Behavioural, haematological, biochemical and histological changes in African Catfish, *Heterobranchus bidorsalis* (Geoffrey Saint-Hilaire, 1809) exposed to Orizoplus®

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**Abstract**

Worldwide, herbicides are used to control weeds to boost crop productivity, however, they could harm non-target species like fish if misapplied. This study evaluates the effect of Orizoplus®; a commonly used herbicide on the behaviour, haematology, blood biochemistry and kidney histology of juvenile catfish, *Heterobranchus bidorsalis*. Juvenile fish weighing 12.6.01±5.43g were exposed to sublethal concentrations of Orizoplus® for 96h. Thereafter, the water quality and behavioural, blood biochemistry and histopathological changes of the fish were monitored. The results showed concentration-dependent changes in behaviour. The white blood cell increased significantly (p<0.05) by 107-112% while red blood cells, haemoglobin, mean cell corpuscular volume, mean corpuscular haemoglobin concentration and mean cell haemoglobin content reduced non-significantly (p>0.05) in the test organisms compared to the control. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) increased significantly (p<0.05), however, changes in the serum glucose, protein and bilirubin were not significant (p>0.05). Histopathological changes in the exposed fish were moderate shrinking of the glomerulus, eosinophilic appearance of the tubules, focal distortion of the glomerulus and severe loss of renal tissues. These show that Orizoplus® is toxic to fish and proper regulation and education are required to guide against misuse.

**Introduction**

Farmers target maximum crop productivity, however, weeds if uncontrolled could thwart this goal and reduce yield (Cheikyula et al 2019). Several articles have shown that uncontrolled weed can reduce crop production through allelopathy, parasitism and competition for resource such as nitrogen, water and light (Teyker et al 1991; Radioglu et al 2005; DiTommaso et al 2016; Colbach et al 2020). Therefore, herbicides are applied before or during planting to eliminate weeds and enhance crop production (Santos et al 1998; Colbach et al 2020; EPA 2022).

Orizoplus® is the trade name of Propanil (3, 4-dichlorophenylanilide) herbicides sold in Nigeria and is commonly used for post emergence weed control. Orizoplus® contains 360g propanil and 200g of 2, 4-dichlorophenoxyacetic acid (2, 4-D) per litre (Okoh 2015; Cheikyula et al 2019). Propanil (3, 4-dichlorophenylanilide) is a selective acylamine herbicide used widely in rice cultivation in many parts of the world (Roberts et al 2009). It may be the most extensively used herbicide for rice production worldwide (Webster et al 2018) and is ranked within the top 20 pesticides used for agriculture in the United States (Valbuena et al 2021). Propanil is made industrially by nitrating of 1, 2-dichlorobenzene, reduction with catalytic hydrogen, and then reacted with acetyl chloride (Wyatt and Warren 2008). Propanil is mobile, with potential for transport via water (Liu 2014). Propanil is rapidly metabolized under anaerobic and aerobic conditions in water and soil matrices. Its half-life in water is 1-4 days and it could still be detected more than 8 days after application (Santos et al 1998).

The second constituent, 2, 4-dichlorophenoxyacetic acid (2, 4-D) is a commonly and widely used herbicide to control broadleaf weeds (Sarikaya and Yilmaz 2003; Okogwu et al 2015). It was the fifth most heavily applied herbicide in the US agricultural sector in 2012 (Freisthler et al 2022). It kills plants selectively by disrupting several enzymatic activities, inhibiting photosynthesis and obstructing water and food transportation (Barbieri 2008). In Nigeria, 2, 4-D is widely used in rice paddies to control weeds and increase yields (Okogwu et al 2015).

