HAEMATOLOGICAL ABERRATIONS AND ELECTROLYTE STABILIZATION IN *Heterobranchus bidorsalis* INDUCED BY RHONASATE 360SL CONTAINING GLYPHOSATE

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**ABSTRACT**

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The aim of the study was to determine the effect of the herbicide, rhonasate 360SL, containing glyphosate (Isopropylamine salt, glycine) in the plasma and organs on *Heterobranchus bidorsalis*. Adult *Heterobranchus bidorsalis* were exposed in four replicates to varying sublethal concentrations of the toxicant (ranging from 0.00 to 3.00 mg L⁻¹) in 14 days semi-static bioassays. Red blood cell (RBC), white blood cell (WBC), haemoglobin (Hb) and thrombocytes (platelets) were statistically significant (p<0.05) the differential count values were insignificant (p>0.05). Muscle and liver electrolytes values stabilizes except K⁺ ions in the muscle that clearly showed a decrease in values as the concentrations increased. These haematological indices could serve as a biomarker/bioindicator of rhonasate 360SL on *Heterobranchus bidorsalis*, a common Niger Delta wetland fish.

INTRODUCTION

It is globally known that pesticides pose a threat to biotic and abiotic environment. Pesticides have been reported to have negative ecological consequences in the environment. Pesticides enter aquatic environment via direct application, aerial drift or run-off from application or accidental release and become rapidly distributed via the action of wind and water (Rand and Petrocelli, 1985). Once in the water, the pesticides residues may either become attached to suspended material, deposited on the bottom sediment or absorbed by organisms where they are detoxified or accumulated (Rand and Petrocelli, 1985). The poisoning by pesticides from agricultural fields and other anthropogenic activities constitute a serious water pollution problem and its environmental long term effect may result in the incidence of poisoning of fish and other aquatic life forms (Joythi and Narayan, 1999). Pesticides at high concentrations are known to reduce the survival, growth reproduction of fish and produce many visible effects on fish (Johnson, 2002). Glyphosate with the chemical name, N-phosphonomethyl glycine is one of the world’s best selling chemical herbicides. Glyphosate (isopropyl amine salt, glycine) is a broad spectrum, non-selective systemic herbicide that kills or suppresses many grasses, forbs, vines, shrubs and trees (Carlisle and Trevors, 1988). Glyphosate on its own is less toxic to aquatic and terrestrial organism as well as the ecosystem, but when combine with other chemicals such as salts called surfactants become toxic in lethal and acute doses, hence it exist as salts combinations. Glyphosate containing herbicides such as rhonasate 360SL, monsanto’s round-up etc. are the most widely used herbicides in Africa, in Nigeria rhonasate is sold in an open market and it is well embraced by local farmers (Patani, 2014). Herbicides can alter fish physiology. A study on the European eel concluded that environmentally relevant concentrations of Round-up can pose a health risk for fish populations (Guilherme, et al., 2009) and found that the herbicide damaged the DNA of the exposed fish. Other effects were observed on interactions between fish and parasites. There is also evidence that glyphosate affects the activity of the operation of the enzyme, acetylcholinesterase, which is vital for the operation of the nervous system. If acetylcholinesterase is not working properly, nerve impulses are not switched off, causing serious health problems and even death (Glue et al., 1997). Glyphosate have been found to suppress the activity of the enzyme in brown mussels (Sandrini et al., 2013) and fish (Menendez et al., 2012; Cattaneo et al., 2011; Glusczak, 2007; Glusczak et al., 2006).

Haematological analyses have been routinely used in determining the physiological state of organisms and are known to be affected by different environmental factors as well as agro-chemicals. It is used as a guide in the diagnosis of many diseases used in evaluating the responses to therapy in organisms (Solomon and Okonmoda, 2012). Studies on fish blood gives the possibility of knowing physiological conditions within the fish long before there is an outward manifestation of diseases. Under stressful condition as well as environmental imbalances some parameters in the fish blood changes in response to the toxicant effect (Shah and Altidag, 2005). The objective of this work was to study the effect of rhonasate 360SL, containing glyphosate on haematological and electrolyte parameters in *Heterobranchus bidorsalis*.

