The Zoologist
2024. Vol. 24: 30-36. http://dx.doi.org/10.4314/tzool.v24i1.6 Print ISSN 1596 972X, Online ISSN 2992-4030

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

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Received: 27 August, 2023
Revised: 03 May, 2024
Accepted: 02 June, 2024

Keywords: Behaviour, Haematology, Biochemical, Histology, *Heterobranchus bidorsalis.*

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; Kadioglu *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-dichloropropionanilide) herbicides sold in Nigeria and is commonly used for post emergence weed control. Orizoplus® contains 360g propanil and 200g of 2, 4-dichlorophynoxyacetic acid (2, 4-D) per litre (Okoh 2015; Cheikyula *et al* 2019). Propanil (3, 4-dichloropropionanilide) is a selective acylanilide 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 nitration 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-dichlorophynoxyacetic 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, haemtology, blood biochemistry and

histology of fish (Ayoola 2008a; Ayoola 2008b; Nwani *et al* 2010; Nwani *et al* 2011; Blahova *et al* 2014; Okogwu *et al* 2015; Madhu 2019; Nwonumara and Okogwu 2020; Salim *et al* 2021).

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 12.6.01±5.43g 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-Dichlorophynoxyacetic 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 (LC_{50}) 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 LC_{50} 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 lethal toxicity test, 10 fish in each group were exposed to three sublethal concentrations of Orizoplus® of 9.19% LC_{50} (4.50µg/l), 11.11% LC_{50} (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).

Heamatological, 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 LC_{50} 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 LC_{50} 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±0.10ºC, the pH decreased from 7.27±0.03 to 6.93±0.09 while the dissolved oxygen (DO) decreased from 5.25±05.50 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).

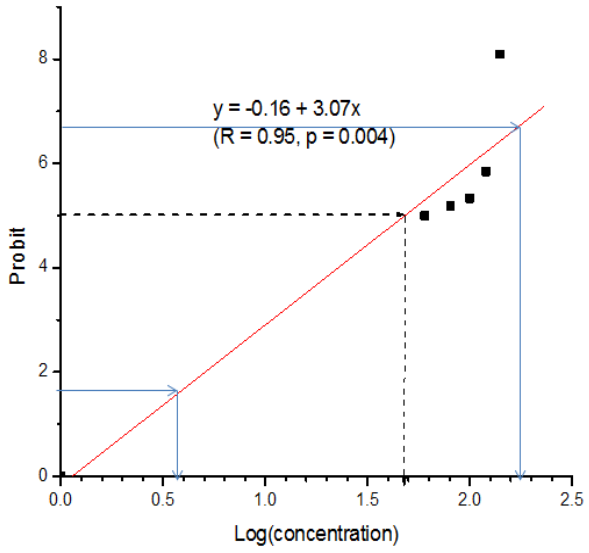

Figure 1. Median Lethal Concentration (LC_{50}) of Orizoplus in *H. bidorsalis.* (LC_{50}=48.98µg/l)

Behavioural changes

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

Concentration of Orizoplus®	Temperature (ºC)	pH	DO (mg/l)	Conductivity (µS/cm)	TDS (mg/l)
Control	27.63±0.09^a	7.50±0.06^a	5.25±05.50^a	760.37±1.45^a	367.33±13.72^a
4.50µg/l	27.70±0.06^b	7.40±0.06^b	5.10±0.12^b	765.00±3.06^b	378.88±5.24^a
5.44µg/l	27.50±0.03^b	7.27±0.03^b	4.50±0.17^b	781.33±4.41^b	394.00±5.00^a
6.10µg/l	28.20±0.10^b	6.93±0.09^b	3.13±0.20^b	812.33±11.67^b	393.67±26.96^a

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

	Concentration of Orizoplus®															
	Control				4.50µg/l				5.44µg/l				6.10µg/l			
Time (h)	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
Hyperactivity	-	-	-	-	+	++	+	+	++	++	+	+	++	++	++	+
Equilibrium status	+++	+++	+++	+++	++	+	++	++	+	+	++	++	+	+	+	++
Swimming rate	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	+
Gulping for air	-	-	-	-	-	+	+	+	+	+	+	+	++	++	++	+++
Convulsion	-	-	-	-	-	-	-	-	+	+	-	-	+	++	++	-
Somersaulting activity	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-
Fin movement	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	+
Opercular movement	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	+

