production of red blood cells has been suppressed. The ability to predict changes in haematological indices accurately for human toxicity is very high. In a toxicity study, male Wistar rats were inhaled dichlorvos and paraquat pesticide for four weeks, resulting in a significant decrease in hemoglobin levels and a significant increase in neutrophil levels and the total white blood cell count [3].

Histological analysis of the bone marrow and spleen of B6C3F mice, which were given dichlorvos five days a week for two years at a dose of up to 40 mg/kg/day, revealed no evidence of gross organ damage. Furthermore, during 52 weeks of inhalation dosages of 4 and 8 mg/kg/day of dichlorvos, 344 male Fischer rats in the NTP study showed a marked increase in neoplastic lesions (leukaemia) in their organs [4]. Male rats given a high dose of dichlorvos showed a notable positive trend for mononuclear cell leukemia that was unrelated to dosage. Another study on the carcinogenicity of 50 Fischer 344 rats given an inhalation dose of 4 or 8 mg of dichlorvos five days a week for 103 weeks revealed a noteworthy rise in neoplasms in the treated male rats' pancreas and hematopoietic system [4]. After receiving a dichlorvos dosage through feed at a rate of 45 and 90 mg/kg/day for 80 weeks, along with a matched control group, fifty Osborne-mental rats displayed several negative clinical symptoms, including rough coats, epitasis, and haematuria. There were numerous proliferative, degenerative, and inflammatory lesions visible. The male rats had a variety of non-neoplastic lesions, including focal follicular cell hyperplasia, myocardial interstitial fibrosis, and clusters of alveolar macrophages in the lungs. Benign endocrine neoplasms were noticed and there was also a high incidence of benign mammary neoplasms in both control and treated rats [5]. The aim of this study was to assess the levels of some hematological parameters in New Zealand white rabbits exposed to 2, 2-dichlorovinyl dimethyl phosphate (Sniper)

2. MATERIALS AND METHODS

2.1 Experimental Animals

A total of thirty-six (24), two-month-old New Zealand white rabbits (*Oryctolagus cuniculus*) that weighed averagely 1.0kg were used for this study. The rabbits were purchased from Department of Biological Science, Rivers State University, Port Harcourt animal house. They were used for inhalation chronic studies. The rabbits were kept in a spacious and well-ventilated cage at room temperature, under natural circadian rhythm and were allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed (Top Feed Finisher Mash, Sapele, Nigeria) and water *ad libitum* from the animal house, department of animal and environmental science, Rivers State University, Port Harcourt. All the animals received humane treatment according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health.

2.2 Procurement and administration of Dichlorvos

1 litre of concentrated solution of dichlorvos (DDVP) insecticide 1000EC (which contains 1000mg of 2-2 dichlorovinyl dimethyl phosphate compound was purchased in Nigeria from Swiss–Nigeria chemical company which is the sole marketing company for dichlorvos in Nigeria). For the chronic inhalation study, 10% of the LD50 dose which is 0.05mg/kg dose of dichlorvos, mixed with 1.0ml of distilled water was administered to the rabbits daily for the stipulated period of 0-30, 0-60 and 0-90 days. The matched control rabbits received only feed and water *ad libitum* during the study. The experimental dose of dichlorvos was mixed with 1.0ml of distilled water, sprayed in the closed cages. The rabbits were transferred into the closed cages that were sprayed with dichlorvos to spend 4 hours daily before returning them back to their normal cages.

2.3 Experimental Design

The rabbits were divided into three (3) groups of four (4) rabbits each with four (4) matched controls. A total of 6 cages were used for this experiment as shown below:

Duration	Chronic inhalation study	Matched control
0-30 days	4	4
0-60 days	4	4
0-90 days	4	4

2.4 Sample Collection, Storage and Analysis

2.4.1 Sample collection

At day 30, 60 and 90 4 rabbits were sacrificed each from the study group and from the matched control group. Blood specimens were collected at each stage, about 5.0mls of blood was collected into EDTA for haematological parameters.

2.4.2 Laboratory Investigation of Parameters

2.4.2.1 Determination of Haematological Indices

Haematological parameters such as packed cell volume, haemoglobin, platelets count, lymphocytes, neutrophils, basophils, eosinophils, monocytes, total white blood cell count as well as red cell indices were analysed with Sysmex XP-300 automated CBC Hematology Analyzer Coulter.

