

# Effects of 2, 2-Dichlorovinyl Dimethyl Phosphate (DDVP) on leukocyte profiles in juveniles and adult sizes of *Tilapia guineensis*

## Abstract

The acute effect of 2, 2-dichlorovinyl dimethyl phosphate (DDVP) also known as Dichlorvos an organophosphate pesticide on leukocytes profiles in juvenile and adult sizes of *Tilapia guineensis* was carried out. The fish was exposed to concentrations of 0.000 (control) 0.025, 0.050, 0.075 and 0.100 mgL<sup>-1</sup> of the chemical in triplicates. The results obtained indicated an increase in the number of leucocytes (leucocytosis), a normal reaction of the fish body, against attacks of foreign substances, such as DDVP, which can alter the normal physiological function in fish. Significant increases in the number of lymphocytes (lymphocytosis) and eosinophils (eosinophilia) combined with significant decreases in monocytes (monocytopenia) and neutrophils (neutropenia), are indicative of changes (infections) that set in after short-term exposure to this chemical. This finding was confirmed by the number of leucocytes in *T. guineensis* that increased in both sizes, to protect the body against infections that may have been caused by the pesticide.

**Keywords:** leucocytes, haematology, toxicity, lymphocytes, catfish, infections

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## Introduction

Most of the vertebrates including fish have five types of white blood cells such as lymphocytes, neutrophils, eosinophils, basophils and monocytes. The shape of each cell type appears to be conserved in living organism, with the exception of the neutrophils.<sup>1,2</sup> In aves and reptilia, the neutrophils take the place of heterophils, and carry out the same immunological functions in the cell of these organisms.<sup>3</sup> While in amphibians, this category of cell is sometimes called heterophils, however it is comparable to a neutrophil. And reptilian families have a sixth type of cell, called an azurophil, which majority of scientists classify with monocytes.<sup>4</sup> Neutrophils/heterophils and lymphocytes constitute about 80% of WBCs in mammals. They are the main phagocytic leukocytes, and multiply rapidly in the system of the fish as a reaction to disease invasion, inflammations, stress and toxicants.<sup>5,6</sup>

Lymphocytes are engaged in a range of immunological activities which include production of immunoglobulin and adaptation of immune defense strategies.<sup>7</sup> The remaining 20% of the leukocytes epitomize an integration of eosinophils which take part in the inflammation process<sup>8</sup> and are connected with defense against parasitic organisms.<sup>9</sup> Monocytes, which are long-lived phagocytic cells, they are linked with defense against invading foreign organisms and bacteria.<sup>10</sup> The functions of basophils are not clearly understood in fish, but are thought to be involved in the inflammation activities in the cell during stress.<sup>11</sup>

Leukocytes profiles are particularly are being utilized in the field of specie biological management because they are easily changed by stress and are associated with stress hormone concentrations. Conversely, Dhabhar et al.,<sup>12</sup> observed that deviation of leukocyte indices from standard parameters were regularly being used to reveal

mammalian hormonal stress responses to environmental changes induced by contaminants. In particular, the changes brought about by stressful exposure such as increases in numbers of neutrophils (neutrophilia) and decreases in lymphocyte numbers (lymphopenia or lymphocytopenia). Many authors have reported changes in leucocytes profiles in some organisms such as humans, farm animals and mammalian laboratory animals exposed to stressful conditions.<sup>13-15</sup> Changes in leucocytes profiles have been observed in a number of fish species treated with toxicants. Leucocytosis was observed in some fish species exposed to a wide range of chemicals.<sup>14-16</sup> Recently, there is a rising awareness on the consequence of agricultural based chemicals and damaging materials on the leucocytes of tilapia species on the basis of their importance as a widely culture fish for food supply in Nigeria. This study is part of our continuing contribution to the physio-ecology of the tilapiine fishes. The objective of this experiment was to evaluate the consequent of sublethal concentrations of 2, 2-Dichlorovinyl Dimethyl Phosphate (Dichlorvos) on leukocyte profiles in juveniles and adult sizes of *Tilapia guineensis*.

