



Induction of microcytic-hypochromic anaemia in *Oreochromis niloticus* (Trewavas, 1983) exposed to sublethal toxicity of 2, 3-dichlorovinyl dimethyl phosphate (sniper 1000ec) under laboratory conditions

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Abstract

Microcytic-hypochromic anaemia was induced in *Oreochromis niloticus* (Trewavas, 1983) at intervals of 1, 14 and 28 days. Experimental fish were exposed to test water separately diluted with sub lethal concentrations of sniper 1000EC of 0, 0.12, 0.15, 0.19 and 0.25 mg/L. Experimental design was a complete randomized design. A 28 day exposure to sub lethal concentrations of the toxicant resulted in microcytic-hypochromic anaemia in the exposed fish on days (1, 14 and 28). Blood dyscrasias attributable to microcytic-hypochromic anaemia was observed with a significant ($p < 0.05$) decrease in haemoglobin, haematocrit, red blood cells, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). White blood cells, lymphocytes, monocytes, Neutrophils, eosinophil and basophil increased significantly ($p < 0.05$) with increasing concentrations of the toxicant. It was concluded that sniper 1000EC induced microcytic-hypochromic anaemia in the exposed fish.

Keywords: toxicity, sniper 1000EC, *Oreochromis niloticus*, microcytic-hypochromic, anaemia

Introduction

Pesticides are recognized as serious pollutants in the aquatic environment with the potential to cause deleterious effects on the biota, especially fish (Verma, *et al.*, 1982) [32]. Over 5.5 billion liters of pesticides are sold around the world each year which consist of herbicides, insecticides, fungicides and rodenticides, but herbicides are sold in largest quantities followed by insecticides, fungicides and rodenticides (MacEwen and Stephenson, 1975) [22]. Organophosphorus pesticides (OPs) are the most commonly used pesticides in the world due to their quick degradation (Eto, 1974) [13]. Indiscriminate disposal of pesticides into water bodies leads to contamination of aquatic environment. They pose a severe threat to aquatic organisms, which form important members of food chain (Satyaparameshwar, *et al.*, 2005). Pollution brings undesirable changes in the environment, which affects the biotic composition of the ecosystem. Most of the pollutants are either emitted to the atmosphere through gases which serve as medium, or are discharges to water bodies, or directly through the introduction of the chemical to attack particular organisms such as in pest control programs (Holden, 1977). In developing countries, the human population is exposed to pesticide compounds through drinking water and via the food supply, including fish (Hayes, 1982). In recent years, extensive use of pesticides has caused many problems due to their adverse effects on the aquatic ecosystem and human health (Hanke, *et al.*, 1983) [15]. According to West and Biney (1991), besides habitat loss and over exploitation, pollution ranked third as the main cause of fish species loss, and that there are three main sources of aquatic pollution in Africa, Urban development, industrial waste and the use of pesticides. Contamination of water by pesticides either directly or

indirectly kills, reduced fish productivity or elevated concentrations of undesirable chemicals in edible fish tissue which are deleterious to humans eating these fishes (Adedeji, *et al.*, 2012) [5, 7]. The effects of pesticides concentrations on haematological parameters on different fish species have been studied by many investigators (Mekawy, *et al.*, 1996) [23] and Abubakar and Abdulsalami, (2013) [2, 3]. Fish blood is being studied increasingly in toxicological research and environmental monitoring as possible indicator of physiological and pathological changes in fishery management and disease investigations (Mulcahy, 1975) [25] as the blood in the gill has direct contact with the water medium and any unfavourable change in water could be reflected in the circulatory system (Adhikari, *et al.*, 2002) [6].

Sniper 1000EC (2, 3-dichlorovinyl dimethyl phosphate), a brand of dichlorvos, is contact acting and fumigant insecticide (Abubakar, 2013) [2, 3]. Like all organophosphates, it kills insects and other target organisms because of its toxicity to the nervous system. This is achieved by inhibition of enzyme acetylcholinesterase (AChE) that breaks down acetylcholine at the receptor site for partial uptake into the nerve terminal. Without functioning AChE, accumulation of acetylcholine results in depolarizing block of muscle membrane, producing rapid twitching of involuntary muscles, convulsions, paralysis and early death.