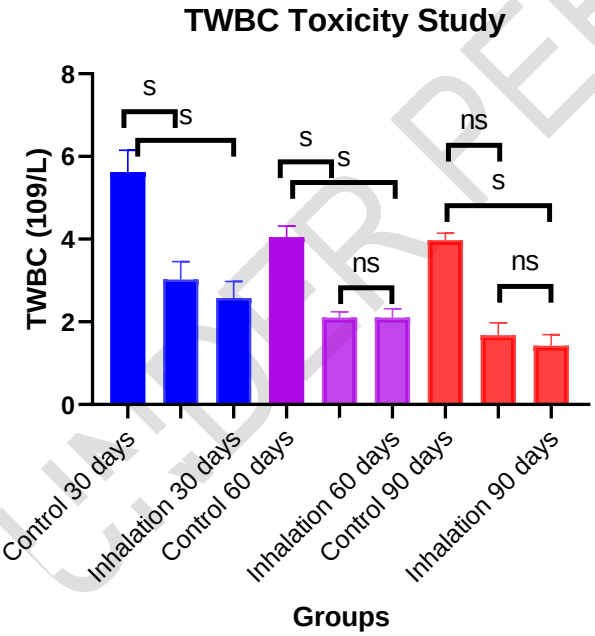
2.5 Statistical Analysis

SPSS version 22.0 of windows statistical package was used to analyze the data generated. The mean ± standard deviation was determined. One-way analysis of variance (ANOVA) with Tukey's Post Hoc test, bar charts were also done using the same statistical package. From the values obtained statistical decision and inferential evaluation were made. A probability (p) value of less than *.05* was considered statistically significant.

3. RESULTS AND DISCUSSION

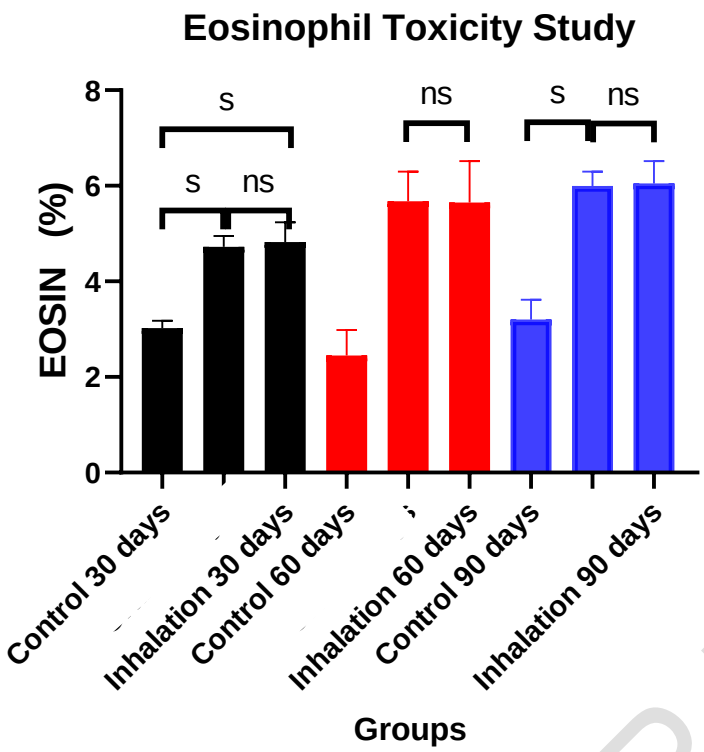
Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on Haematological Parameters (Mean ±SD)