## Materials and method

The experimental fish were procured from recruitment ponds at African Regional Aquaculture Center, Buguma, and Port Harcourt, Nigeria. The experimental fish were later moved to the laboratory in a synthetic container and were later acclimatized for a period of 1 week. Two life stages of the experimental fish comprising of juvenile of mean weight of 65.33±2.12 g and adult size of mean weight 105.38±2.79g used in the study were randomly selected into five experimental treatment levels, comprising of 10 fish in each level. Each of the experimental treatment level was replicated three times with 10 fish per replicate. The fish in experimental treatment levels

1 and 2 were subjected to 0.025 and 0.005mg/l DDVP, respectively. While the fish in experimental treatment levels 3 and 4 were treated with 0.075 and 0.100mg/l DDVP, respectively. The fish in the fifth experimental group which served as the control was exposed to dechlorinated water only. The experimental duration was 12 days in a static bioassay system. The water and the chemical were renewed daily to sustain steady concentration and avoid the accrual of wastes and food remains.

The fish were fed twice daily at 3% body weight with a commercial feed throughout the experimental period. The blood was collected from the fish at the end of the experimental period. Standard methods of blood collection in fish as described Gabriel et al.,<sup>17</sup> were used. The leucocytes counts were evaluated in the laboratory by using improved Neubauer haemocytometer after dilution in the blood in 1:100 ratio using Shaw's solution. Data obtained from the study were subjected to two-way analysis of variance (ANOVA) test at 0.05% probability level, using statistical package for the social sciences (SPSS) version 22. Differences among means where existed was done using Tukey test. Physico-chemical parameters of the water in experimental during the study were determined: Water temperature was evaluated with mercury in glass thermometers, water pH was done with pH meter (Model 3013, Jenway, China), while Salinity was performed with hand held refractometer (Atago products, Model H191, Japan). The values of dissolved oxygen, nitrite and ammonia were assessed by method described by APHA (1998).

## Result

The results of physico-chemical parameters in the experimental tanks during the exposure period are presented in Table 1. The values of temperature, pH and salinity were within the same range in all concentrations of DDVP. While ammonia, dissolved oxygen and nitrite increased significantly. However, the dissolved oxygen reduced with increasing concentrations of the chemical. The effects of DDVP on the total leucocytes and differential white blood cell obtained in both juvenile and adult sizes of *T. guineensis* are presented in Tables 2 & 3 respectively. The result of this study indicated a significant increase ( $P < 0.05$ ) in the white blood cell counts in the exposed fish than the control. The result obtained in the study further revealed that lymphocytes, neutrophils, and monocytes, were the dominant category of white blood cells discover in the tangential blood of *T. guineensis*. These were categorized as granulocytes or granulocytes, depending on the presence or absence of granules in their cytoplasm. The lymphocytes are on the whole the prominent type of leucocytes in the blood of *T. guineensis*. The values of lymphocytes increased tremendously as the concentrations of the chemical increased in both sizes of the fish. Conversely, the values of monocytes and neutrophils consistently decreased as the concentration of the chemical increased. These alterations were more pronounced at 0.075 and 0.100mg/l concentrations of DDVP- treated fish.

**Table 1** Physico-chemical parameters of water in experimental tanks (Mean±SD)

Parameters	Concentrations of DDVP (mg/l)				
	0	0.025	0.05	0.075	0.1
Temperature (°C)	27.11±1.21 <sup>a</sup>	27.14±1.22 <sup>a</sup>	27.11±1.01 <sup>a</sup>	27.61±1.21 <sup>a</sup>	27.62±1.11 <sup>a</sup>
pH	6.37±1.01 <sup>a</sup>	6.29±1.11 <sup>a</sup>	6.38±1.81 <sup>a</sup>	6.44±1.17 <sup>a</sup>	6.71±1.01 <sup>a</sup>
Ammonia (mg/l)	0.37±0.01 <sup>a</sup>	0.44±0.02 <sup>ab</sup>	0.47±0.02 <sup>b</sup>	0.47±0.02 <sup>b</sup>	0.51±0.22 <sup>c</sup>
Dissolved Oxygen (mg/l)	6.69±0.02 <sup>a</sup>	6.48±0.22 <sup>a</sup>	5.52±0.24 <sup>ab</sup>	4.13±0.32 <sup>b</sup>	4.16±0.03 <sup>b</sup>
Nitrite (mg/l)	0.04±0.01 <sup>a</sup>	0.08±0.01 <sup>b</sup>	0.08±0.01 <sup>b</sup>	0.09±0.01 <sup>c</sup>	0.09±0.01 <sup>c</sup>
Salinity (ppt)	14.41±1.21 <sup>a</sup>	14.24±1.04 <sup>a</sup>	14.18±1.01 <sup>a</sup>	14.49±1.19 <sup>a</sup>	14.63±1.44 <sup>a</sup>

Means within the row with different superscripts are significantly different ( $p < 0.05$ ).