Tilapia has become the shining star of aquaculture across the globe (Waleed, 2012) [34]. Arrington (1998) [9] describes *Oreochromis niloticus* as the best species for culture among the tilapia family with squat shape. Trewavas (1982) recognized *Oreochromis niloticus* as macrophages and herbivorous used in irrigation channels and dams to control weed. Fagbenro (2002) [19] stated that tilapia species are of

major economic importance in tropical and sub-tropical countries throughout the world, particularly in Africa where farms stock mixed-sex tilapia in production ponds. They are disease resistant, highly prolific; feed on wild variety of foods, tolerant of poor water quality with low dissolved oxygen level (Fagbenro, 2002) [19]. Tilapia is one of the fisheries resources that suffers from environmental effects and needs to be protected because world production of tilapia exceeds two million tones per year far exceeding the harvest of Atlantic salmon and secondary only to carp as a culture food fish (FAO, 2005) [18].

There is paucity of information on toxicity of sublethal concentrations of sniper 1000EC on tilapia fish despite its indiscriminate use by the fish farmers.

The aim of the present study was to evaluate microcytic-hypochromic anaemia in *Oreochromis niloticus* under laboratory conditions.

Materials and Methods

Procurement of test fish

Juveniles of *Oreochromis niloticus* (mean body weight 7.05±1.02; mean standard length 9.60±0.38cm) were obtained from a reputable fish farm in Minna and brought to the laboratory. The fishes were kept in the glass aquaria to observe any visible pathological symptoms. Before introducing into the aquarium, fishes were treated with 0.1% potassium permanganate (KMnO₄) solution to obviate any dermal infection.

Acclimation of test fishes

Fishes were acclimatized to laboratory conditions for a period of two weeks. No mortality was recorded during acclimation period. The fishes were fed with pelleted feed containing 35 % crude protein at 3% body weight per day. Daily ration was divided into three portions and fed thrice per day. After acclimatization, fishes were kept in different concentrations of sniper 1000EC in different aquaria. The test solutions were renewed fortnightly.

Sources of sniper 1000ec and its exposure

Sniper 1000EC (2, 3-dichlorovinyl dimethyl phosphate) was purchased from Minna central market. Renewal toxic test method (APHA, 1992) was used. Fishes were exposed to sublethal concentrations for 28 days. Control fish were also maintained under identical conditions without the toxicant.

Experimental Design

The experimental design was a complete randomized design. A total of one hundred and fifty (150) juvenile of *Oreochromis niloticus* were randomly distributed into the tanks at a stocking rate of 10 fish per tank. The fifteen (15) tanks were assigned to 5 treatments (control inclusive). In order to determine the LC₅₀, the *O. niloticus* were exposed to four different concentrations of sniper 1000EC for 96hr. LC₅₀ value obtained using EPA Probit Analysis programme version 1.5 was 3.81mg/l and one fifteen (1/15), one twenty (1/20), one twenty fifth (1/25) and one thirty (1/30) were taken as sublethal using the method of Abubakar (2013) [2, 3] to produce 0, 0.12, 0.15, 0.19 and 0.25mg/L respectively.

Collection of blood

Blood samples were collected from both the control and experimental fish at intervals of 1, 14 and 28days. The fish were stunned with a gentle knock on the head. The stunned fish was placed in a trough and blood was taken by caudal venous puncture using 23GX 11/4 (0.6 x 32 mm) syringe. The blood was put into EDTA vials and taken to Medical diagnostic laboratory in Minna for analysis using methods described by Blaxhall and Daisley (1973) [10] at a wavelength of 540µm. The haematological parameters analyzed were Haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC) and its differential counts.

