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Biochemical, Haematological and Growth Responses of a Catfish, *Heterobranchus longifilis* (Valenciennes 1840) Exposed to Monocrotophos

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Abstract: Toxicological effects of monocrotophos (MCP) on some haematological, biochemical and growth parameters of *Heterobranchus longifilis* were investigated using static renewal bioassay. Based on the result of presumptive test, fish were exposed to both acute concentrations (0.00, 0.67, 0.72, 0.77, 0.82, 0.87µg/l) and sub-lethal concentrations (0.00, 0.20, 0.25, 0.30, 0.35µg/l) for 96 h and 28 days respectively. Fishes were sacrificed at the end of each exposure period for haematological, biochemical and growth analyses. In both the lethal and sub-lethal exposures, the physicochemical properties (biological oxygen demand, pH and temperature) of the water were within the NESREA Standard whereas the conductivity and dissolved oxygen were higher than the NESREA Standards. The 96 h LC_{50} of MCP to *H. longifilis* was 0.69µg/l. During acute and sub-lethal treatments, there were significant increases ($P < 0.05$) in the levels of white blood cell (WBC), serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and superoxide dismutase (SOD) when compared to the control, whereas the red blood cell (RBC), haemoglobin (HB), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) significantly decreased but with indefinite pattern of effect on lactate dehydrogenase (LDH) activity. In both the acute and sub-lethal exposure studies, activities of SOD and LDH in gills and liver increased significantly compared to the control, while ALT and AST decreased significantly. LDH decreased in the liver during acute exposure, Growth significantly reduced with increasing MCP concentrations. These findings showed that exposure to monocrotophos caused haematological and biochemical alterations in *H. longifilis*, which might lead to tissue/organ impairment and ultimately reduce the chances of survival of the fishes.

KEYWORDS: Biochemical parameters; Growth; Haematology; *Heterobranchus longifilis*; Monocrotophos

1.0 Introduction

Aquatic ecosystems have been a major repository for hazardous and toxic anthropogenic wastes generated from agricultural, industrial, domestic, sewage and drainage sources. Globally, this has been a serious concern particularly in developing countries. Organophosphorous pesticides (OPs) are popular and easily biodegradable insecticides used in the world. Their identification as having a short half life and ability to biodegrade make them a better alternative to the highly persistent and lipophilic organochlorines. However, they have greater acute toxicity and may portend serious danger to biota on exposure to high concentrations. Most OPs are highly soluble in water and can easily contaminate aquatic ecosystems, thus posing serious risk to aquatic biota.

Monocrotophos (Dimethyl (E)-1-methyl-2-methylcarbamoylvinylphosphate), popularly called Azodrin is an OP insecticide widely used in agriculture to eradicate insect pests of cotton, tobacco, rice, sugarcane, sorghum and vegetables (Maniyar *et al*., 2011). It is a broad-spectrum contact and systemic insecticide and acaricide but also used as avicide against birds in rice fields. MCP is one of the organophosphate pesticides that have received a great deal of regulatory pressure all over the world owing to its toxic effects on animals and humans.

Nigeria is one of the largest consumers of MCP in Africa and the indiscriminate use of this pesticide could cause serious ecological

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imbalance as many non-target organisms may be destroyed. Pesticides use and management in Nigeria raise a serious concern given the paucity of adequate information on their hazards and toxicity levels to organisms. Up till now, Nigeria is yet to conform to the FAO's international code of conducts on the use of pesticides. Although leading countries of the world such as USA, Australia, European countries and some Asian countries like China, Philippines, Thailand etc have banned the use of MCP (www.pan-uk.org/pestnews/actives/monocrot.htm accessed November 2013) due to its negative impact on the environment and its unacceptable health risk, its production and usage still persists in developing country like India (Maniyar *et al*., 2011). Similarly, its importation into Nigerian markets and other developing countries that have flair for cheap pesticides still continues.

Fish species serve as major test organisms in ecotoxicological assessment because of their link to man in the food chain. Fish's blood, gill, liver and kidney are known to be the primary target of pesticidal action where changes in the activities of enzymes and eventual pathological damage can easily be detected. The main route of entry of any pesticide is the gills from where it is transported to various parts of the body via the blood stream. The blood, gill, liver and kidney, therefore, provide a good medium for toxicological studies.