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

		Concentrations of Orizoplus®		
Parameters	Control	4.50µg/l	5.44µg/l	6.10µg/l
RBC ($\times 10^6$)	9.67±0.86[a]	9.42±0.87[a]	8.66±0.66[a]	8.94±0.56[a]
WBC ($\times 10^3$)	8750±6.80[a]	9400±7.20[b]	9800±8.50[b]	9600±8.20[b]
Hb (%)	6.90±0.56[a]	7.30±0.60[a]	6.50±0.45[a]	6.50±0.34[a]
PCV(%)	32.00±1.03[a]	32.04±0.98$_a$	30.03±0.87[a]	31.02±1.05[a]
MCH (pgcell/l)	7.12±0.90[a]	7.75±0.84[a]	7.51±0.70[a]	7.27±0.67[a]
MCV (flcell/l)	33.09±1.00a	33.97±1.54a	34.64±1.02a	34.68±1.12a
MCHC gdl/l)	21.56±0.44a	22.81±0.38a	21.67±0.54a	20.97±0.42a

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

		Concentrations of Orizoplus®		
Parameters	Control	4.50µg/l	5.44µg/l	6.10µg/l
AST (IU/L)	48.50 ± 0.95[a]	58.20 ± 0.55[b]	50.40 ± 0.65[a]	54.20 ± 0.60[b]
ALP (IU/L)	60.00 ± 1.26[a]	69.20 ± 1.22[b]	74.40 ± 1.08[b]	68.10 ± 0.96[b]
ALT (IU/L)	43.40 ± 0.38[a]	50.40 ± 0.55[b]	48.00 ± 0.86[b]	49.20 ± 0.56[b]
Glucose (mg/dl)	50.50 ± 0.43[a]	51.00 ± 0.60[a]	50.20 ± 0.65[a]	54.05 ± 0.70[a]
Protein (mg/dl)	6.60 ± 0.13[a]	6.90 ± 0.14[a]	6.80 ± 0.20[a]	5.80 ± 0.16[a]
Bilirubin (mg/dl)	5.05 ± 0.11[a]	5.94 ± 0.15[a]	5.66 ± 0.16[a]	5.92 ± 0.18[a]

