HAEMATOXICITY, CONDITION AND ORGAN INDICES OF *Heterobranchus bidorsalis* TREATED WITH CYMBUSH UNDER LABORTAORY CONDITIONS

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ABSTRACT

Heterobranchus bidorsalis (mean length 31.50 ± 2.32 cm SD; mean weight 241.25 ± 30.39 g SD) were exposed to cypermethrin (5.00, 7.50, 10.00, 12.50 and 15.00 ppb) and a control (0.00 ppb) for 23 days. Fulton's condition was assessed at the beginning and end of the experiment. Organ (liver, kidney, heart and spleen) indices and haematological variables: packed cell volume (PCV) haemoglobin, Hb; leucocrit, erythrocyte sedimentation rate (ESR) white blood cells (WBC) thrombocytes, red blood cells (RBC) and their indices-Mean corpuscular haemoglobin, MCH; Mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were determined. The haematological responses to the cymbush exposure were very variable relative to the exposure concentrations. However, there was a decline in the PCV, WBC and lymphocytes (P < 0.05) values in exposed fish compared to their control values. Highest decline in the blood variables, except in the value of lymphocytes occurred at 150 ppb. Hb, leucocrit, eosinophil and neutrophil values in exposed fish were very variable. Thrombocytosis was the most blood responsive variable (25 units at 150 ppb, above the control). ESR value was generally raised in the exposed fish compared with the control (1.75 ± 0.48 mm/hr). RBC indices (MCHC, MCH and MCV) in exposed fish did not vary (P ≥ 0.05) from the control. Fish condition generally declined from the initial values but final condition was raised above the control in almost all the concentrations (P > 0.05). The changes in the organ weights in exposed fish were variable except in the kidney. Results from this study indicate that exposure to sub lethal levels of cymbush impacted mainly on the blood variables with minimal changes in the condition and organ indices of the exposed fish. Hence, indiscriminate application of the insecticide should be checked to reduce contamination of the aquatic environment to the barest minimum.

Keywords: gonadosomatic index, pyrethroids, agrochemical, biomarkers

INTRODUCTION

There is an increase in the use of pesticides in agricultural production, animal husbandry, post harvest technology and public health. Cymbush (cypermethrin, a pyrethroid pesticide) is a composite, broad spectrum, noncumulative and fast-acting neurotoxin used in Nigeria to control insects on crops and household pests. It is manufactured by Zeneca Inc., USA and was first marketed in 1977. The agrochemical has various trade names such as demon, ammo and cynoff. It has eight isomers and every of its container is a combination of the various isomers (Cox, 1996). The agrochemical is indiscriminately applied in the field and indoors to control pests without strict regulations in many parts of the country.

Contamination of the aquatic environment with the agrochemical can occur through rain flood, wind drift from field applications, precipitation and run offs to ponds, lakes, and rivers (Richardson, 1998). Excessive use of pyrethroids has led to contamination of the environment and water resources with poisoning and mass mortalities recorded in some cases (Agnihothrudu, 1988). This endangers all aquatic life forms directly and humans indirectly (Hill, 1989). Besides, the contamination of the aquatic environment with the agrochemical could result from direct applications (at low doses) to control fish parasites such as *Argulus* spp., eradicate the larvae of mosquitoes and frogs

during pond preparations for fish stocking (Adham *et al*., 2002).

According to USEPA, (1989) cymbush is highly toxic to fish and has a slow clearance rate especially from the adipose tissues after a single application which suggests potential bioaccumulation. Sensitivity of fish to pyrethroids could be due to a number of factors: (1) the slow metabolism and elimination of these compounds. The half lives for elimination of several pyrethroids in rainbow trout are usually more than 48 hrs compared with 6 - 12hrs for birds and mammals (Bradbury and Coats, 1989). (2) pryethroids are lipophilic and can be readily absorbed into and retained in the body of fish from very low concentrations in water (Dhawan and Kaur, 1996) and (3) it disrupts normal functioning of the nerves through repetitive and prolongation of nerve impulse action causing excitability and convulsion in exposed organisms (Ramadan, 1988). These may explain the reason why the chemical is reportedly very lethal to fish species with very low 96 hr L C_{50} value: juvenile *Tilapia guineensis* – 125 ppb (Chindah *et al*., 2002), *Clarias gariepinus* - 34 ppb (Gabriel and Kparobo, 2005) and 5.99 µg/l for alpha-cypermethrin for adult *Oreochromis niloticus* (Sarikaya, 2009).