The genus, *Heterobranchus* is endemic to Africa and constitutes one of the main genera of economic value as food fish. *Heterobranchus longifilis* is harvested all year round in Nigerian rivers, lakes and swamps. The fish commands high market price because of its big size and is sought after because of its delicacy and good flesh. *H. longifilis* is widely cultivated in Nigeria as a result of its fast growth rate and ability to eat any available food in its environment. Despite the wide distribution of *H. longifilis* in Africa waters, the species has been observed to be declining through much of its range. For example, in northern Africa, water pollution through agriculture, domestic, commercial and industrial wastes has been implicated as one of the major threats to its survival (Azeroual, *et al*., 2010). However, not much is known on the widespread ecological threats to the species generally in Africa region and hence categorized by International Union for Conservation of Nature as "Least Concern" (Azeroual, *et al*., 2010).

The realization of the polluting and potential public health effects that could emanate from pesticides application, have prompted a number of investigations on the toxicity of MCP on finfish (Rao, 2004; Agrahari *et al*., 2007; Yaji and Auta, 2007; Maniyar *et al*., 2011), insect (Rao *et al*., 2005); daphnia (Wang *et al*., 2009); rat (Sunmonu and Oloyede, 2010). Rao (2004) observed that both MCP and its thiol analogs, 2-butenoic acid-3-(diethoxyphosphinothionyl) methyl ester (RPR II) and 2-butenoic acid-3-(diethoxyphosphinothionyl) ethyl ester (RPR-V) were highly toxic to *Oreochromis mossambicus* but the analogs were 65-fold more toxic. Yaji and Auta (2007) reported reduction in growth rate and food utilization in *Clarias gariepinus* exposed to sub-lethal concentrations of MCP. Agrahari *et al*. (2007) reported that MCP exposure induced significant biochemical changes in the blood plasma of *Channa punctatus*. MCP has also been reported to induce physiological stress in *Cyprinus carpio* resulting in differential oxygen consumption at higher concentrations (Maniyar *et al*., 2011). All these investigations further showed that various degrees of physiological alterations could occur following exposure of fish species to MCP. However, despite all these, information on the potential toxic effects of MCP on tropical finfish species is limited and none so far to the best of our ability has been reported on *H. longifilis*. The acute median lethal concentration (LC_{50}) values (the value at which 50% of the test organism would be killed by the toxicant) had been determined for many fish species and findings showed that the values varied among different species and in same species under different conditions (Pandey *et al*., 2008). However, there is dearth of information on the 96 h LC_{50} of MCP for tropical cultivable freshwater fish species. Available information is only restricted to the report by Thangnipon *et al* (1995) on *Oreochromis niloticus* (4.9 mg/l) and Rao (2004) on *O. mossambica* (11.51 mg/l). The situation with 96 h LC_{50} of MCP in *H. longifilis* is still obscured and this may have jeopardized the understanding of the potential health risk the

pesticide may portend on *H. longifilis* and other aquatic animals in African waters.

In Nigeria, fish is one of the commonest sources of protein. Thus pollution of water body, the home of fish, deserves greater attention. Since the health status of aquatic organisms is determined by the nature of aquatic ecosystem, knowledge about the effects of environmental toxicants discharged into water bodies before they affect aquatic biota become imperative. Information on such effects can be utilized by environmental managers, ecologists and conservationists for the formulation and implementation of ecosystem remediation and protection policies. This study was therefore undertaken to evaluate the acute (96 h LC_{50}) and sub-lethal toxicity of MCP on the behavioural changes and some haematological, biochemical and growth parameters of *H. longifilis* upon exposure to MCP.

2.0 Materials and Methods

2.1 Monocrotophos

Analytical grade of monocrotophos with 98% purity was purchased from Sigma-Aldrich (St Louis, USA) and used without further purification for the experiment.