Figure 1: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on TWBC



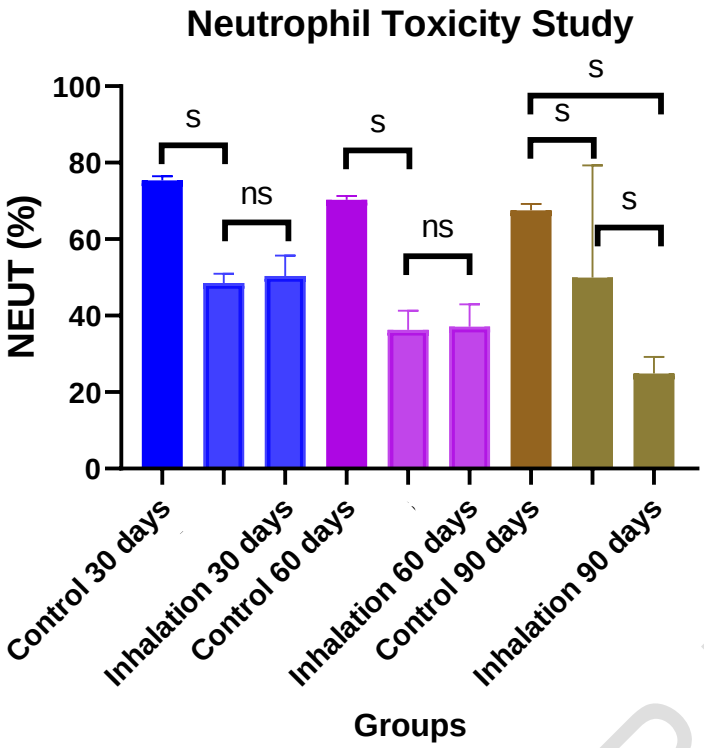
Means ±SD of experimental groups with different superscripts are significantly different from each other at p<0.05.

Figure 2: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on Eosinophil



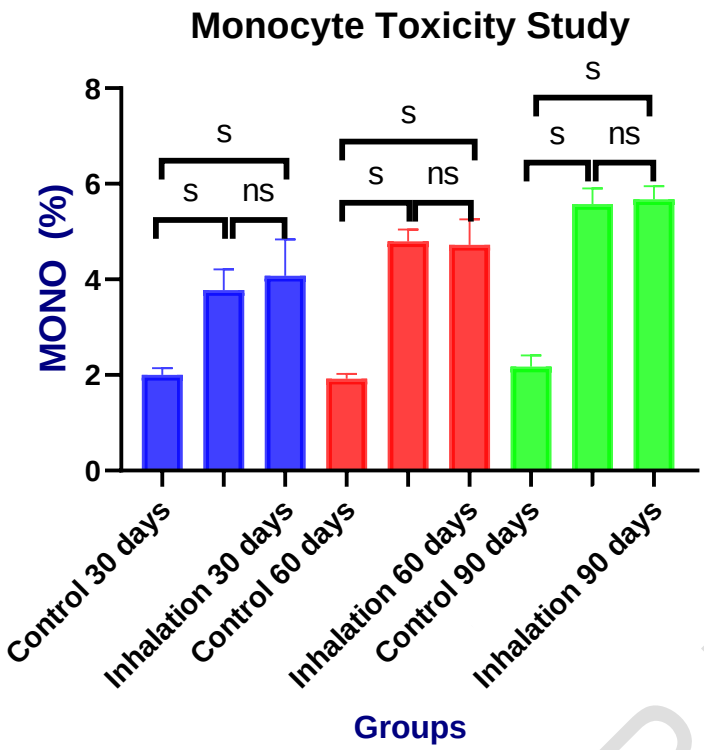
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 3: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on Neutrophil



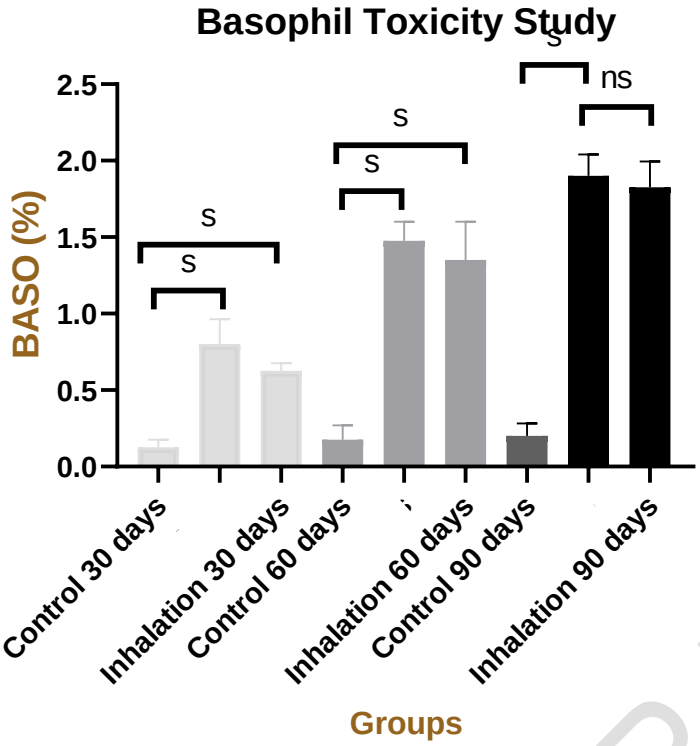
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 4: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on Monocyte