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

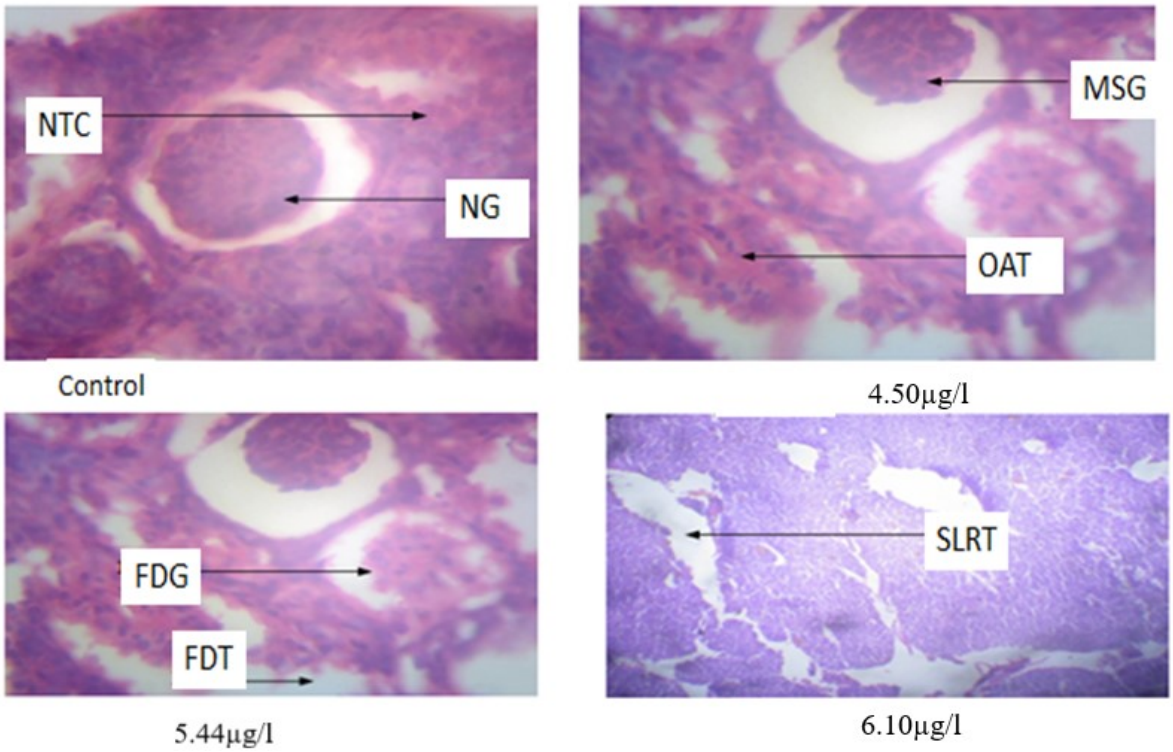

Plate 1. Histopathological changes in *H. bidorsalis* exposed to different concentrations of Orizoplus®. NG = normal glomeruli, NTC = normal tubular cells, MSG = moderate shrinking of the glomerulus, OAT= eosinophilic in the tubules, FDG = focal distortion of the glomerulus, FDT = focal distortion of 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), Ayaoola (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 opercula 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 nilotichus* 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 heamatological 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.

References

APHA. 2012. *Standard methods for the examination of water and wastewater* (21st ed.) American Public Health Association, Washington DC, 541pp.

Ayoola, S.O. 2008a. Toxicity of glyphosate herbicide on Nile Tilapia (*Oreochromis niloticus*). *Afr. J. Agric. Res. 3*: 825-834.

Ayoola, S.O. 2008b. Histopathogical effects of glyphosate on juvenile of African catfish (*Clarias gariepinus*). *Am-Eurasian J. Agric. Environ. Sci. 3*(*4*): 362-367.

Barbieri, E. 2008. Effect of 2,4-D herbicide (2,4-dichlorophenoxyacetic acid) on oxygen consumption and ammonium excretion of juveniles of *Geophagus brasiliensis* (Quoy & Gaimard, 1824) (Osteichthyes, Cichlidae). *Ecotoxicology. 18*:55-60.

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr. Comp. Biol. 42*: 517-525.

Blahová, J., Modra, H., Sevcikova, M., Marselek, P., Zelnikova, L. and Svobodova, M. 2014. Evaluation of biochemical, haematological, and histopathological responses and recovery ability of common carp (*Cyprinus carpio*) after acute exposure to atrazine herbicide. *Biomed. Res. Int. 14*: 1-8.

Cheikyula, J.O., Garba, A.A. and Omuwa, J.W. 2019. Acute Toxicity of Orizo-Plus. The Water Flea, *Moina Species*. *J. Res. For. Wildl. Environ. 11*(*4*): 20-22.

Colbach, N., Petit, S., Chauvel, B., Deytieux, V., Lechenet, M., Munier-Jolain, N. and Cordeau, S.

2020. The pitfalls of relating weeds, herbicide use, and crop yield: Don't fall into the trap! A Critical Review. *Front. Agron*. 2: https://doi.org/10.3389/fagro.2020.615470.

DiTommaso, A., Averill, K.M., Hoffmann, M.P., Fuchsberg, J.R., and Losey, J.E. 2016. Integrating insect, resistance, and floral resource management in weed control decision-making. *Weed Sci*. 64: 743–756. https://doi.org/10.1614/WS-D-16-00052.1.

Dogan, D. and Can, C. 2011. Haematological, biochemical, and behavioral responses of *oncorhynchus mykiss* to dimethoate. *Fish Physiol. Biochem. 37*: 951-958.

Du Preez, G., Fourie, H., Daneel, M., Miller, H., Höss, S., Ricci, C., Engelbrecht, G., Zouharand Wepener, V. 2020. Oxygen consumption rate of *Caenorhabditis elegans* as a high-throughout endpoint of toxicity testing using the Seahorse XF^{e}96 Extracellular Flux Analyzer. *Sci. Rep*. *10*: https://doi.org/10.1038/s41598-020-61054-7.