2.2 Heterobranchus longifilis and handling

Juvenile *Heterobranchus longifilis* (average weight: 9.46 ± 0.77 g and average length 11.12 ± 0.80 cm) of the same brood stock were obtained from the hatchery of National Institute for Freshwater Fisheries Research (NIFFR), Kainji, Nigeria, and transported in oxygenated plastic bags containing water from the hatchery to the laboratory. Prior the trip, no food was administered to the specimen as well as on arrival at the laboratory until the next day; so as to minimize mortality. In the laboratory, the water from hatchery was replaced by well aerated borehole water and fish were held in a large tank of 800 litres capacity, maintained at 24-27^{o}C using a 300 Watt AZOO submersible thermometer with thermostat. The acclimatization was done for 14 days. Fish were fed twice daily at 9.00 am and 4.00 pm with commercial feed (pellets) at 3% body weight. Unconsumed food and faecal wastes were siphoned out regularly and water was changed every two days to reduce the risk of mortality due to accumulation and contamination of waste materials. Feeding was stopped 24 hours before the commencement of the experiment. The fish were checked for any infectious disease by monitoring any physical changes that include swimming activities.

2.3 Determination of water quality parameters

Water quality parameters such as dissolved oxygen (DO), temperature and pH were monitored throughout the experimental period using a digital CS-C933T Electrochemistry multimetre (Topac Instrument, Inc., USA). Conductivity was measured using conductivity meter (Eutech EC Testr 11 Pocket tester) and BOD was determined using a BOD metre (Aqualytic Sensor System, AL606). These probing instruments were dipped into water in the aquaria and the readings taken accordingly.

2.4 Behavioural response, lethal toxicity test and determination of 96 h LC_{50} value of MCP to H. longifilis

Based on the result of the range finding test, five varying definitive concentrations, 0.67, 0.72, 0.77, 0.82, and 0.87µg/l were prepared. Ten acclimatized fishes each of equal weight and size were introduced into each aquarium containing the different concentrations of the MCP and three replicates each of the set of concentration were made. The fishes were monitored for 96 hours. The test was carried out using a static renewal method to keep the toxicant's concentration constant (FAO, 1986). The behaviour of the fishes was monitored and those which did not respond to gentle prodding were considered dead (Rand and Procelli, 1985). Mortality was recorded for each concentration at 24, 48, 72 and 96 hours of exposure to the toxicant and dead fishes were removed from each aquarium immediately. The lethal concentration at which 50% of *H. longifilis* died (LC_{50}) was calculated for 96 hours of exposure using trimmed Spearman Karber Method (Hamilton *et al*., 1977). At the end of 96 hours, fishes were randomly collected from each

concentration and blood samples were collected for haematological and biochemical assays; after which the gill and liver were carefully removed for enzyme assay.

2.5 Chronic test and determination of growth parameters

A known volume (0.1) ml of MCP was introduced into 40 litres of borehole water and mixed thoroughly to make a stock solution of 1.0 µg/l. Of this, four different concentrations (0.20, 0.25, 0.30 and 0.35µg/l) were prepared and each introduced into ten litres of water. Ten fishes each were introduced into each concentration and their weight, using Ohaun compact digital weighing balance (Mettler Instrument), in each of the concentration recorded as initial weight (W_1). A common control was set-up for each of the concentration. Three replicates of the four set of concentrations and that of the control were also made. Fish were fed twice daily (0900 and 1600 hours) with commercial feed pellets at 3% of their body weight. Each aquarium containing the fishes was cleaned and the MCP was added every three days, to maintain a constant concentration throughout the exposure period. The fishes were weighed at the end of the experiment and recorded as final weight (W_2). Growth in this study was expressed as absolute weight gain, percentage weight gain and specific growth rate. Specific growth rate (SGR) was computed from the following expression:

$$SGR = 100 \times \ln W_2 - \ln W_1/t$$

where:

W_1 = initial weight of *H. longifilis* in each concentration;
W_2 = final weight of *H. longifilis* in each concentration; and
t = test duration in days.

The fish were analysed after 28 days.

2.6 Collection of blood and determination of haematological parameters

Blood samples were collected after 96 hours and 28 days for both acute and chronic tests respectively. Fish were lacerated at the ventral part near the caudal artery after which samples of blood was collected using heparinised capillary tubes swiftly covered with plasticin (to prevent air which can aid clotting of the blood from entering the tubes). RBC and WBC counts were estimated using an Electronic Cell Counter (Coulter Electronic Limited, England), standardized by an improved Neubauer haemocytometer after the blood had been diluted with Dacie fluid (Dacie and Lewis, 2001) and stained with brilliant cresyl blue. For the RBC count, a dilution of 1: 2000 was used, while for WBC count, a dilution of 1: 1, 500 was adopted. Haemoglobin (HB) concentration of the blood was carried out using the cyanomethalomoglobin reagent (Blaxhall and Daisley, 1973). Haematocrit (PCV) was determined by the microhaematocrit method (Nelson and Morris 1989). Erythrocytes related indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using the expressions reported by Dacie and Lewis (2001).