Haematological tests

Haemoglobin

The sahil's-Hellige haemoglobin determination was performed as follows: The sallied pipette was filled slightly above the 20mm³ mark, the pipette was wiped with a soft absorbent tissue to remove excess blood and the volume was adjusted to exactly 20mm³ by blotting the tip. The blood was expelled into a calibrated (transmission) test tube containing 10.0 milliliters of 0.1N hydrochloric acid, and the pipette was raised several times in the acid solution. The sample was allowed to stand for not less than 3 minutes before reading the values in the colorimeter. The intensity of color was measured at a wavelength of 540µm and was recorded as percent transmission.

$$\text{Calculation: } X \% = \frac{\text{XC14 gm haemoglobin per 100 ml of blood.}}{100}$$

Determination of pack cell volume

Pack Cell Volume (PCV) was carried out by micro-westgreen method as described by Blaxhall and Diasely (1973) [10]. The blood sampled from the severed caudal peduncle was drawn into micro-haematocrit tube. The tubes were sealed with wax and centrifuged for 5 - 6 minutes. The PCV was measured with the aid of a microhaematocrit reader and expressed as the volume of the erythrocytes per 100cm³.

Red blood cell count

The standard RBCC diluting pipette and a 1:200 dilution were used for the red blood cell count. Blood was drawn just beyond the 0.5 mark on the pipette. The tip of the pipette was wiped with a soft absorbent tissue to adjust the volume to exactly the 0.5 mark. The pipette was immediately filled to the 101 mark with Hendricks diluting fluid. Partial rotation of the pipette while being filled assured the complete mixing of the blood and diluting fluid, and prevented clotting. With its ends gripped between the thumb and second finger, the pipette was then shaken for 30 to 60 seconds. After the pipette had been shaken, a few drops of the diluted blood were expelled from it. Control of over flow of fluid was maintained by replacing the index finger over the bulb end of the pipette. The haemocytometer (counting chamber) was a Neubauer, the pipette was held to the edge between the cover slip and the chamber, and capillary action drew the diluted suspension of

cells into the chamber. The haemocytometer was then placed under the light microscope, and the cells were counted. The haemocytometer is divided into ruled areas 1mm², with the centre square millimeter divided into 25 groups of 16 small squares. The cells within the boundaries of five of these small squares were counted. Each corner plus the center group were counted when the red blood cell count was computed, the number of cell counted in all five squares was multiplied by 106, this gave the total number of cells per cubic millimeter (mm³) of blood (Mallum *et al.*, 2015).

Erythrocyte Indices

Calculated mean values that reflect the size, weight and haemoglobin content of individual erythrocytes.

Means Corpuscular Volume (MCV) express the average volume of the individual erythrocyte and is calculated from the formula.

$$MCV = \frac{\text{Haematocrit}}{\text{Erythrocyte count (millions/Cu.mm)}} \times 10$$

Minot and Murphy, (2007)

It is expressed in femtoliter (fl).

Means Corpuscular Haemoglobin (MCH) is the amount of haemoglobin by weight, in the average erythrocyte and is calculated thus:

$$MCH = \frac{\text{Haemoglobin (gm/100ml)}}{\text{Erythrocyte count (millions/Cu.mm)}} \times 10$$

Minot and Murphy, (2007)

It is expressed in pictogram (pg).

Means Corpuscular Hemoglobin Concentrations (MCHC) is the concentration of haemoglobin in the average erythrocyte and is calculated thus:-

$$MCHC (\text{g } 100\text{ml}^{-1}) = \frac{\text{Haemoglobin (gm/ 100ml)}}{\text{Haematocrit}} \times 100$$

Minot and Murphy, (2007)

It is expressed in gram deciliter (gdL⁻¹).