Elebe, F. A. 2022. Acute toxicity of Primextra Gold on freshwater fish *Clarias gariepinus* (Burchell 1822). Zoologist. 20: 101-107. http://dx.doi.org/10.4314/tzool.v20i1.13.

Elebe, F.A., Uka. U.N., Uhuo, C.A. and Mgbabu, C.N. 2018. Acute toxicity of dragon (Paraquate Dichloride) exposed to African freshwater fish, *Heterbranchus bidorsalis* (Geoffrey Saint-Hilaire, 1809). *Middle-East J. Sci. Res. 26*(*2*): 227-234.

EPA (Environmental Protection Agency). 2022. Herbicides, Overview, https://www.epa.gov/caddis- vol2/herbicides. Accessed 09 May, 2022.

Freisthler, M.S., Robbins, C.R., Benbrook, C.M. 2022. Association between increasing agricultural use of 2,4-D and population biomarkers of exposure: findings from the National Health and Nutrition Examination Survey, 2001–2014. *Environ. Health 21*(*23*). https://doi.org/10.1186/s12940-021-00815-x.

Gjin, N. 2021. Aspartate aminotransferase and cardiovascular disease-a narrative review. *J. Lab. Prec. Med.* 6:6-23.

Kadioglu, I., Yanar, Y., and Asav, U. 2005. Allelopathic effects of weeds extracts against seed germination of some plants. *J. Environ. Biol. 26:* 169-173.

Liu, J. 2014. Propanil in *Encyclopedia of Toxicology* (3rd ed.) https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/propanil. Accessed 05 June 2022.

Madhu, S. 2019. Behavioural responses in effect to chemical stress in fish: A review. *Int. J. Fish. Aquat. Stud. 7*(*1*): 01-05.

Nwani, C.D., Nagpure, N.S., Ravindra, K., Basdeo, K., Pavan, K. and Lakra, W.S. 2011. Mutagenic and genotoxic assessment of atrazine- based herbicide to freshwater fish *Channa Punctatus* (Bloch) using micronucleus test and single cell gel 35electrophoresis. *Environ. Toxicol. Pharmacol. 31*: 314-322.

Nwani, C.D., Naresh, S.N., Ravindra, K., Basdeo, K., Pavan, K. and Wazir, S.L. 2010. Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish *Channa punctatus* (Bloch). *Int. Aquat. Res. 2*: 105-111.

Nwani, C.D., Udu-Ibiam, F., Okoh, O., Uche-Obasi, R.C., Ogbonyealu, A., and O. UduIbiam, 2013. Acute toxicity of chloroacetalinide herbicide butachlor and effects on behavior in freshwater fish *Tilapia zilli*. *Afr. J. Biotech. 12*: 499-503.

Nwonumara GN and Okogwu O.I. 2020. Oxidative and biochemical responses as indicators of chemical stress in *Clarias gariepinus* (Burchell, 1822) juvenile exposed to glyphosate. *Sci. Afr. 8*: https://doi.org/10.1016/j.sciaf.2020.e00373.

Ochei, J.O. and Kolhatkar, A.A. 2008. *Medical Laboratory Science: Theory and Practice*, Tata McGraw-Hill Publishing Company Limited, New York, 745pp.

OECD (Organization for Economic Cooperation and Development) 2014. *Guideline for the testing of chemicals: Fish, acute Toxicity test.* OECD: Paris, 21pp.

Okogwu, O. 2016. Changes in muscle biochemistry, myoglobin and cytoglobin in goldfish *Carassius auratus* exposed to hypoxia-reoxygenation and microcystin RR. *Zool. Ecol*. *26*(*1*): 57-61. http://dx.doi.org/10.1080/21658005.2016.114270.

Okogwu, O.I. Elebe, F.A. and Nwonumara, G.N. 2022. Combinations of cypermethrin and dimethoate alter behavior, hematology and histology of African Catfish, *Clarias gariepinus Environ. Anal. Health Toxicol*. *37*(*4*): https://doi.org/10.5620/eaht.2022028.

Okogwu, O.I. Xie, P., Zhao, Y., and Fan, H. 2014. Organ-dependent response in antioxidants, myoglobin and neuroglobin in goldfish (*Carassius auratus*) exposed to MC-RR under varying oxygen level. *Chemosphere 112*: 427-434.