2.7 Preparation of serum and tissue supernatants and determination of biochemical parameters

At the end of 96 hours and 28 days exposure respectively, the fishes were sacrificed by medullar transection and dissected within three minutes on ice. An aliquot, 0.5 ml of the blood was centrifuged at 350 rpm for 5 minutes using a refrigerated centrifuge to obtain the serum used for the biochemical analyses. The gills and liver were quickly removed from the test fish and the control fish. A known weight (1 g) each of the tissues was homogenized in 5ml of 0.25 M of sucrose solution in ice cold condition. The homogenates were centrifuged at 14,700 x *g* for 20 minutes at 4^{o}C to obtain the tissue supernatants. Tissue and serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the method of Reitmann and Frankel (1957). Superoxide dismutase (SOD) was assayed using the procedure described by Misra and Fridovish (1972) while lactate dehydrogenase (LDH)

activity was assayed following the procedure highlighted by DGKC (1970). Determination of protein concentration in the serum was done using the method described by Lowry *et al* (1951). The glucose content of the blood was determined using the procedure described by Trinder (1969).

2.8 Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA). Significant difference between the treatment means was determined at $P < 0.05$ using Duncan Multiple Range test (Duncan, 1955).

3.0 Results

The water quality parameters determined did not show any significant difference ($P > 0.05$) between the various concentrations of MCP-contaminated water and the control and all the parameters were within the National Environmental Standards and Regulattions Enforcement Agency (NESREA) (2011) standard, except dissolved oxygen whose range of concentration (6.30-7.50 µg/l) was higher than the NESREA Standard (Table 1).

During the acute exposure, the fishes in the control group exhibited normal behaviour compared with those exposed to varying concentrations of the toxicant; where changes in behaviour such as loss of equilibrium, respiratory distress, jumping for air, reluctance in feeding, congregation at the bottom, erratic swimming, increase in opercula ventilation, skin darkening, copious amount of mucus on gill surface and inability to respond to gentle prodding were noticed. These behavioural responses were more pronounced at higher MCP concentrations (0.77, 0.82 and 0.87µg/l) and started by 38 minutes after the introduction of the fish into the MCP.

The percentage mortality of *H. longifilis* exposed to acute concentrations of MCP at 24 h interval is shown in Table 2. While no death of experimental animal was observed in the control, mortality tend to increase with concentration of MCP and exposure period; except in concentrations range of 0.77 to 0.87 µg/l at intervals of 24 and 48 hours and similarly between 48 and 72 hours of exposure at 0.67 µg/l of MCP. A mortality rate of 33.3% occurred at 0.87 µg/l of MCP after 96 hours of exposure. The median lethal concentration (LC_{50}) of MCP for *H. longifilis* was estimated to be 0.80, 0.77, 0.73 and 0.69µg/l after 24, 48, 72 and 96 hours of exposure respectively (Table 2). The lowest observable effect concentration (LOEC) and no observable effect concentration (NOEC) were estimated to be 0.25µg/l and 0.20µg/l respectively. The maximum allowable toxicant concentration (MATC) was 0.22 µg/l.

Changes in the blood profiles of *H. longifilis* during acute and chronic exposures showed that RBC, HB, PCV and protein decreased significantly ($P < 0.05$) from their respective control values, with the lowest recorded in the group of fishes that were exposed to the highest concentration of MCP. Whereas the WBC count and the glucose level of fish in both the acute and chronic exposures showed a significant ($P < 0.05$) increase when compared with the control group (Table 3), all the other haematological indices of RBC, HB, PCV, MCV, MCH and MCHC decreased significantly ($P < 0.05$) when compared with their respective control values. However, decrease in MCH values were disproportionately more than those of MCV and MCHC (Table 3).