Fish plays an important role in the food chain and therefore investigation of pesticide effects on fish has a diagnostic significance in the evaluation or examination of the adverse effects of pesticides to humans.

Fish blood responds sharply to pollution induced stress (Patil and Kulkarini, 1993) and therefore the study of changes in fish blood is of diagnostic importance. Studies involving several fish species have shown that cypermethrin affects various physiological processes in fish even at very low concentrations resulting in changes in haemtology (Adhikari *et al.,* 2004*;* Jee *et al.,* 2005; Velisek *et al.,* 2006). This is because blood variables readily respond to pesticide-induced stress and are good biomarkers of pesticide toxicosis (Ramesh and Saravanan, 2008).

Toxicants have also been found to cause changes in the weight and various abnormalities in the liver, gill and kidney of fish (Adams *et al.*, 1996). Lower gonadosomatic index and condition have been reported in European flounder, *Platichthys flesus* as a result of exposure to aquatic pollution (Kleinkauf *et al*., 2004). Changes in the weight of important organs of fish are good indicators of their health status and thus have been used by a number of authors to assess the effects of xenobiotics (Adams *et al.*, 1996). Such changes in the weight of important organs of fish if they occur in important commercial species like the clariids may give an indication of their health status.

This study was undertaken to assess the effect of cymbush, a synthetic pyrethyroid pesticide under laboratory conditions on the haematological parameters and organ indices of the catfish, *Heterobranchus bidorsalis*, an important commercial fish species in both open water fisheries and aquaculture in many tropical countries and Nigeria in particular.

MATERIALS AND METHODS

Heterobranchus bidorsalis (mean length 31.50 ± 2.32 cm SD; mean weight 241.25 ± 30.39 g SD) was obtained from a private farm and transported to the Laboratory, Department of Chemistry, Rivers State University of Science and Technology, Port Harcourt. They were acclimated individually to laboratory conditions for seven days in a 30 l aquarium with 10 l effective volume. The top of the aquaria were covered with perforated lid to avoid escape of fish. The fish was fed daily with a 35% crude protein diet at one percent biomass. The length and weight of the fish was recorded before exposure. The technical grade of cymbush (100 mg/l) was purchased off shelf from an outlet, Dizengoff Nig. Ltd., Port Harcourt. Details of the preparation of exposure concentrations, characteristics of borehole dilution water and exposure procedures are as reported in Gabriel *et al*. (2009). The fish was exposed to graded concentrations of cymbush (5.00, 7.50, 10.00, 12.50 and 15.00 ppb) and a control for 23 days. At the end of the experimental period blood samples were collected from the fish by kidney puncture behind the anal fin using a 2G hypodermic syringe and needle. Samples thus collected were stored in EDTA embedded bottles for haematological studies. The weight and length of the fish were taken. The fish was then killed with a blow on the head and the organs of interest (liver, kidney, heart and spleen) excised for organ indices assessment.

These organs were chosen because of the crucial roles they play in the physiological and biochemical management of toxicants in fish.

Standard haematological procedures (Brown, 1980) were employed in the determination of the various blood parameters. Packed cell volume (PCV) was determined with microhaematocrit after centrifuging at 3000 rpm for 10 minutes. Haemoglobin (Hb) was assessed by the cyanomethaemoglobin method and erythrocyte sedimentation rate (ESR) by the micro-Wintrobe method. Total white blood cell (WBC) and red blood cells (RBC) counts were determined manually with Neubauer haemacytometer using Nath-Herricks solution as diluents. White cell differential counts were done on blood film stained with May-Grunwald-Giemsa stain. The mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were also calculated. Thrombocytes were analyzed with haemacytometer which was placed under a microscope focused under high power and then counted in two large chambers. The organ indices and Fulton's condition factor of experimental fish were calculated with the methods described by Adams *et al*. (1996) as shown below:

Organ index = Weight of organ x 100/Weight of fish

Fulton's Condition = Weight of fish x $100/L^3$

L = Total length

Data obtained were subjected to a one way analysis of variance (ANOVA) and where differences existed among means, they were separated by Duncan multiple range test (Zar, 1996).