**Table 2** Leucocytes profiles in juveniles of *T. guineensis* exposed to different concentrations of DDVP (Mean±SD)

Parameters	Concentrations of DDVP (mg/l)				
	0	0.025	0.05	0.075	0.1
WBC (cellsx10 <sup>9</sup> )	15.78±1.04 <sup>a</sup>	17.56±1.08 <sup>a</sup>	24.67±0.99 <sup>b</sup>	27.11±1.88 <sup>b</sup>	32.11±1.99 <sup>c</sup>
Thrombocytes (%)	130.67±2.77 <sup>c</sup>	120.82±3.23 <sup>c</sup>	112.67±2.23 <sup>b</sup>	93.88±1.25 <sup>a</sup>	95.43±2.12 <sup>a</sup>
Neutrophils (%)	22.39±2.12 <sup>a</sup>	20.22±1.12 <sup>b</sup>	18.61±1.19 <sup>b</sup>	14.08±1.21 <sup>a</sup>	11.21±2.61 <sup>a</sup>
Lymphocytes (%)	59.61±1.01	63.36±1.03 <sup>c</sup>	68.61±1.17 <sup>ab</sup>	77.03±1.89 <sup>b</sup>	82.89±1.21 <sup>b</sup>
Monocytes (%)	18.00±1.12 <sup>a</sup>	16.42±1.08	13.78±1.17 <sup>ab</sup>	9.09±1.62 <sup>b</sup>	6.68±3.11

Means within the row with different superscripts are significantly different ( $p < 0.05$ ).

**Table 3** Leucocytes profiles in adults of *T. guineensis* exposed to different concentrations of DDVP (Mean±SD)

Parameters	Concentrations of DDVP (mg/l)				
	0	0.025	0.05	0.075	0.1
WBC (cellsx10 <sup>9</sup> )	17.99±1.06 <sup>a</sup>	19.91±1.18 <sup>a</sup>	27.61±0.9 <sup>b</sup>	36.11±1.64 <sup>c</sup>	40.11±1.27 <sup>c</sup>
Thrombocytes (%)	160.72±2.88	130.8±3.87	126.61±2.17	114.61±1.77	109.6±1.21
Neutrophils (%)	26.00±2.72 <sup>c</sup>	22.72±1.18 <sup>b</sup>	20.61±1.19 <sup>b</sup>	17.88±1.99 <sup>a</sup>	14.01±2.99 <sup>a</sup>
Lymphocytes (%)	55.61±1.92 <sup>a</sup>	62.36±1.03 <sup>b</sup>	68.01±1.17 <sup>b</sup>	73.33±1.89 <sup>bc</sup>	80.89±1.28 <sup>d</sup>
Monocytes (%)	20.39±1.15 <sup>c</sup>	15.42±1.08 <sup>b</sup>	12.08±1.17 <sup>b</sup>	10.01±1.62 <sup>b</sup>	5.08±3.43 <sup>a</sup>

Means within the row with different superscripts are significantly different ( $p < 0.05$ ).

## Discussion

White blood cells are vital cells in the immune structure in any living organism, because of their major defensive activities. The increase in number of WBCs may play an important role in immunological defense systems during exposure to chemicals such as DDVP and appears to be associated with increased circulatory levels of granulocytes, which are known to respond for phagocytosis of foreign body in the system of the fish.<sup>18</sup> The white blood cells acts in response promptly to the alterations in the system of the fish due to toxicants transformation. During toxic exposure period of the fish to DDVP, the White blood cell counts were elevated considerably with increasing concentration of the chemical. An indication that the fish can build up a defensive apparatus to surmount the lethal stress.