**MATERIALS AND METHODS**

Thirty-five African catfish (*Heterobranchus bidorsalis*), mean weight 97.00±0.3SD and mean length 14.02±0.20 cm were obtained from Ajimmy fish farm, a private fish farm at Okaka in Yenagoa, Bayelsa State. They were
transported in a 30 litres trough to the wet laboratory (ecotoxicology unit) of the department of biological sciences, Niger Delta University, where the assays were conducted from May to July, 2014. Fishes were acclimatized individually in a rectangular aquarium for ten days during which they were fed once a day (9:00-11:00 hrs) with 35% crude protein at 1% biomass.

**General bioassays technique**

Sublethal concentrations of rhonasate 360SL containing glyphosate (isoprophyl amine salt, glycine) in the form of 480gl for the assay (1.00, 2.00, and 3.00ppm) were determined based on the range finding test (Inyang, et al., 2010). These were prepared by transferring 0.06, 0.125 and 0.188 mls of the original concentration of the toxicant and making it up to 30L with borehole water in the test aquaria. The diluent was water (30L). Four replications of each treatment level (concentration) and control were set up by introducing fishes individually into each aquarium. The exposure period lasted 21 days during every 48 hours. The physico-chemical characteristics of the water used for fish bioassay was carried out using standard methods of APHA (1998) and the following values were obtained, temperature 26.70 – 26.15 °C, pH 6.36-6.37, Dissolved oxygen 5.50 – 7.05 mg l⁻¹, alkalinity 1.25 – 12.30 mg l⁻¹, conductivity 99.55 – 136.00 μs cm⁻¹ and turbidity, 0.26 – 0.49 NTU.

**Haematological and electrolyte assay technique**

After 14 days exposure period, blood samples for haematological analysis were collected from each fish (behind the anal fin) with 23G size needle and syringe. Fish were not fed prior to blood collection samples were preserved in EDTA bottles (Inyang, 2008). Fish were sacrificed after blood collection and dissected for the collection of the liver and muscle. Additionally, 0.5g of each organ was macerated (grounded) with pestle and mortar. Deionized water was used for preservation and stabilization. Samples were centrifuged at the rate of 3,000 rpm for 15 minutes. The supernatants were then removed and stored in plain bottles at – 2 °C for analysis. The activities of sodium (Na⁺), Potassium (K⁺) and Calcium (Ca++) were assayed using Logaway et al., (2006) and APHA (1998).

**Data analysis**

The data were subjected to analysis of variance (ANOVA). Where differences exist, Duscan multiple range test were used to test for significant difference (p<0.05) between treatments (Wahua, 1999).

Table 1: Changes in some selected haematological indices of *Heterobranchus bidorsalis* exposed to sublethal concentrations of rhonasate 360SL after 14 days exposure.

<table>
<thead>
<tr>
<th>Conc. of Rhonasate mg l⁻¹</th>
<th>RBC (×10⁶ g⁻¹)</th>
<th>WBC (×10³ g⁻¹)</th>
<th>Plat (×10⁴ g⁻¹)</th>
<th>Hb (g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>2.25±0.00⁣a</td>
<td>203.00±10.20⁣a</td>
<td>53.00±1.10⁣a</td>
<td>9.40±0.06⁣a</td>
</tr>
<tr>
<td>1.00</td>
<td>2.15±0.01⁣ab</td>
<td>205.00±7.30⁣ab</td>
<td>21.50±0.09⁣ab</td>
<td>9.20±0.04⁣ab</td>
</tr>
<tr>
<td>2.00</td>
<td>2.30±0.00⁣b</td>
<td>211.00±5.01⁣b</td>
<td>31.50±0.03⁣b</td>
<td>9.18±0.00⁣b</td>
</tr>
<tr>
<td>3.00</td>
<td>1.60±0.01⁣b</td>
<td>179.00±3.60⁣b</td>
<td>31.00±0.03⁣b</td>
<td>5.80±0.02⁣b</td>
</tr>
</tbody>
</table>