RESULTS

Generally the responses of the blood variables to different concentrations of cymbush were very variable and not directly concentration-dependent. There was a non-concentration dependent decline in the PCV, WBC and lymphocytes ($P < 0.05$) values in the treated fish compared to their respective control values -PCV, 24.25 ± 1.09 l/l; WBC, 13.78 ± 2.56 $x10^9$cells/l and lymphocytes, 81.05% (Table 1). All the blood variables had the highest decline at 15 ppb cymbush, except lymphocytes. Hb values in some of the treated groups (10 ppb, 5.57 ± 1.30 g/dl; 5 ppb, 4.80 ± 3.48 g/dl) were lower ($P < 0.05$) than the control value, 8.08 ± 0.40 g/dl (Table 1). Leucocrit values increased slightly from 5 ppb to 125 ppb (0.042 - 0.50 units) above the control value (1.25 ± 0.83%) but declined thereafter. Higher neutrophil values, 1.75 - 2.25 units above the control (18.50 ± 5.55%) were recorded at 5 and 15 ppb, respectively. Lower values relative to the control were recorded at 7.5 to 12.5 ppb with a minimum (10.67 ± 2.05%) at 10 ppb (Table 1). Eosinophil values were gradually raised at 5 ppb to a maximum, 21.75 ± 1.75% at 125 ppb but declined beyond this concentration (Table 1). Monocytes were absent in the control group and those exposed to 15 ppb cymbush. The values of monocytes and eosinophil in some of the treatment groups differed ($P \leq 0.05$) from the control values.

The toxicant caused thrombocytosis in the treated fish with a much higher value, 25 units at 15 ppb above that in the control, whereas that in the other concentrations were 6 - 12 units above that in the control (25.00×10^9 cells/l). Treatment with cymbush led to increased ESR values in the treated fish compared with the control (1.75 ± 0.48 mm/hr) except at 5 ppb. MCHC, MCH and MCV values did not show any variation from the control (Table 1). Liver weight was raised in all the concentrations ($P > 0.05$). Kidney weight was also raised in all the concentrations except at 12.5 ppb and both increment and decline were recorded in the weight of the heart and viscera of exposed fish relative to the control (Table 2). Fish condition generally declined from the initial values but was raised above the control in almost all the concentrations in the final condition at the end of the exposure (Table 2).

Table 1: Haematological Parameters of *Heterobranchus bidorsalis* exposed to cymbush for 23 days

Conc. of cymbush (ppb)	PCV	Hb	RBC	Leucocrit (%)	Total WBC	Neutrophils (%)	Lymphocytes (%)
0.00	24.25 ±1.09^{a}	8.08 ±0.40^{a}	2.55	1.25 ±0.83^{ab}	13.78 ±2.56^{a}	18.5 ±5.55^{a}	81.5 ±5.55^{a}
5.00	24.25 ±8.81^{a}	8.10 ±2.92^{a}	2.62	1.75 ±0.83^{a}	8.78 ±4.61^{ab}	20.25 ±1.05^{a}	76.75 ±7.99^{ab}
7.50	21.75 ±5.35^{ab}	7.23 ±1,79^{a}	2.30	1.75 ±0.43^{a}	11.60 ±5.75^{a}	17.25 ±5.36^{a}	74.50 ±11.41^{ab}
10.00	16.67 ±4.25^{b}	5.57 ±1.30^{b}	1.34	1.67 ±1.06^{a}	7.77 ±3.04^{ab}	10.67 ±2.05^{b}	78.67 ±7.54^{a}
12.50	21.75 ±1.79^{ab}	7.23 ±0.61^{a}	2.26	1.00 ±0.00^{ab}	6.90 ±4.21^{b}	17.00 ±7.71^{a}	68.75 ±6.06^{ab}
15.00	14.50 ±1.05^{bc}	4.80 ±3.48^{bc}	1.49	1.00 ±0.10^{ab}	6.28 ±4.51^{b}	20.75 ±1.03^{a}	74.75 ±7.82^{ab}