Overall, herbicides are necessary agricultural tools for enhancing crop production. Unfortunately, due to extensive use and careless handling consequent to application by non-professionals, herbicides can reach aquatic habitats (Nwonumara and Okogwu 2020). On entering the aquatic ecosystems, they imperil organisms by direct toxicity or indirectly by reducing water quality. Many studies have reported direct toxicity of herbicides to fish. These studies have shown that herbicides affect the behaviour, haemotology, blood biochemistry and

Indirectly, herbicides and other xenobiotics can affect aquatic organisms by altering the physicochemical variables of water. Reports have shown that herbicides can reduce pH and dissolved oxygen levels in water (Okogwu et al 2015; Elebe 2022). This can lead to water acidification and hypoxia, conditions that can cause massive fish kill (Okogwu 2014; Okogwu 2016; Okoh 2018).

This study aims to evaluate the effect of sublethal concentrations of Orizoplus® on water physicochemistry and behaviour, haematological, biochemical and histological parameters of Heterobranchus bidorsalis in order to deepen understanding on the toxicity of herbicides to non-target species. In addition, the results will assist policy makers to make appropriate environmental laws for proper application of herbicides and sustainable management of aquatic ecosystem.

Materials and methods
Experimental fish and herbicides
Heterobranchus bidorsalis juveniles weighing 12.60±1.43g were procured from a fish farm in Abakaliki and then transported in an aerated plastic container to the laboratory of Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. The fish were acclimated for two weeks before commencement of the experiment during which they were fed commercial diet at 3% of their body weight. The herbicide, Orizoplus®, containing 200g of 2, 4-Dichlorophyosnoxyacetic acid and 360g of propanil, manufactured by Proficol- Columbia was used for the experiment.

Experimental design
After the period of acclimation, several tests were conducted to determine the concentrations of the herbicide that produce a range of effects in accordance with OECD (2014) recommendations. Thereafter, experiments were conducted to determine the median lethal concentration (LC50) of Orizoplus® at 96 hours. Six concentrations of Orizoplus®, 0.00 (control), 70.00, 100.00, 130.00, 160.00 and 190.00µg/l were used for the test based on the results of the range finding test. The experiment was performed in triplicate plastic tanks of 60×30×30cm (L×B×H) dimensions containing 10 fish each in 20 litres of water for each treatment. During the experiment, dead fish were swiftly removed to prevent additional fouling of the water. The LC50 value of Orizoplus on H. bidorsalis was calculated using the probit analysis approach as described by OECD (2014) and Okogwu et al (2022).

Sublethal test
After the period of acclimation, 10 fish in each group were exposed to three sublethal concentrations of Orizoplus® of 9.19% LC50 (4.50µg/l), 11.11% LC50 (5.44µg/l) and 12.45% (6.10µg/l) and a control group (0.00µg/l) in 20l of water. The physicochemical parameters of the water (pH, conductivity, total dissolved solids, dissolved oxygen and temperature) were measured using Hanna digital instruments in accordance to APHA (2005). The fish behaviour such as swimming rate, hyperactivity, convulsions, equilibrium status, somersaulting, and operculum and fin movement, were noted in both exposed and control groups at 24, 48, 76, and 96 hours after exposure following the methods of OECD (2014).

Haematological, biochemical and histopathological analyses
At the end of the 96-hour exposure, blood was collected from two fish from each tank (six fish per group) from the caudal vein and transferred to plain tubes and tubes containing anticoagulant, potassium salt of ethylene diamine tetraacetic acid (EDTA) and sodium fluoride. Blood in the plain tubes was used for biochemical analysis, whereas blood in the EDTA tubes was used for haematological analysis and blood in the fluoride tubes was used specifically for glucose measurement. The fish were then dissected, and the kidneys removed and processed for histopathological examinations as described in Si-Tayeb et al (2010) and Elebe (2022).

The blood parameters, red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), and haemoglobin (Hb) concentration were assayed according to the method of Ochei and Kolhatkar (2008). The wintrobe indices; mean cell corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and mean cell haemoglobin content (MCH) were derived from the main indices.