Okogwu, O.I., Anionwo, Q., Anoke, D.C. and Ugwuezi, P.O. 2015. Behavioural, haematological and histopathological changes in the African Catfish, *Clarias gariepinus e*xposed to 2,4-Dichlorophenoxyacetic Acid (2,4-D). *Nig. J. Biotech. 30*: 26-35.

Okoh, F.A.A. 2015. Behavioral responses of african freshwater fish *Clarias gareiepinus* to different concentrations of a commercial herbicide, Orizoplus, *J. Biol. Agric. Healthcare. 5*(*8*): 59-63.

Ramesh, M., Rinivasan, R. and Saravanan, M. 2009. Effect of atrazine (Herbicide) on blood parameters of common carp *Cyprinus carpio* (Actinopterygii: Cypriniformes). *Afr. J. Environ. Sci. Technol. 3*(*12*): 453-458.

Reitman, S. and Frankel, S. 1957. A colorometric method for the determination of serum glutamic

oxaloacetic and glutamic pyruvic transaminase. *Am. J. Clin. Pathol. 28*: 56-63.

Roberts, D.M., Heilmair, R., Buckley, N.A., Dawson, A. H., Fahim, M., Eddleston, M. and Eyer, P. 2009. Clinical outcomes and kinetics of propanil following acute self-poisoning: a prospective case series. *BMC Clin. Pharmacol. 9*: https://doi.org/10.1186/1472-6904-9-3.

Robertson-Brayan, O. 2004. *pH Requirements of Freshwater Aquatic Life*. Technical Memorandum. Robertson-Brayan Inc. California, 15pp.

Salim, A.M., Dauda, A.B. and Yusuf, M.A. 2021. Behavioural responses of african catfish (*Clarias gariepinus* Burchell, 1822) juveniles Exposed to acute concentrations of butachlor (herbicide). *FUDMA J. Sci. 5*(*1*): 472-476.

Santos, T.C.R. Rocha, J.C., Alonso, R.M., Martìnez, E., Ibañez, C. and Barceló, D. 1998. Rapid degradation of propanil in rice crop fields. *Environ. Sci. Technol. 32*(*22*): 3479–3484.

Sarakiya, R. and Yalmaz,R. 2003. Investigation of acute toxicity and the effect of 2,4-D (2, 4-dichlorophenoxyacetic acid) herbicide on the behaviour of the common carp (*Cyprinus carpio* L. 1758; Pisces, Cyprinidae). *Chemosphere 52*: 195-201.

Shalaby, A.M.E. 2007. Effect of EDTA on toxicity reduction of cadmium in relation to growth, some haematological and biochemical profiles of Nile tilapia (*Oreochromis niloticus*) *J. Fish. Aquat. Sci. 2*(*2*):100-109.

Si-Tayeb, K, Lemaigre, F.P. And Duncan, S.A. 2010. Organogenesis and development of the liver. *Dev. Cell*. *18*: 175-189.

Teyker, R., Hoelzer, H., and Liebl, R. 1991. Maize and pigweed response to nitrogen supply and form. *Plant Soil*. *135*: 287–292. http//doi.org/10.1007/BF00010918.

Valbuena, D., Cely-Santos, M., Obregón, D. 2021. Agrochemical pesticide production, trade and hazard: Narrowing the information gap in Colombia. *J. Environ. Manage. 286*: https://doi.org/10.1016/j.jenvman.2021.112141.

Velisek, J., Svobodov, Z. and Piackova, V. 2008. Effects of metribuzin on rainbow trout (*Oncorhynchus mykiss*) *Vet. Med. 53*(*6*): 324-332.

Velisek, J., Svobodova, Z., Piackova, V. and Sudova, E. 2009. Effects of acute exposure to metribuzin on some hematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.) *Bull. Environ. Contam. Toxicol. 82*(*4*):492–495.

Webster, E., Telo G., Blouin, D. and Mcknight, B. 2018. Imazethapyrplu propanil mixtures in imidazolinone-resistant rice. *Weed Technol. 32*(*1*): 45-51.

Wyatt, S. and Warren, P. 2008. *Organic Synthesis: The Disconnection Approach* (2nd ed.). Wiley-Blackwell, Oxford, 25pp.

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