Serum, gill and liver enzymes activities of *H. longifilis* following exposure to acute and chronic concentrations of MCP (Table 4) revealed a significantly higher ($P < 0.05$) elevation of ALT and AST activities in the serum, but with significant ($P < 0.05$) reduction in the gill and liver supernatants. The levels of SOD in the serum, gill and liver of the test fish in both exposures increased significantly as the concentration of MCP increased. However, in all the tissues, the activity of LDH did not follow a definite trend. The levels of LDH decreased with increasing concentration of MCP in the liver of fish during acute bioassay, while the reverse was the case in the gill of fish from the same assay. In the chronic test, however, the activity of the enzyme increased significantly ($P < 0.05$) in all the tissues; with peak value observed in the highest concentration of MCP.

At the end of the experiment, the weight gain and the percentage weight gain of the fish (Table 5) significantly ($P < 0.05$) reduced as concentra-

Table 1: Water quality parameters during exposure of *H. longifilis* to Monocrotophos

MCP Concentration (µg/l)	Water Quality Parameters				
	BOD (mg/l)	**Conductivity (µ/s)**	**Dissolved Oxygen (mg/l)**	**pH**	**Temperature (^{0}C)**
Acute (96 hours)					
0 (Control)	22.42 ± 0.0[a]	0.85 ± 0.01[a]	6.30 ± 0.02[a]	6.00 ± 0.02[a]	24.00 ± 0.01[a]
0.67	23.21± 0.02[a]	0.85 ± 0.03[a]	6.40 ± 0.01[a]	6.01 ± 0.02[a]	23.04 ± 0.02[a]
0.72	24.00 ± 0.02[a]	0.86 ± 0.01[a]	7.50 ± 0.10[a]	5.92 ± 0.01[a]	24. 04 ± 0.01[a]
0.77	24.03 ± 0.01[a]	0.86 ± 0.02[a]	7.30 ± 0.02[a]	5.89 ± 0.03[a]	23. 64 ± 0.02[a]
0.82	23.10 ± 0.00[a]	0.89 ± 0.00[a]	7.30 ± 0.02[a]	5.78 ± 0.12[a]	24.34 ± 0.03[a]
0.87	24.01 ± 0.00[a]	0.89 ± 0.02[a]	6.40 ± 0.02[a]	5.83 ± 0.02[a]	24. 19 ± 0.02[a]
NESREA Standard	**30.00**	**ND**	**4.00**	**6.00-9.00**	**<40.00**
Chronic (28 days)					
0 (Control)	19.00 ± 0.12[a]	0.71 ± 0.03[a]	6.70 ± 0.01[a]	6.42 ± 0.02[a]	23.42 ± 0.01[a]
0.20	19.42 ± 0.01[a]	0.71 ± 0.03[a]	6.80 ± 0.02[a]	6.50 ± 0.02[a]	23.03 ± 0.02[a]
0.25	19.02 ± 0.02[a]	0.70 ± 0.01[a]	6.70 ± 0.02[a]	6.70 ± 0.02[a]	24.04 ± 0.01[a]
0.30	19.20 ± 0.01[a]	0.71 ± 0.03[a]	6.70 ± 0.00[a]	6.49 ± 0.03[a]	24. 21 ± 0.03[a]
0.35	19. 45 ± 0.10[a]	0.72 ± 0.02[a]	6.70 ± 0.00[a]	6.66 ± 0.02[a]	24.00 ± 0.02[a]
NESREA Standard	**30.00**	**ND**	**4.00**	**6.00-9.00**	**<40.00**

Values are means ± SEM of three determinations. Means along the same column with same superscripts are not significantly different (P > 0.05).