The biochemical parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) activities were measured using the technique described in the Randox kit by Reitman and Frankel (1957) and Elebe (2022). Total protein, glucose, bilirubin levels in the plasma were determined as detailed in Elebe (2022).

The kidneys for histopathological analyses were collected from six fish per group and preserved in 10% normal saline in airtight container for one week. Thereafter, the tissues were processed as detailed in Elebe (2022) according to the methods of Si-Tayeb et al (2010) and then mounted in D.P.X. for histopathological examination under the microscope.

Data analysis
The LC50 was determined by Finney’s Probit Analysis (Sarikaya and Yilmaz 2003). One-way analysis of variance (ANOVA) was used to test the difference in the variables measured between the control and treatments. Values were considered significant at p<0.05. All statistical analyses were carried out using Statistical Package for Social Science, SPSS version 21.1.

Results
Median lethal concentration
The LC50 of Orizoplus® was estimated as 48.98µg/l by the Probit technique (Figure 1).
Physicochemical variables of test water
The temperature increased from 27.50±0.03 to 28.20±0.10°C, the pH decreased from 7.27±0.03 to 6.93±0.09 while the dissolved oxygen (DO) decreased from 5.25±0.55 to 3.13±0.20mg/l compared to the control. Total dissolved solid (TDS) and conductivity were 393.67±26.96mg/l and 812.33±11.67µS/cm, respectively in the highest treatment. The temperature, pH, DO and conductivity varied significantly (p<0.05) between test water and the control (Table 1).

The fish in the control group exhibited normal behaviour throughout the acute toxicity bioassay, but the fish in the toxicant doses showed uncoordinated activity such as vigorous fin and opercula movement, erratic swimming convulsions and somersaulting as well as air gulping. The behaviours were both time and concentration dependent. These behaviours were classified as none, mild, moderate or strong based on the degree of reactions observed (Table 2).

Heamatological, biochemical and histopathological changes All exposures showed a non-significant (p>0.05) decrease in RBC, PCV and Hb levels when compared to the control. In contrast, the value of WBC increased significantly (p<0.05) in all treatments when compared to the control (Table 3).

The AST decreased from 58.20±0.55 to 50.40±0.65, the ALP decreased from 69.20±1.22 to 68.10±0.96 and ALT decreased from 50.40±0.55 to 49.20±0.56. The Glucose value, protein and bilirubin showed no significant difference from the control. The kidney of fish from the control group showed normal glomeruli, tubular cells and renal architecture. However, fish exposed to different concentrations of Orizoplus® exhibited different abnormalities in the kidney (Plate 1). In the group exposed to 4.50µg/l, glomerulus shrinkage (MSG) and eosinophilic appearance of the tubules were observed. The kidney of the 5.44µg/l group showed distortion of the glomerulus (FDG) and tubules, while the 6.10µg/l group showed sever pathological damages such as loss of glomeruli and substantial loss of kidney tissue (Plate 1).

**Table 1:** Physicochemical parameters of the experiment water

<table>
<thead>
<tr>
<th>Concentration of Orizoplus®</th>
<th>Temperature (ºC)</th>
<th>pH</th>
<th>DO (mg/l)</th>
<th>Conductivity (µS/cm)</th>
<th>TDS (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.63±0.09a</td>
<td>7.50±0.06a</td>
<td>5.25±0.55a</td>
<td>760.37±1.45d</td>
<td>367.33±13.72d</td>
</tr>
<tr>
<td>4.50µg/l</td>
<td>27.70±0.06b</td>
<td>7.40±0.06b</td>
<td>5.10±0.12b</td>
<td>765.00±3.06b</td>
<td>378.88±5.24d</td>
</tr>
<tr>
<td>5.44µg/l</td>
<td>27.50±0.03b</td>
<td>7.27±0.03b</td>
<td>4.50±0.17b</td>
<td>781.33±4.41b</td>
<td>394.00±5.04a</td>
</tr>
<tr>
<td>6.10µg/l</td>
<td>28.20±0.10b</td>
<td>6.93±0.09b</td>
<td>3.13±0.20b</td>
<td>812.33±11.67b</td>
<td>393.67±26.96d</td>
</tr>
</tbody>
</table>