Total leucocytes count

Leucocytes were counted using Shaw's solution A and B. The blood was drawn up to the 0.5 mark, solution A was added to fill the bulb of the pipette approximately half filled, and mixed. Then, the pipette was removed from solution A and filled to the mark 101 with solution B. The pipette was then shaken as in the erythrocyte count. A few drops were expelled and the haemocytometer was filled in the manner described previously. For comparison of the total number of leucocytes, the cells in the four large squares noted by the large cycle were counted. The total number of cells counted multiplied by 500, determined the total number of leucocytes per cubic millimeter (mm³) of blood (Hesser, 1960).

Statistical Analysis

Data were analyzed with One-Way analysis of variance (ANOVA) procedure using Statistical Package for Social Sciences (SPSS) version 16.0 for window. Statistical significance of difference among means was compared using Turkey (HSD) test.

Results

Alteration in blood attributable to sublethal concentrations of sniper1000EC were observed at intervals of days (1, 14 and 28) after exposure.

Haematological parameters of the exposure concentrations and duration in *Oreochromis niloticus*.

Exposure of *Oreochromis niloticus* to sublethal concentrations of sniper 1000EC for 28 days resulted in microcytic hypochromic anaemia. Haemoglobin, haematocrit and red blood cell decreased (pancytopenia) with increase in concentrations of sniper 1000EC at all levels compared with their control. The values of Erythrocyte indices of MCV, MCH and MCHC for the exposed groups were significantly ($p < 0.05$) lower than their respective control along different levels (0.12, 0.15, 0.19 and 0.25mg/L). There were insignificant increase ($p > 0.05$) in the values of WBC as evidence in immune response. Differential white blood cells analysis revealed no pronounced joint disorder with insignificance different between neutrophil and lymphocyte of the exposed groups and their controls ($p > 0.05$). The values for the exposed groups of monocyte, eosinophil and basophil fluctuated relative to their control groups (Table 1 and 2).

Table 1: Haematological parameters of *Oreochromis niloticus* exposed to sublethal concentrations of sniper 1000EC (Mean \pm SD)

| Parameters | Concentration(mg/L) | | | | |
|---|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | Control | 0.12 | 0.15 | 0.19 | 0.25 |
| Hb(gdL ⁻¹) | 4.7 \pm 0.16 ^a | 3.9 \pm 0.13 ^b | 3.7 \pm 0.15 ^b | 3.5 \pm 0.13 ^b | 3.1 \pm 0.12 ^b |
| PCV (%) | 21.6 \pm 0.22 ^a | 20.0 \pm 0.36 ^a | 19.3 \pm 0.25 ^a | 18.7 \pm 0.27 ^a | 17.9 \pm 0.32 ^b |
| RBC($\times 10^{12}$ Cells L ⁻¹) | 2.21 \pm 0.05 ^a | 2.19 \pm 0.02 ^a | 2.16 \pm 0.03 ^a | 2.13 \pm 0.17 ^a | 2.03 \pm 0.17 ^a |
| MCV(fl) | 97.25 \pm 0.83 ^a | 91.09 \pm 1.09 ^b | 89.67 \pm 1.29 ^b | 85.13 \pm 1.94 ^b | 75.36 \pm 5.56 ^c |
| MCH (pg) | 21.39 \pm 0.69 ^a | 18.21 \pm 0.26 ^b | 17.19 \pm 0.56 ^b | 15.90 \pm 1.06 ^c | 13.24 \pm 0.25 ^d |
| MCHC (gdL ⁻¹) | 21.92 \pm 0.77 ^a | 19.72 \pm 0.61 ^b | 19.11 \pm 0.73 ^b | 18.44 \pm 0.70 ^b | 17.45 \pm 0.61 ^c |
| WBC($\times 10^9$ Cells L ⁻¹) | 3581 \pm 2.00 ^a | 3583 \pm 2.23 ^a | 3587 \pm 1.73 ^a | 3590 \pm 1.90 ^a | 3593 \pm 1.86 ^a |
| Neutrophils (%) | 71.9 \pm 0.66 ^a | 71.6 \pm 0.60 ^a | 71.6 \pm 0.87 ^a | 71.9 \pm 0.93 ^a | 71.7 \pm 0.83 ^a |
| Lymphocytes (%) | 23.7 \pm 0.35 ^a | 24.1 \pm 0.49 ^a | 23.6 \pm 0.73 ^a | 23.7 \pm 0.69 ^a | 23.7 \pm 0.79 ^a |
| Monocytes (%) | 3.3 \pm 0.53 ^a | 3.1 \pm 0.37 ^a | 3.2 \pm 0.46 ^a | 3.0 \pm 0.66 ^a | 3.1 \pm 0.64 ^a |
| Eosinophils (%) | 0.5 \pm 0.17 ^a | 0.7 \pm 0.31 ^a | 0.8 \pm 0.18 ^a | 0.9 \pm 0.27 ^a | 0.9 \pm 0.38 ^a |
| Basophils (%) | 0.4 \pm 0.15 ^a | 0.5 \pm 0.17 ^a | 0.6 \pm 0.32 ^a | 0.7 \pm 0.11 ^a | 0.6 \pm 0.40 ^a |