MCP- Monocrotophos; BOD- Biological Oxygen Demand; ND- Not Determined; NESREA: National Environmental Standards and Regulations Enforcement Agency

Table 2: Mortality of *H. longifilis* following exposure to Monocrotophos

Duration of Exposure (h)	MCP concentration (µg/l) Mortality (%)						LC_{50}
	0 (Control)	**0.67**	**0.72**	**0.77**	**0.82**	**0.87**	
24	0	1 (8.33)	3 (14.29)	3 (16.67)	6 (22.22)	6 (20.00)	0.80 (0.77-0.84)
48	0	3 (25.00)	5 (23.81)	3 (16.67)	6 (22.22)	6 (20.00)	0.77 (0.70-0.84)
72	0	3 (25.00)	6 (28.57)	5 (27.77)	7 (25.93)	8 (26.67)	0.73 (0.67-0.79)
96	0	5 (41.67)	7 (33.33)	7 (38.89)	8 (29.63)	10 (33.33)	0.69 (0.65-0.74)

Values in parentheses denotes the mortality of *H. longifilis* in percentage while those for LC_{50} are range of values

Table 3: Haematological parameters of *H. longifilis* exposed to Monocrotophos

Concentration of MCP (µg/l)	Blood Parameters								
	RBC × 10^{12}/l	HB (g/dl)	PCV (%)	MCV (µl)	MCH (pg)	MCHC (g/dl)	WBC x10^{9}/l	Total Protein (g/l)	Glucose (mmol/L)
Acute (96 h)									
0 (Control)	3.20 ± 0.00^{f}	10.50 ± 0.00^{f}	32.01 ± 0.01^{f}	13.89 ± 0.04^{f}	35.55 ± 0.05^{e}	3.28 ± 0.00^{e}	2.04 ± 0.07^{a}	110.00 ± 0.00^{e}	4.90 ± 0.00^{a}
0.67	3.00 ± 0.11^{e}	10.30 ± 0.07^{e}	31.01 ± 0.04^{e}	11.73 ± 0.09^{e}	33.91 ± 0.03^{d}	3.32 ± 0.01^{e}	2.40 ± 0.00^{b}	102.00± 0.21^{d}	5.80 ± 0.07^{b}
0.72	2.70 ± 0.14^{d}	8.60 ± 0.14^{d}	29.00 ± 0.10^{d}	10.77 ± 0.06^{d}	31.85 ± 0.01^{c}	2.96 ± 0.05^{d}	2.41 ± 0.07^{b}	96.01 ± 0.01^{c}	6.51 ± 0.07^{c}
0.77	2.60 ± 0.07^{c}	8.20 ± 0.00^{c}	28.02 ± 0.02^{c}	10.74 ± 0.01^{c}	31.53 ± 0.08^{c}	2.92 ± 0.06^{c}	2.60 ± 0.07^{c}	96.00 ± 0.00^{c}	7.90 ± 0.07^{d}
0.82	2.30 ± 0.12^{b}	7.80 ± 0.14^{b}	27.00 ± 0.00^{b}	10.30 ± 0.02^{b}	28.27 ± 0.02^{b}	2.88 ± 0.08^{b}	2.68 ± 0.21^{d}	85.01 ± 0.07^{b}	8.00 ± 0.00^{e}
0.87	1.80 ± 0.07^{a}	6.40 ± 0.14^{a}	25.01 ± 0.21^{a}	10.00 ± 0.02^{a}	26.58 ± 0.01^{a}	2.55 ± 0.08^{a}	2.90 ± 0.00^{e}	79.00 ± 0.00^{a}	8.20 ± 0.07^{f}
Chronic (28 days)									
0 (Control)	3.10 ± 0.00^{d}	10.60 ± 0.00^{d}	30.00 ± 0.00^{d}	9.67 ± 0.01^{c}	34.01 ± 0.01^{d}	3.53 ± 0.03^{c}	2.49 ± 0.07^{a}	108.00 ± 0.00^{d}	6.00 ± 0.00^{a}
0.20	3.00 ± 0.00^{c}	10.00 ± 0.14^{d}	29.00 ± 0.01^{c}	9.66 ± 0.03^{c}	33.03 ± 0.03^{c}	3.44 ± 0.08^{b}	2.59 ± 0.07^{b}	97.50 ± 0.07^{c}	6.60 ± 0.07^{b}
0.25	3.00 ± 0.07^{c}	9.41 ± 0.00^{c}	28.01 ± 0.04^{c}	9.33 ± 0.00^{b}	31.36 ± 0.03^{b}	3.36 ± 0.02^{b}	2.59 ± 0.04^{b}	85.02 ±0.04^{b}	6.81 ± 0.28^{c}
0.30	2.80 ± 0.07^{b}	8.10 ± 0.04^{b}	26.00 ± 0.07^{b}	9.28 ± 0.05^{b}	31.15 ± 0.00^{b}	3.11 ± 0.01^{a}	2.70 ± 0.00^{c}	84.68 ± 0.06^{b}	7.18 ± 0.07^{d}
0.35	2.70 ± 0.01^{a}	7.95 ± 0.07^{a}	24.96 ± 0.04^{a}	9.23 ± 0.04^{a}	30.07 ± 0.02^{a}	3.06 ± 0.04^{a}	2.80 ± 0.01^{d}	76.46 ± 0.04^{a}	7.40 ± 0.07^{e}