Values with different alphabetic superscripts differ significantly (p<0.05) between different concentrations of Orizoplus®

**Table 2:** Behavioural changes in *H. bidorsalis* exposed to different concentrations of Orizoplus® and control

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>4.50µg/l</th>
<th>5.44µg/l</th>
<th>6.10µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td>-</td>
<td>-</td>
<td>++</td>
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<tr>
<td>48h</td>
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<tr>
<td>72h</td>
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<td>96h</td>
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<tr>
<td>120h</td>
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<tr>
<td>144h</td>
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<tr>
<td>168h</td>
<td>-</td>
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<tr>
<td>192h</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

**Keys:** - = None, + = Mild; ++ = Moderate; +++ = Strong, TC = Toxicant concentration
Table 3: Haematological parameters (mean±SE) of juvenile H. bidorsalis exposed to different concentrations of Orizoplus® and control

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Concentrations of Orizoplus®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4.50µg/l</td>
</tr>
<tr>
<td>RBC (×10⁶)</td>
<td>9.67±0.86a</td>
<td>9.42±0.87a</td>
</tr>
<tr>
<td>WBC (×10³)</td>
<td>8750±6.80a</td>
<td>9400±7.20b</td>
</tr>
<tr>
<td>Hb (%)</td>
<td>6.90±0.56a</td>
<td>7.30±0.60a</td>
</tr>
<tr>
<td>PCV(%)</td>
<td>32.00±1.03a</td>
<td>32.04±0.98a</td>
</tr>
<tr>
<td>MCH (pg cell/l)</td>
<td>7.12±0.90a</td>
<td>7.75±0.84a</td>
</tr>
<tr>
<td>MCV (fcell/l)</td>
<td>33.09±1.00a</td>
<td>33.97±1.54a</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>21.56±0.44a</td>
<td>22.81±0.38a</td>
</tr>
</tbody>
</table>

Values with different alphabetic superscripts differ significantly (p<0.05) between different concentrations of Orizoplus®

Table 4: Biochemical parameters (mean±SE) of juvenile H. bidorsalis exposed to different concentrations of Orizoplus® and control

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Concentrations of Orizoplus®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4.50µg/l</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>48.50 ± 0.95a</td>
<td>58.20 ± 0.55b</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>60.00 ± 1.26a</td>
<td>69.20 ± 1.22b</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>43.40 ± 0.38a</td>
<td>50.40 ± 0.55b</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>50.50 ± 0.43a</td>
<td>51.00 ± 0.60a</td>
</tr>
<tr>
<td>Protein (mg/dl)</td>
<td>6.60 ± 0.13a</td>
<td>6.90 ± 0.14a</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>5.05 ± 0.11a</td>
<td>5.94 ± 0.15a</td>
</tr>
</tbody>
</table>

Values with different alphabetic superscripts differ significantly (p<0.05) between different concentrations of Orizoplus®

Plate 1. Histopathological changes in H. bidorsalis exposed to different concentrations of Orizoplus®. NG = normal glomeruli, NTC = normal tubular cells, MSG = moderate shrinking of the glomerulus, OAT = eosinophilic in the tubules, FDG = focal distortion of the glomerulus, FDT = focal distortion of tubules and SLRT = sever loss of renal tissue

Discussion

The present study revealed a dose-dependent significant (p<0.05) rise in temperature, which is in agreement with the findings of Nwani et al (2010), Nwani et al (2011) and Okogwu et al (2015). Temperature can increase the toxicity of xenobiotics such as herbicides in water. The dissolved oxygen level decreased as the concentration of herbicide increased. This trend is consistent with reports of Barton (2002), Sarikaya and Yilmaz (2003), Ayoola (2008a), Ayoola (2008b), Dogan and Can (2011), Nwani et al (2013), Okoh (2015), Okogwu (2015) and...
Du Preez et al. (2020). Oxygen depletion could lead to hypoxia, which could harm fish and other aquatic organisms (Du Preez et al. 2020).