Means of parameters with the same superscripts along the rows are not significantly different at $p>0.05$.

Hb – Haemoglobin; PCV – Packed cell volume; RBC – Red

Blood Cell count; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular Haemoglobin; MCHC – Mean Corpuscular Haemoglobin Concentration; WBC – White Blood Cell count.

Table 2: Haematological parameters of *Oreochromis niloticus* at the various duration of exposure to sniper 1000EC (Mean \pm SD)

| Parameters | Duration of exposure (Days) | | | |
|---|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | control | 1 | 14 | 28 |
| Hb(gdL ⁻¹) | 4.7 \pm 0.16 ^a | 3.4 \pm 0.21 ^b | 3.6 \pm 0.10 ^c | 4.3 \pm 0.53 ^d |
| PCV (%) | 21.6 \pm 0.22 ^a | 18.9 \pm 0.30 ^b | 19.0 \pm 0.21 ^c | 20.8 \pm 0.30 ^d |
| RBC($\times 10^{12}$ L ⁻¹) | 2.21 \pm 0.05 ^a | 2.12 \pm 0.12 ^c | 2.15 \pm 0.11 ^b | 2.19 \pm 0.01 ^d |
| MCV(Fl) | 97.25 \pm 0.83 ^a | 82.2 \pm 2.12 ^b | 86.3 \pm 3.18 ^c | 94.7 \pm 1.00 ^d |
| MCH (pg) | 21.39 \pm 0.69 ^a | 15.24 \pm 1.03 ^b | 16.55 \pm 0.62 ^c | 19.77 \pm 0.39 ^d |
| MCHC (gdL ⁻¹) | 21.92 \pm 0.77 ^a | 18.21 \pm 1.18 ^b | 19.03 \pm 0.37 ^c | 20.74 \pm 0.49 ^d |
| WBC($\times 10^9$ L ⁻¹) | 3581 \pm 2.00 ^a | 3585 \pm 1.62 ^f | 3588 \pm 1.96 ^b | 3587 \pm 2.26 ^c |
| Neutrophils (%) | 71.9 \pm 0.66 ^a | 72.1 \pm 0.42 ^a | 71.5 \pm 0.77 ^a | 71.7 \pm 1.24 ^a |
| Lymphocytes (%) | 23.7 \pm 0.35 ^a | 23.7 \pm 0.55 ^a | 24.8 \pm 0.38 ^a | 23.7 \pm 0.85 ^a |
| Monocytes (%) | 3.3 \pm 0.53 ^a | 2.8 \pm 0.26 ^a | 3.4 \pm 0.69 ^a | 3.2 \pm 0.64 ^a |
| Eosinophils (%) | 0.5 \pm 0.17 ^a | 0.9 \pm 0.30 ^a | 0.7 \pm 0.20 ^a | 0.7 \pm 0.29 ^a |
| Basophils (%) | 0.4 \pm 0.15 ^a | 0.5 \pm 0.23 ^a | 0.6 \pm 0.25 ^a | 0.6 \pm 0.20 ^a |

Means of parameters with the same superscripts along the rows are not significantly different at $p>0.05$.