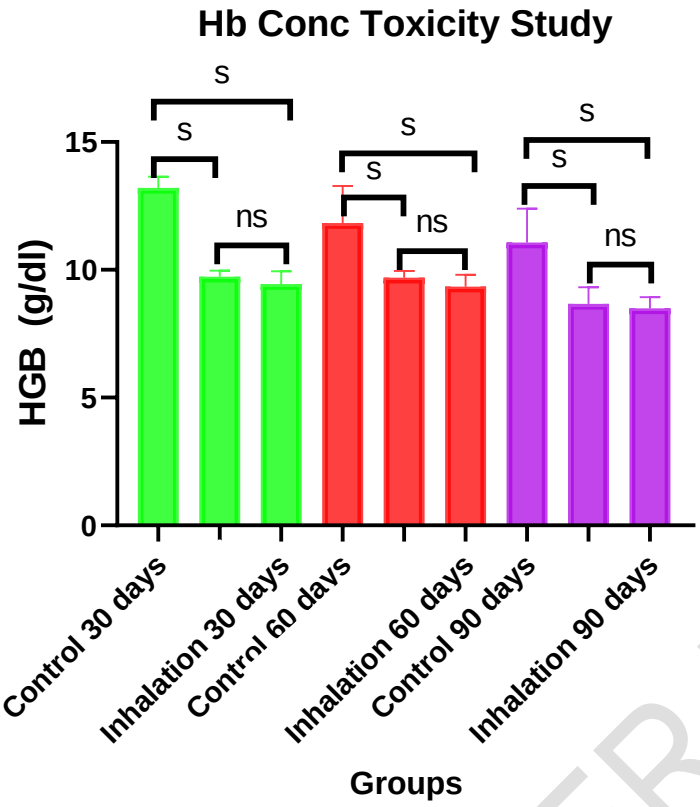
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 5: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on Basophil



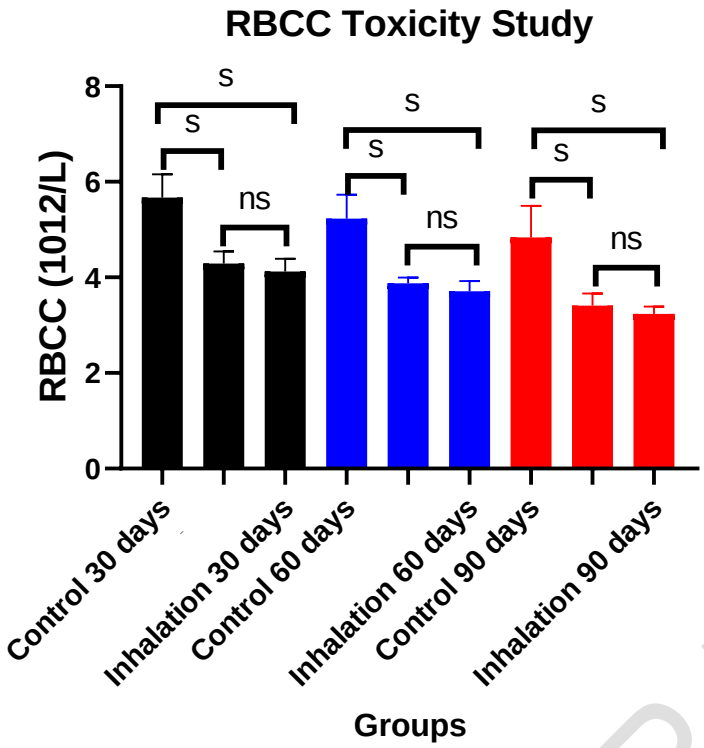
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 6: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on Hb