Means within column different superscript are significantly different (p<0.05). RBC (Red blood cells), WBC (White blood cells, Plat (platelets), Hb (hemoglobin)

Table 2: Changes in some selected haematological indices (differential count) of *Heterobranchus bidorsalis* exposed to sublethal concentrations of rhonasate 360SL after 14 days exposure.

<table>
<thead>
<tr>
<th>Conc. of Rhonasate (mg l⁻¹)</th>
<th>Neut</th>
<th>Lymph</th>
<th>Eosin</th>
<th>Mono</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>0.00±0.00⁣a</td>
<td>98.00±0.02⁣a</td>
<td>0.01±0.00⁣a</td>
<td>0.20±0.00⁣a</td>
</tr>
<tr>
<td>1.00</td>
<td>0.00±0.00⁣b</td>
<td>90.00±0.01⁣b</td>
<td>0.00±0.00⁣b</td>
<td>0.20±0.01⁣b</td>
</tr>
<tr>
<td>2.00</td>
<td>0.10±0.00⁣b</td>
<td>98.00±0.02⁣b</td>
<td>0.00±0.00⁣b</td>
<td>0.10±0.01⁣b</td>
</tr>
<tr>
<td>3.00</td>
<td>0.10±0.01⁣b</td>
<td>97.00±0.41⁣b</td>
<td>0.00±0.00⁣b</td>
<td>0.10±0.04⁣b</td>
</tr>
</tbody>
</table>

Means within column different superscript are significantly different (p<0.05). Neut (Neutrophils), Lymph (Lymphocytes), Eosino (Eosinophils) and Mono (Monocytes).

Table 3: Results of the muscle and liver electrolytes values of *Heterobranchus bidorsalis* exposed to sublethal concentrations of rhonasate 360SL after 14 days.

<table>
<thead>
<tr>
<th>Conc. of Rhonasate (mg l⁻¹)</th>
<th>Na⁺ (mmol l⁻¹)</th>
<th>K⁺ (mmol l⁻¹)</th>
<th>Ca⁺ (mmol l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td>Muscle</td>
<td>Muscle</td>
<td>Muscle</td>
</tr>
<tr>
<td>0.00</td>
<td>3.50±0.86⁣a</td>
<td>7.50±0.30⁣a</td>
<td>14.03±0.12⁣a</td>
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<tr>
<td>1.00</td>
<td>6.00±0.10⁣a</td>
<td>8.50±0.00⁣a</td>
<td>16.25±0.06⁣a</td>
</tr>
<tr>
<td>2.00</td>
<td>5.00±0.22⁣a</td>
<td>8.00±0.01⁣a</td>
<td>10.10±0.11⁣a</td>
</tr>
<tr>
<td>3.00</td>
<td>5.00±0.05⁣a</td>
<td>8.50±0.00⁣a</td>
<td>9.85±0.01⁣a</td>
</tr>
</tbody>
</table>

Means within column different superscript are significantly different (p<0.05).
RESULTS AND DISCUSSION

Haematological parameters

There was observed fluctuations in values of various blood parameters due to exposure of fishes to rhonasate 360SL. The highest concentration (0.30 mg l⁻¹) unveiled a profound reduction in values. The red cell (RBC) reduced from 2.25 gl l⁻¹ to 1.60 gl l⁻¹ while the white blood cells reduced from 203.50 to 179.35 gl l⁻¹. The xenobiotic may have interfered with the process of neural transmission, blocking of ionic channels. Similar result was also obtained when Clarias gariepinus were exposed to diazinon (an organophosphate insecticide), Inyang (2008), Omoniyi et al. (2002) also reported reduction in haematological parameters such as haematocrit, haemoglobin, leucocytes and RBC when they exposed Clarias gariepinus to tobacco leaf extract. The reduction in blood parameters may imply that the primitive stem cells responsible for blood production in the experimental fish have been hampered due to the toxic effect of the toxicant. This reduction effect could result in anaemia or leucopenia (Low WBC) as reported by Dixon and Dick (1985).