The findings of this experiment revealed that DDVP had considerable consequence on the white blood cell and differential white blood cell count in *T. guineensis* during exposure. In line with the submissions of Nussey et al.,<sup>15</sup> the observed trend of leucocytosis in the DDVP treated fish signified a physiological adaption of the fish to contamination, triggered by the chemical. Some trials conducted by scientists indicated that leucocytosis was observed in fish subjected to contamination by pesticides.<sup>19,20</sup> The elevation in the percentage of lymphocytes in *T. guineensis* treated with DDVP is in consonant with the previous report of Akinrotimi et al.,<sup>21</sup> in Claries gariepinus fish treated with cypermethrin. Moreover, Akinrotimi et al.,<sup>22</sup> also observed that the proportion of lymphocytes in *Tilapia guineensis* subjected to industrial effluents increased tremendously when compared to the control values. Similarly, lymphocytosis was equally reported in *C. albopunctatus* exposed to Gamelan 20 in the laboratory.<sup>14</sup> In addition to this, leucocytopenia was equally observed in Coho salmon exposed to industrial effluents from Kraft pulp mill.<sup>23</sup>

The proportion of monocytes as well as neutrophils in *T. guineensis* in this study, decreased with increasing concentrations of the chemical, this result agrees with the findings of Nussey et al.,<sup>15</sup> in tilapia fish exposed to copper in the laboratory. This trend of monocytopenia and neutropenia in fish subjected to contaminants, was equally observed in *Tilapia guineensis* abruptly transfered to lower salinities<sup>24</sup> and different water pH.<sup>25</sup> Reduction in neutrophils was reported in *Labeo rohita* exposed to cypermethrin and carbofuran<sup>26</sup> as well as in *Heteropneustes fossilis* treated with alaclor and rogor.<sup>27-29</sup> In this study, the basophiles and eosinophils was absent in leucocytes of *Tilapia guineensis* exposed to DDVP. This agreed with report of Gabriel et al.,<sup>11</sup> who noted that the percentage of basophiles and eosinophils are very low in fish leucocytes. They are sometimes not present in some species of fish.

## Conclusion

In conclusion, the levels leucocytosis, lymphocytosis, monocytopenia, and neutropenia observed in this study, are clear manifestations of stress and associated infection in *T. guineensis* exposed to sublethal levels of DDVP concentrations. Fish have suitable immune systems, with complete depiction of all known basic mechanisms of innate and adaptive immunity, although with some specializations and distinct characteristics. The key feature of the immune system in many species of fish, as exemplified by *Tilapia guineensis* exposed to DDVP is that the leucocytes have the capacity to combat to a certain limit, some foreign invasions in the system of the fish. Moreover, information on the changes in the white blood cells in fish could be applied as analytical tool in evaluation and prediction of contamination in aquatic environment as reflected in the system of the fish.

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## Conflict of interest

Authors declare no conflict of interest.

## References

- Alexander JB, Ingram GA. Noncellular nonspecific defense mechanisms of fish. *Annual Review of Fish Disease*. 1992;2:249–279.
- Akinrotimi OA, Amachree D. Changes in haematological parameters of *Tilapia guineensis* exposed to different concentrations of detergent under laboratory conditions. *Journal of Aquatic Science*. 2016;31(1):95–103.
- Anderson DP. Immunostimulants, adjuvants and vaccine carriers in fish: applications to aquaculture. *Annual Review of Fish Disease*. 1992;2:281–307.
- Hawkey CM, Dennett TB. *Color Atlas of Comparative Veterinary Hematology*. Iowa State University Press, Ames, Iowa; 1989.
- Dalmo RA, Bogwald J.  $\beta$ -glucans as conductors of immune symphonies. *Fish Shellfish Immunol*. 2008;25(4):384–396.
- Cain KD, Jones DR, Raison RL. Characterisation of mucosal and systemic immune responses in rainbow trout (*Oncorhynchus mykiss*) using surface plasma resonance. *Fish Shellfish Immunol*. 2000;10(8):651–666.
- Campbell TW. Clinical pathology. In: Mader DR, editor. *Reptile Medicine and Surgery*. 1996;248–257.
- Jain NC. *Essentials of Veterinary Hematology*. Blackwell Publishing, Philadelphia; 1993.