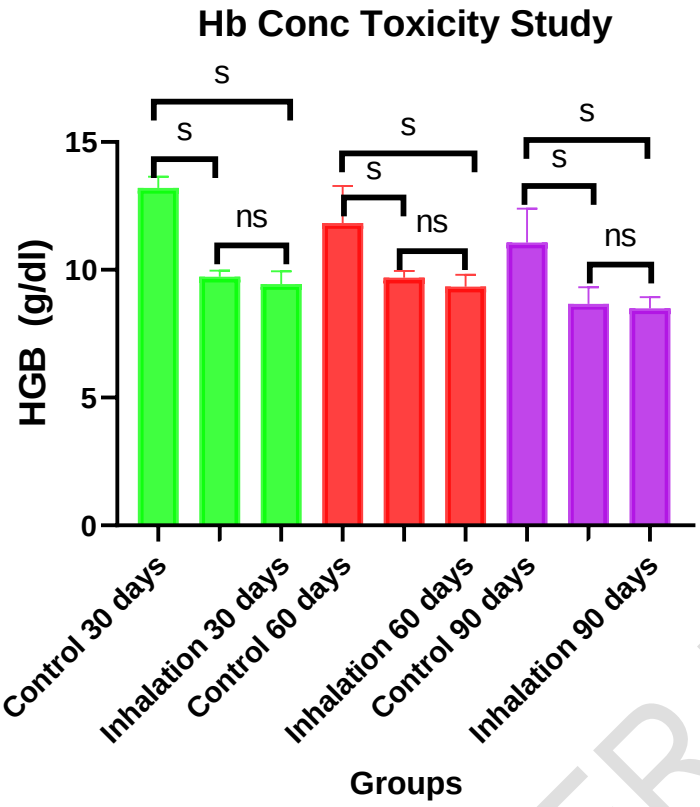
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 7: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on RBCC



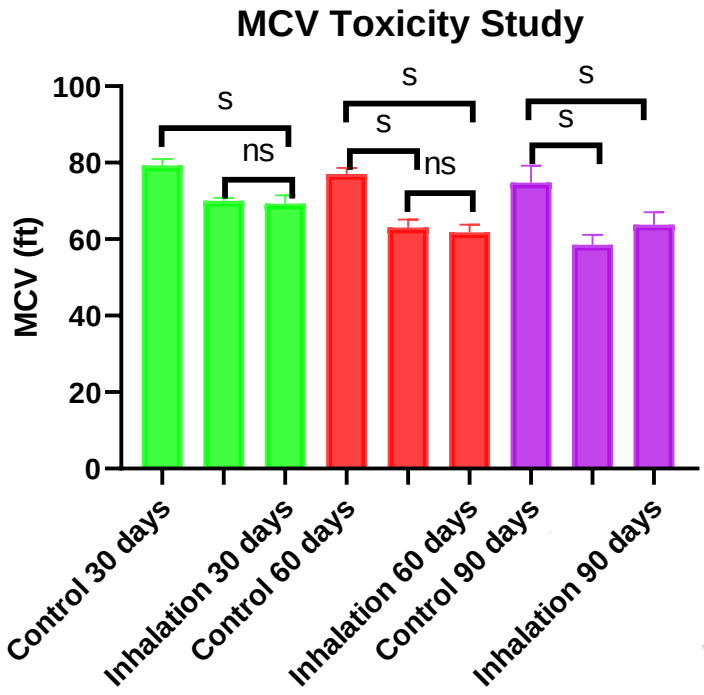
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 8: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on Hb Conc