Hb – Haemoglobin; PCV – Packed cell volume; RBC – Red Blood Cell count; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular Haemoglobin; MCHC – Mean Corpuscular Haemoglobin Concentration; WBC – White Blood Cell count.

Discussion

This study clearly demonstrated that sublethal toxicity of Sniper 1000EC induced microcytic hypochromic anaemia in *O. niloticus* under laboratory conditions. According to the findings, the test chemical could be ranked toxic (Wagner, *et al.*, 1995) [33]. This results demonstrated that *O. niloticus* is sensitive to Sniper 1000EC. Abubakar and Abdulsalami (2013) [2, 3] observed microcytic-hypochromic anaemia in *Clarias gariepinus* exposed to sublethal toxicity of Sniper 1000EC. Reduction in erythrocyte count, haematocrit value and haemoglobin content of *O. niloticus* may be attributed to such factors as blood haemorrhage due to an equilibrium of osmotic pressure inside and outside blood cells (Heath, 1987) [17], and haemodilution of blood due to damage and bleeding of fish organs (Movotny and Beeman, 1990) [24]. The reduction in blood parameters was an indication of anaemia caused by this toxicant as the concentration increased. A decrease in the concentration of haemoglobin in blood is usually caused by the effect of pollutant in gills as well as decrease in oxygen carrying capacity; which also suggests anaemia in tilapia (Abubakar, 2013) [2, 3]. Haematological indices (RBC count, concentration of haemoglobin and haematocrit) have been reported to indicate secondary responses of an organism to pollutants (O'Neal and Weirich, 2001) [28]. Decrease in MCV, MCH and MCHC were indication of Microcytic-hypochromic anaemia (Abubakar, 2013) [2, 3]. Microcytic-hypochromic anaemias are characterized by abnormally small red blood cells with insufficient haemoglobin content (Lee, 1993) [21]. The anaemic exposure could be as a result of destruction of RBC which is an indication of lack of iron (Abubakar, 2013) [2, 3] or haemodilution as reported by Sampath *et al.* (1993). Iron plays

a principal role in erythropoiesis, as it is necessary for proliferation and maturation of red blood cells and for haemoglobin synthesis (Abubakar, 2013) [2, 3]. Joshi *et al.* (2002) [20] made a similar observation on blood parameters of *C. batrachus* exposed to Lindane and Malathion which are pesticides. The pesticide stress caused the microcytic anaemic condition by destroying mature erythrocytes, resulting in a reduced RBC, and disrupting iron- synthesizing mechanisms (Adhikari, *et al.*, 2002) [6]. Microcytosis may be due to decrease in haematocrit during exposure. Similar pattern has been detected in *Labeo umbratus* after exposure to various pollutants (Van Vuren, 1986) [31]. Changes in WBC and differential counts have been reported to play important roles in the state of health of *C. gariepinus* (Ezeri, 2001; Omoregie and Oyebanji, 2002) [14, 27]. Changes in neutrophils, eosinophils and basophils indicated stress condition in the fish and similar reports have been made by several authors including Johansson-Sjoberg *et al.* (1978) and Anyanwu *et al.* (2007) [8].

Conclusion

The abnormalities in haematological parameters of *O. niloticus* as a result of exposure to sublethal concentrations of sniper 1000EC under laboratory conditions resulted in microcytic-hypochromic anaemia attributable to abnormally small red blood cell, haemodilution and impaired haemoglobin synthesis.

Recommendations

1. Proper education of farmers on the danger of Sniper 1000EC to the environment is required,
2. Attempts should be made to monitor and control the usage of Sniper 1000EC by local fishermen,
3. Manufacturing industries should look into ways of reducing the potency of Sniper 1000EC to non-target organisms such as fish,
4. Manufacturers should also be compelled to state categorically the effect of Sniper 1000EC to non-targeted aquatic organisms.

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