PCV - Packed cell volume (l/l), Hb - Haemoglobin (g/dl), RBC-Red blood cells (x10^{6} cells/l), WBC-White blood cells (x10^{9} cells/l). Means with the different superscript in the same column are not significantly different ($P < 0.05$).

Table 1 continued

Conc. of cymbush (ppb)	Eosinophils (%)	Monocytes (%)	ESR mm/hr	Thrombocytes x10^{9} cell/*l*	MCHC (g/dl)	MCH (pg)	MCV (fl)
0.00	0.00 ±0.00^{d}	0.00 ±0.00^{c}	2.50 ±0.10^{b}	25.00 ±0.00^{d}	33.00 ±0.00^{a}	31.75 ±0.87^{a}	95.75 ±0.87^{a}
5.00	1.00 ±0.12^{c}	2.00 ±0.72^{b}	1.75 ±0.15^{c}	31.25 ±1.83^{c}	33.50 ±1.00^{a}	31.50 ±1.00^{a}	94.75 ±1.64
7.50	6.25 ±1.08^{b}	2.00 ±0.72^{b}	4.25 ±0.97^{a}	31.25 ±1.83^{c}	32.50 ±1.00^{a}	31.25 ±0.83	94.75 ±1.64^{a}
10.00	8.67 ±3.37^{a}	1.50 ±0.29^{bc}	3.00 ±0.82^{ab}	50.00 ±2.41^{a}	33.33 ±0.47^{a}	31.00 ±0.00^{a}	94.00 ±0.82
12.50	10.50 ±0.90^{a}	3.75 ±0.68^{a}	3.00 ±0.82^{ab}	37.50 ±1.25^{b}	33.00 ±0.00^{a}	32.00 ±0.00^{a}	95.75 ±0.43^{a}
15.00	4.5 ±0.78^{b}	0.00 ±0.00^{c}	4.75 ±0.24^{a}	50.00 ±3.06^{a}	32.25 ±0.43^{a}	32.00 ±0.71^{a}	95.00 ±1.22^{a}

ESR - Erythrocyte sedimentation rate, MCHC - Mean corpuscular haemoglobin concentration, MCH - Mean haemoglobin concentration, MCV - Mean haemoglobin concentration. Means with the same superscript in the same column are not significantly different ($P < 0.05$).

Table 2: Condition factor and organ indices of *Heterobranchus bidorsalis* exposed to various concentrations of cymbush for 23 days

Conc. of cymbush (ppb)	Initial Condition	Final Condition	Renatosomatic index	Hepatosomatic index	Cardiosomatic index	Viscerosomatic index
0.00	0.72 ± 0.18	0.71 ± 0.20	0.67 ± 0.11	0.82 ± 0.33	0.16 ± 0.02	0.075 ± 0.02
5.00	0.75 ± 0.07	0.74 ± 0.15	0.69 ± 0.09	0.90 ± 0.12	0.15 ± 0.01	0.088 ± 0.01
75.0	0.75 ± 0.12	0.70 ± 0.26	0.74 ± 0.43	0.95 ± 0.11	0.17 ± 0.04	0.073 ± 0.02
10.00	0.83 ± 0.07	0.80 ± 0.11	0.72 ± 0.16	1.07 ± 0.47	0.16 ± 0.02	0.083 ± 0.01
12.50	0.82 ± 0.29	0.79 ± 0.28	0.65 ± 0.31	1.02 ± 0.13	0.18 ± 0.02	0.10 ± 0.01
15.00	0.81 ± 0.04	0.75 ± 0.03	0.71 ± 0.09	0.85 ± 0.09	0.16 ± 0.01	0.07 ± 0.02