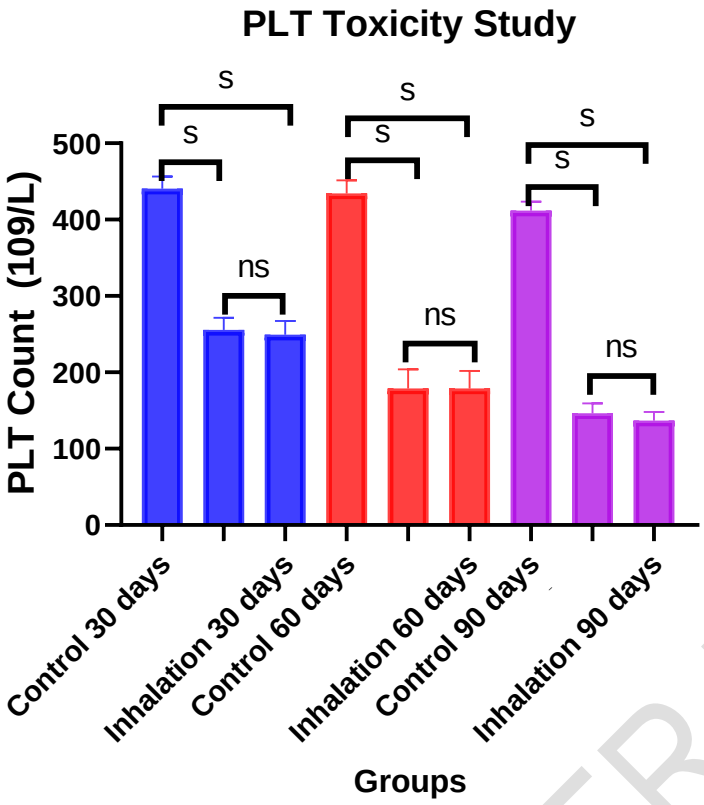
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 9: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on MCV



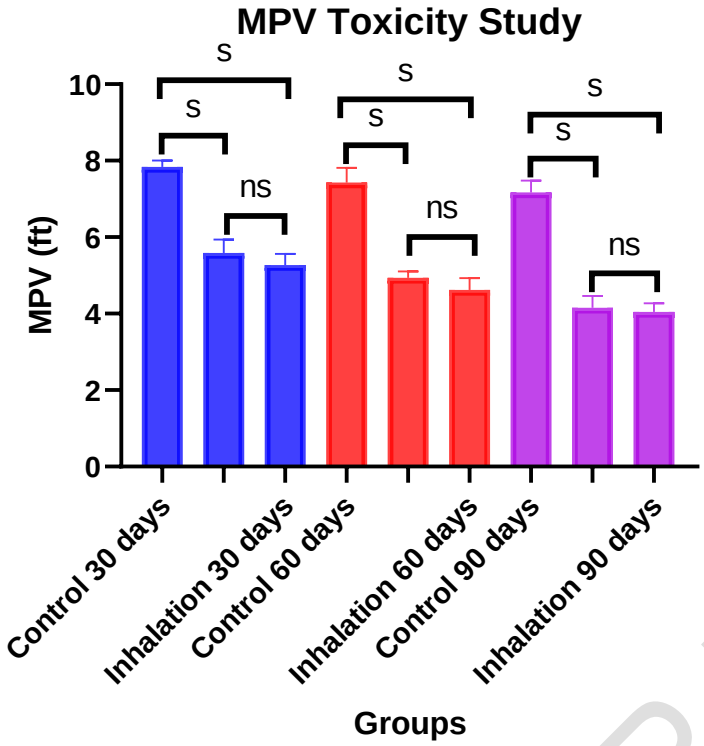
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 10: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on PLT



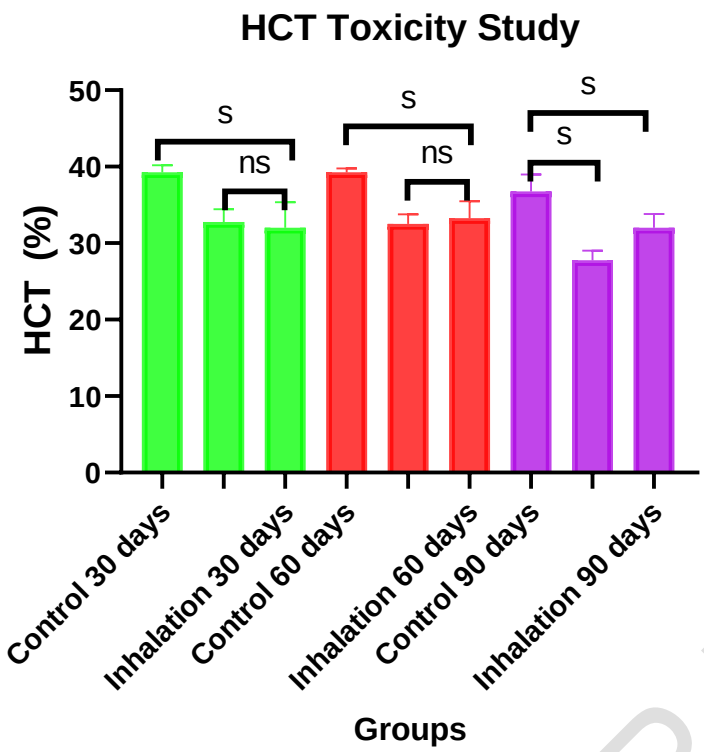
Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 11: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on MPV



Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

Figure 12: Effect of Duration of Intoxication of Rabbits with Dichlorvos by Inhalation Administration on HCT



Means ±SD of experimental groups with different superscripts are significantly different from each other at $p<0.05$.

The result of this study revealed that the administration of dichlorvos on the rabbits caused a significant reduction in the mean level of the experimental rabbits' haemoglobin at $P < 0.05$ when compared with the matched control level. Dichlorvos can affect the haematopoietic system through restraining synthesis of haemoglobin by hindering various key enzymes involved in the haeme synthesis pathway, such as the enzyme aminolevulinic acid dehydratase (ALAD). Dichlorvos has also been known to cause suppression and damage, which contributes to the reduction of the lifespan of the erythrocytes. Dichlorvos increases the fragility of cell membranes. The effect of these processes results in anaemia [6]. Significant reduction was observed in the mean values of RBC'S, PCV, Hb, T.WBC, platelets, neutrophils, MCV and MPV of the rabbits that were exposed to dichlorvos by inhalation route at $P < 0.05$ as the duration of dichlorvos treatment progressed from 30-90 days. Again, significant increases were observed in the mean values of eosinophils, basophils, lymphocytes and monocytes in a duration dependent manner from 30-90 days.

Haematological indices are of diagnostic significance in routine clinical evaluation for state of health. The reduction in the haematocrit values was an indication of an anaemic condition. The mean values of the haematological indices between the dichlorvos treated rabbit and the non-dichlorvos treated rabbits were significantly different ($P < 0.05$). The anaemic condition could be

because of haemolysis which was beyond the capability of the bone marrow to compensate for the loss. Haematuria and deproteinization processes initiated by the dichlorvos induced oxidative stress and lipid peroxidation. The erythrocytopenia that was recorded in this study could be associated with the suppressing effect of dichlorvos on erythropoiesis. The toxic effects of dichlorvos on the haemotopoietic cells in the bone marrow could be associated with the metabolites of dichlorvos that were released in high concentration which could have hindered the normal mechanism that regulate blood cell formation [7].

Reports of Brown et al. [8] and Adeoti et al. [9] and Kanu, et al. [10] all corroborated with the results of the present study. They observed significant decreases in the haematological indices of albino rats that were exposed intraperitoneally to sublethal doses of dichlorvos. In the study of Brown [8], it was reported that the development of hypochromic anaemia which occurred because of a fall in the iron level of the blood due to oxidative stress. Exposure to dichlorvos induces oxidative stress through the generation of reactive oxygen species (ROS). This occurs when the production of free radicals due to exposure to harmful chemical/substance is beyond the protective capabilities of the antioxidants. Antioxidant activities disruption by the pesticides results in alteration in oxidative state. When toxicants are inhaled, they are transported by blood to various organs including the kidney and liver where they may cause deleterious effects. Haematological parameters can be used as a pathological and physiological indicator of one's state of health [10].

The mean values of T. WBCs in the dichlorvos treated rabbits differed significantly from the control at day 30, 60 and 90 groups ($P<0.05$). The reduction was in an exposure duration dependent. Decrease in the total WBC with increase in the duration of dichlorvos exposure could be associated with the effects of the dichlorvos on the immune system. The effect on the immune system caused an immunosuppressive effect, which is suggestive of an impairment of the ability of the leucocytes to respond to antigenic mitogenic stimuli due to rapid proliferation.