In this study, the fish exhibited different behavioural changes that were concentration dependent. Some of the behaviours such as air gulping, vigorous fin and operculum movement and erratic swimming are signs of asphyxiation attributable to low levels of dissolved oxygen. Introduction of toxicants into an aquatic ecosystem may reduce the dissolved oxygen content and consequently inhibit respiration (Okogwu et al. 2014). This probably explains some of the behavioural changes observed in the test fish, which were also earlier reported by Okogwu et al. (2015). During the study, the pH decreased with an increase in herbicide concentration. Similar inverse proportional relationships between the pH of test medium and the concentration of the toxicant have been recorded by other researchers (Ayoola 2008a; Ayoola 2008b; Nwani et al. 2010; Nwani et al. 2011; Okogwu et al. 2015). Increased acidity of the water could be harmful to the fish because fish thrive in water with pH of 6.5 to 8.5 (Robertson-Brayan 2004).

The behavioural responses observed in this study (hyperactivity, mild swimming rate, convulsions, somersaulting activity, fin movement, and operculum movement), are consistent with published toxicological responses of fish to herbicide (Sarikaya and Yilmaz 2003; Okogwu et al. 2015; Okogwu et al. 2022)

Reduction in RBC, PCV, Hb and their derivatives (MCH, MCV, and MCHC) observed in the herbicide exposed fish have been previously reported by Shalaby (2007), Ramesh et al. (2009), and Okogwu et al. (2015). The increase in WBC is likely a defence mechanism against the toxicity of the herbicide (Velisek et al. 2008; Velisek et al. 2009; Okogwu et al. 2022). The increase in the activities of the liver enzymes AST and ALT can be related to necrosis-induced hepatic cell injury, whereas the increased level of ALP may be caused by cholestasis (Gjin 2021). These are probably due to detoxification response to the toxicity of xenobiotics. Changes in the levels of glucose, protein and bilirubin did not follow any discernible pattern. This contradicts well defined concentration dependent decline in these parameters in Clarias gariepinus exposed to the herbicide, primextra (Elebe 2022). This shows that the response of fish to herbicides depend on the type of herbicide and fish.

The histopathological damages observed in the kidneys of exposed fish are consistent with the findings of Okogwu et al. (2015) for C. gariepinus exposed to 2, 4-D, Ayoola (2008a) for Oreochromis niloticus exposed to glyphosate and Olufayo and Alade (2012) for H. bidorsalis exposed to cypermethrin. Pathological damages such as loss of glomeruli, congestion of kidney vessels and intra-renal haemorrhages could adversely affect the normal functioning of the kidney, leading to the death of fish. The survival rate, biological activity, osmoregulation, reproduction and buoyancy may all be impacted by histopathological abnormalities in important organs, which may ultimately compromise stock recruitment and conservation of H. bidorsalis in the wild.

Conclusion
The findings of this study revealed that the water quality declined with increased concentration of Orizoplus®. The haematological parameters (except WBC) of the exposed fish, H. bidorsalis followed the same pattern and severe histological damages were also observed in the kidney. This shows that this widely used herbicide is hazardous to aquatic ecosystem. There is therefore urgent need to safeguard aquatic environments from herbicide runoffs. Regulated use of these herbicides, restricted application by trained professionals and enforcing protective regulations are advocated as effective measures to preserve the quality of water bodies and conserve aquatic flora and fauna.

Acknowledgement
I am profoundly grateful to the Department of Applied Biology, Ebonyi State University for providing the facility used for this study.

Conflict of Interest
The author declares no conflict of interest.

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