White blood cells decrease is contrary to the conventional belief that it usually increase as a result of antitoxic response against foreign bodies which always result in profound production of antibodies to fight the lethal effect of the toxicant (Inyang, 2008). The possible explanation is that the host immune system has been overpowered or eventually destroyed by the xenobiotic, hence exposure of Heterobranchus bidorsalis to rhonasate may have caused a depression in the established total immune response against a variety of antigenic substances. Haemoglobin is the chemical substance responsible for the red pigmentation of the blood. Its function is tremendous as it is responsible for carrying oxygen in the blood. The dose dependent reduction in values was observed. The toxicant may have disrupted the synthetic pathway of the compound (haemoglobin) by affecting the activity the enzymes involved in the synthesis of haemoglobin. Gaafet al. (2010) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological conditions in fish exposed to toxicants.

Thrombocytes (platelets) are nucleated cells which are responsible for blood clothing in fish. Decreased values observed in this study may signify the effect on thrombocyte production. Toxic substances, as observed by Calbreath (1994) to bone marrow, spleen, kidney and other haematopoietic organs may cause decreases in the number of circulating thrombocytes, reticulocytes and leucocytes, a condition known as pancytopaenia. This seems to be the situation observed in this study. The immunological parameters (differential count) values were not significant. The values stabilizes as the concentration of the toxicant increased (Table 2) evidence that the toxicant did not affect these parameters or the probe organism was able to metabolize this xenobiotic and reduce less toxic substance.

Electrolytes (ions)

Sodium and potassium are essential for the activity of many enzymes and have been implicated in the transport of Adenosine triphosphate (ATP) which participates in several metabolic processes. Sodium and potassium ATpase are located in the cell membrane, both are involved in the active transport of Na⁺ and K⁺ across the cell membrane (Rajano, 1980). The present result unveiled a clear stabilization of values in both organs (Muscle and liver), Na⁺ and Ca²⁺ electrolytes in the organs were not significant, akin to potassium in the liver of the experimental organism, except K⁺ in the muscle. The stabilization of values according to Ogamba et al., (2011) could be a stress induced response occasioned by the chronic exposure of fish to toxicants which may have activated certain physiological and metabolic mechanisms that could lead to a rapid uptake of electrolytes from water, food material and reduction of ion-efflux. Ca⁺, K⁺ and Na⁺ ions functionally participate in maintaining normal irritability of the heart, muscles and nerves as well as selective permeability of cell membrane. Therefore the significant decreases (p<0.05) in concentrations of K⁺ in the muscle of the probe organism indicated toxic effect on the pesticide tested. Additionally, the ion changes observed are presumably the result of temporary ionic imbalances associated with increased muscular activities of the probe organism and subsequent alterations in ionic fluxes across the muscle membrane due to exposure to rhonasate 360SL.

CONCLUSION

This research has unveiled the toxicity of rhonasate 360 SL, containing glyphosate on haematological parameters in Heterobranchus bidorsalis. These haematological parameters could serve as a biomarker of sublethal effects of rhonasate 360SL, containing glyphosate in the aquatic environment, hence the use of this xenobiotic close to aquatic environment should be done with caution.

REFERENCES


Shah, S. L. and Attingdag, A. 2005. Alterations in the immunological parameters of teh (Tincatinca) after acute and chronic exposure to lethal and sublethal treatments with mercury, Cadmum and lead. Turkish journal of Veterinary and Animal Sc 29:1163 -1168.