Bone marrow depression in mice, rats, guinea pigs, rabbits, pigs and humans that were exposed to xenobiotic was observed. The main manifestation was a reduction in the number of one or more of these formed elements of blood which include platelets, RBC's, WBC's and haemoglobin [9]. Differential leucocytes count showed significant decrease ($P < 0.05$) in the levels of neutrophils, MPV, MCV and platelets as the duration of dichlorvos treatment increased; while significant increase was observed in the levels of lymphocytes, monocytes, basophils and eosinophils as the duration of dichlorvos exposure to the rabbits increased. Low platelets count with low MPV were indicators of bone marrow disorders which could slow down the production of platelets. This result revealed that dichlorvos exposure could cause the suppression of the bone marrow activity which could result in low megakaryocytes production. This corroborated with the result obtained by Singh and Srivastava [11] who revealed reduced levels of platelets, MPV, total erythrocyte sedimentation rate and mean corpuscular haemoglobin concentration in the teleost's that were exposed to dichlorvos.

Increased level of eosinophils and basophils with increase in the duration of dichlorvos treatment in the study may suggest an allergic disorder caused by the toxicant; while the significant lymphocytosis observed with increase in the duration of dichlorvos treatment on the

rabbits was suggestive of the response of the immune system to the toxicant. It could also suggest that the body was responding to an inflammatory condition caused by the toxicant assault on the system.

4. CONCLUSION

Dichlorvos caused a huge alteration in some haematological parameters of rabbits and the severity of the changes increased with the duration of exposure.

ETHICAL APPROVAL

Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

REFERENCES

1. WHO: World Health Organization. International Programme on chemical safety. WHO recommended classification of pesticide by hazards and guidelines to classification 1994-1995 UNEP/ILO/WHO. 1992.
2. Hertz – Picciott, Sass, J. B., Engel, S., Bennett, D. H., Bradman, A., Eskenazi, B., Lanphear, B. & Whyatt, R. Organophosphate exposures during pregnancy and child neurodevelopment: Recommendations for essential policy reforms. PLOS med, 2018; 15 (10): 1-8.
3. Oluwatoyin, T. A., Donatus, C. B., Matthew, O. W. & Justice. O. O. Implication of Acute, Sub-Chronic & Chronic Exposure to Different Pesticides via Inhalation on Male Wister Rats. *Bioengineering &BioScience*, 2017; 3(40): 74 - 85.
4. NTP: National Toxicology Programme. *Toxicology & Carcinogenesis Studies of Dicholorvos* (CAS No. 62-73-7) in F344/ N Rats and B6C3F1 Mice (gavage studies) NTP Technical Reports: 342. 1989.
5. Ezike, C. O. Acute Toxicity of Clarias Garie Pinus (Burchell, 1822) Exposed to 2, 2-Dichlorvos Vinyl Dimethyl Phosphate. *International Journal of Fisheries and Aquatic Studies,* 2017; 5(5): 100 -5.
6. Flora, G., Gupta, D. & Tiwari, A. Toxicity of Lead: A Review with Recent Updates. *Interdisciplinary Toxicology,* 2012; 5: 47 - 58.
7. Edem, V. F., Akinyoola, S. B. & Olaniyi, J, A. Haematological Parameters of Wistar Rats Exposed to 2-2 Dichlorovinyl Dimethyl Phosphate. *Asian Journal of Exp. Science*, 2012; 3: 838 - 41.
8. Brown, H., Kenanagha, B. & Onwuli, D. O. Haemato–Pathological Effect of Dichlorvos on Blood Picture & Liver Cells of Albino Rats. *Journal of Toxicology & Environmental Health Sciences*, 2015; 7(2): 18 – 23.
9. Adeoti, O. T., Belonwu, D. C., Wegwu, M. O. & Osuoha, J. O. Implication of Acute, Subchronic and Chronic Exposure to Different Pesticides via Inhalation on Male Rats. *Journal of Bioengineering and Bioscience*, 2017; 5(4): 74 - 85.

10. Kanu, K. C. &Ijioma, S. N. Haematological, Biochemical and Antioxidant Changes in Wister Rats Exposed to Dichlorvos based Insecticide Formulation Used in Southeast Nigeria: *Toxics,* 2016; 4(4), 28-31.
11. Singh, N. &Strivastava, A. Haematological Parameters as Bioindicators of Insecticide Exposure in Teleosts. *Ecotoxicology*, 2010; 19: 838-54.