DISCUSSION

The responses of the blood variables of the fish to cymbush exposure were very variable, similar to the trends reported in fishes exposed to various toxicants (Reddy and Bashmohideen, 1989; Das and Mukherjee, 2000). The responses of the various blood variables was a reflection of the impact of the agro-chemical on the different haematopoietic organs involved in the production of the blood components. At some concentrations their production was enhanced, whereas the reverse was the case at some other: an indication of the mode of action of the chemical on the mechanism of production of the blood components in the organs which may be concentration - dependent. In those concentrations where the PCV values declined, oxygen transport will be reduced limiting distribution to the various tissues of the organism thereby impairing the rate of the metabolic processes leading to low energy production (Ahmad *et al.*, 1995; Atamanalp and Yanik, 2003). However, at those concentrations with raised PCV values, there will be enhanced oxygen transport and associated metabolic activities. Decrease in the values of Hb, PCV, WBC, MCH and MCH were recorded in rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb (Atamanalp and Yanik, 2003). Conversely, Das and Mukherjee (2000) observed an increase in the Hb, erythrocytes and leucocytes in *Labeo rohita* fingerlings exposed to an organophosphate, quinalphos.

Decrease in Hb levels recorded in the treated fish may result from increased haemolysis or reduction in the rate of its synthesis (Reddy and Bashimohideen, 1989). A fall in PCV value may be due to the incorporation of the toxicant into the blood cells, affecting both their size and volume. Reduction in PCV and Hb reflect an anaemic condition resulting from haemodilution and impaired osmoregulation across the gill epithelia (Sampath *et al*., 1993) and the destruction of erythrocyte or inhibition of its production (Wintrobe, 1978). Reduction in PCV value may also show the extent of cell shrinkage and the possible interference of the agro-chemical in the physiology of RBC when compared to the value of MCV (Atamanalp *et al*., 2002).

The changes in the haematology variables associated with oxygen transport may have grave implications for the general wellbeing of the exposed fish.

Raised leucocrit values in the fish exposed to lower concentrations of the agrochemical (5 - 10 ppb) indicated that these were more stressed than those in the higher concentrations (12.5 – 15 ppb). The explanation for this trend in response is difficult as increasing concentrations of the insecticide is expected to cause increased stress response with a corresponding rise in leucocrit level. Interestingly Ariweriokuma (2010) made similar observation in *Clarias gariepinus* exposed to sublethal levels of the agro-chemical.

Information on trends in response pattern of leucocrit in fish exposed to pyrethroids and agrochemicals generally is scarce but fluctuating pattern in the leucocrit value similar to what was observed in this study was also made *Tinca Tinca* under heavy metal poisoning (Shah and Altindag, 2005). This suggests that the leucocrit level as a measure of stress may not always be directly concentration - dependent.

However, stress is known to cause hormonal changes which decreases the effectiveness of inflammatory response and also impairs the production and release of antibodies (Ajani, 2008). Disrupted inflammatory response could pre-dispose the fish to secondary affections by opportunistic pathogens that may cause diseases and may reduce the chances of escape from predators.

Leucocytosis or leucopaenia in fish exposed to the toxicant can result from decrease or an increase in the number of lymphocytes, eosinophils and thrombocytes (Shah and Altindag, 2005). Since WBC is concerned with the immune response in fish, a reduction in their number under sublethal concentrations of cymbush indicates immunodepression with the attendant vunerability to pathogens and other adverse environmental factors. Leucopenia was specifically attributed to decrease in number of lymphocytes in rainbow trout under metal poisoning (Kotsanis *et al*. (2000). The leucopaenia recorded in the experimental fish may be due to some other factors than specifically to lymphocytes as the number remained almost unchanged. Wepener *et al*. (1992), observed that decreases in the mean cellular lifespan and impaired proliferative capacity of cells were caused by a decline in lymph capacity of cells giving rise to lymphopaenia and necrosis of leucopoietic tissue. Impairment of haematopoietic cells in the kidney and the accumulation of

lymphocytes in lymphoid tissues or destruction of corticosteroid hormones are further implicated as being responsible the same physiological condition (Angeilidis *et al.*, 1987).