9. Hrubec TC, Cardinale JL, Smith SA. Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis hybrid*). *Vet Clin Pathol*. 2000;29(1):7–12.
10. Maxwell MH, Robertson GW. The avian heterophil leucocyte: a review. *Worlds Poultry Science Journal*. 1998;54(2):155–178.
11. Gabriel UU, Ezeri GNO, Opabunmi OO. Influence of sex, sources, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). *African Journal of Biotechnology*. 2004;3(9):463–467.
12. Dhabhar FS, Miller AH, McEwen BS, et al. Stress induced changes in blood leukocyte distribution—role of adrenal steroid hormones. *J Immunol*. 1996;157(4):1638–1644.
13. Arma NR, Hirono I, Aoki T. Characterization of expressed genes in kidney cells of Japanese flounder *Paralichthys olivaceus* treatment with ConA/PMA and LPS. *Fish Pathology*. 2004;39(4):189–196.
14. Mgbenka BO, Oluah NS, Umeike I. Effect of gammalin 20 (Lindane) on the Differential white blood cell counts of the African catfish *Clarias albopunctatus*. *Bull Environ Contam Toxicol*. 2003;71(2):248–254.
15. George ADI, Akinrotimi OA, Nwokoma UK. Haematological Changes in African Catfish (*Clarias gariepinus*) Exposed To Atrazine and Metolachlor in the Laboratory. *Journal of Fisheries Science Com*. 2017;11(3):48–54.
16. Sanchez C, Babin M, Tomillo J, et al. Quantification of low levels of rainbow trout immunoglobulin by enzyme immunoassay using two monoclonal antibodies. *Vet Immunol Immunopathol*. 1993;36(1):65–74.
17. Nussey G, Van Vuren JHJ, Du Prezz HA. Effect of copper on the differential white blood cell counts of the *Mossambique tilapia* (*Oreochromis mossambicus*) *Comparative Biochemistry and Physiology*. 1995;111(3):381–388.
18. Matthiessen P. Haematological changes in fish following aerial spraying with endosulfan insecticide for testse fly control in Botswana. *Journal of Fish Biology*. 1981;18(4):461–469.
19. Gabriel UU, Akinrotimi OA, Ariweriokuma SV. Alterations of selected electrolytes in organs of African catfish, *Clarias gariepinus* treated with cypermethrin. *Advances in students Research*. 2012;2(1):53–60.
20. George ADI, Akinrotimi OA. Influence of Sex on Haematological Response of *Clarias gariepinus* Juveniles Treated with Atrazine and Metalochlor. *Trends Green Chemistry*. 2017;3:1–6.
21. Nte ME, Edun OM, Akinrotimi OA. Biochemical Changes in Mudskipper (*Periophthalmus papilio*) exposed to sodium bromide. *International Journal of Advanced Research in Medical & Pharmaceutical Sciences*. 2018;3(2):1–6.
22. Bowden TJ. Modulation of the immune system of fish by their environment. *Fish Shellfish Immunol*. 2008;25(4):373–383.
23. Akinrotimi OA, Gabriel UU, Ariweriokuma SV. Haematotoxicity of cypermethrin to African catfish *Clarias gariepinus* under laboratory conditions. *Journal of Environmental Engineering and Technology*. 2012;1(2):20–25.
24. Akinrotimi OA, Orlu EE, Gabriel UU. Haematological Responses of *Tilapia guineensis* treated with industrial effluents. *Applied Ecology and Environmental Sciences*. 2013;1(1):10–13.
25. Mcleay DJ. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus Kisutch*) to stressors including sublethal concentration of pulp mill effluent and zinc. *Journal of Fish Research Board of Canada*. 1975;32(12):2357–2364.
26. Akinrotimi OA, Agokei EO, Aranyo AA. Changes in haematological parameters of *Tilapia guineensis* exposed to different salinity levels. *Journal of Environmental Engineering and Technology*. 2012;1(2):4–12.
27. Akinrotimi OA, Opara JY, Ibemere IF. Changes in haematological parameters of *Tilapia guineensis* exposed to different water pH *Environment Innovation in Science and Engineering*. 2012;2:9–14.
28. Adhikari S, Sarkar B, Chatterjee A, et al. Effect of cypermethrin and carbofuran on certain hematological parameters and prediction of recovery in a freshwater teleost, *Labeo rohita* (Hamilton). *Ecotoxicol Environ Saf*. 2004;58(2):220–222.
29. Agarwal K, Chaturvedi LD. Anomalies in blood corpuscles of Heteropneustes fossilis induced by alachlor and rogor. *Adv Bios*. 1995;14:73–80.