The variable pattern of response of the RBC indices (MCHC, MCH and MCV) under cypermehtrin toxicosis was related to that in the RBC. Prolonged reduction of haemoglobin is deleterious to oxygen transport, leading to blood dyscrasia and degeneration of the erythrocytes, which may be ascribed to a pathological condition in fish exposed to toxicants (Buckley *et al*., 1976). Its reduction suggests an appreciable decline in haemotopoiesis, a condition which may lead to various types of anaemia (Das and Mukherjee, 2000).

The number of thrombocytes determines the volume of blood circulating within the organism. Thrombocytosis observed in the treated fish suggests a haemodilution mechanism within the organism by which the blood volume is increased thus diluting the effect of the toxicant. This will reduce clotting time which may be advantageous to the fish in the event of any injury during exposure to the toxicant.

Thrombocytes help in building resistance to induced toxicities arising from external factors by increasing the concentration of WBC in the organism and are involved in the blood clotting mechanism during injury. ESR is a non- specific haemotological variable which indicates the presence and intensity of a diseased state (Wintrobe, 1978). Increased ESR has been observed by Gabriel *et al*. (2004) in sick *Clarias gariepinus* compared to apparently healthy ones, Onusiriuka and Ufodike (2000) *C. gariepinus* exposed to extracts of the plants *Blighia sapida* and *Kigelia africana*. ESR values are always raised along with tissue destruction, as well as acute infections and heavy metal poisoning (Blaxhall and Daisley, 1973). This suggests the exposed fish was stressed by the agrochemical compared with those in the control.

The condition factor is the overall plumpness of the fish. Although the holding condition in the laboratory affected the condition of the fish initially ($P > 0.05$), this was normalized by the end of the exposure period possibly by acclimation to the holding condition. Laboratory holding condition has been shown to impair the condition of experimental fish due to restricted feed supply and available space (Gabriel *et al*., 2010). Besides, diseases (Rehulka, 2003) and environmental contaminants (Adams *et al*., 1996) are also implicated in causing a decline in fish condition. Organosomatic indices as health assessment indices are qualitative indicators of general fish health where the individual matrix within the index is assigned a numerical value based on the degree of the severity or damage incurred by the organ or tissue from environmental stressors (Adams *et al*., 1996) although the variation in organ weight may not be directly dose-dependent. Cox (1996) reported that feeding of laboratory animals with cymbush resulted in weight loss and increase in the liver and kidney weights as was observed in the treated fish. The decrease in weight of organs of exposed fish may be due to the interference of cymbush with the normal metabolism of the fish.

Liver enlargement recorded in exposed fish is associated with hypertrophy or hyperplasia of liver cells, a condition commonly associated with pesticide toxicity (Sloof *et al*., 1983) or from increased lipid storage (Klaunig *et al*., 1979). The liver is the organ responsible for all the processes associated with the management of toxins in the fish. Therefore the increase in size may be a mechanism to cope with increased demand on its functions: detoxification, bio-transformation and other processes involved with effective and efficient management of the toxicant to reduce its toxic effects in the exposed fish. Increased liver weight under the toxicant exposure could result from increase in the production of endoplasmic reticulum for protein synthesis (Andersson *et al*., 1988).

The kidney and spleen are the haematopoietic organs in fish. Increase in the kidney size in particular is because of erythrocyte swelling, which is related to intracellular osmotic disorders and stress (Brucka-Jasatrzebska and Protosowicki, 2005). Cellular membrane damage of these organs may account for the reduction in lymphocyte counts due to the disintegration of immature lymphocytes in these organs (Williams *et al*., 1990). The heart is responsible for the distribution of blood in the body of animals and swelling may be to cope with the volume of blood produced in the spleen and kidney that were enlarged. However, this is not without serious clinical implications for the fish.

The present study strongly suggests that the agrotoxin impacted negatively on some of the blood variables of *H. bidorsalis*. Hence, continuous and unchecked exposure of the fish to the agrochemical

may greatly impair its yield especially in the open fisheries and a decline in the availability of this important source of protein.

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