Values are means ± SEM of three determinations. Means along the same column with different superscripts are significantly different ($P < 0.05$).
RBC = Red Blood Cell; HB = Haemoglobin; PCV = Packed Cell Volume; MCH = Mean Corpuscular Haemoglobin; MCV = Mean Corpuscular Volume; Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; and WBC= White Blood Cell

Table 4: Effect of monocrotophos on the selected enzyme activity of the gill, liver and serum of *H. longifilis*

Concentration of MCP (μg^{-1})	Serum				Gill				Liver			
	Enzyme Activities*											
	ALT	AST	LDH	SOD	ALT	AST	LDH	SOD	ALT	AST	LDH	SOD
Acute (96 h)												
0 (Control)	0.03 ± 0.00^{a}	0.04 ± 0.00^{a}	0.06 ± 0.00^{e}	0.09 ± 0.00^{a}	0.27 ± 0.00^{a}	0.30 ± 0.00^{a}	0.23 ± 0.00^{a}	0.40 ± 0.00^{a}	4.25 ± 0.00^{f}	4.13 ± 0.00^{f}	1.22 ± 0.28^{a}	0.50 ± 0.00^{a}
0.67	0.06 ± 0.00^{b}	0.11 ± 0.00^{b}	0.10 ± 0.01^{a}	0.14 ± 0.00^{b}	0.38 ± 0.14^{b}	0.33 ± 0.07^{b}	0.29 ± 0.01^{b}	0.61 ± 0.02^{b}	3.81 ± 0.06^{e}	3.92 ± 0.05^{e}	1.08 ± 0.01^{b}	1.20 ± 0.08^{b}
0.72	0.09 ± 0.00^{c}	0.16 ± 0.01^{c}	0.15 ± 0.01^{d}	0.19 ± 0.01^{c}	0.42 ± 0.07^{b}	0.41 ± 0.07^{c}	0.31 ± 0.02^{b}	0.80 ± 0.06^{c}	3.58 ± 0.04^{d}	3.52 ± 0.03^{d}	0.99 ± 0.01^{c}	1.40 ± 0.07^{c}
0.77	0.13 ± 0.01^{d}	0.20 ± 0.01^{d}	0.18 ± 0.01^{c}	0.20 ± 0.01^{c}	0.48 ± 0.07^{c}	0.41 ± 0.00^{c}	0.35 ± 0.08^{c}	0.81 ± 0.07^{c}	3.16 ± 0.05^{c}	3.08 ± 0.06^{c}	0.92 ± 0.02^{d}	1.41 ± 0.02^{c}
0.82	0.18 ± 0.00^{e}	0.25 ± 0.01^{e}	0.21 ± 0.01^{f}	0.25 ± 0.02^{d}	0.50 ± 0.08^{d}	0.46 ± 0.14^{d}	0.48 ± 0.07^{d}	1.00 ± 0.09^{d}	2.89 ± 0.03^{b}	2.96 ± 0.02^{b}	0.91 ± 0.01^{d}	1.60 ± 0.01^{d}
0.87	0.21 ± 0.01^{f}	0.30 ± 0.01^{f}	0.26 ± 0.01^{b}	0.31 ± 0.01^{e}	0.53 ± 0.02e	0.48 ± 0.00^{e}	0.54 ± 0.01^{e}	1.01 ± 0.05^{d}	2.60 ± 0.04^{a}	2.60 ± 0.04^{a}	0.90 ± 0.00^{d}	1.81 ± 0.01^{e}
Chronic (28 days)												
0 (Control)	0.04 ± 0.00^{a}	0.05 ± 0.00^{a}	0.20 ± 0.00^{b}	0.20 ± 0.00^{a}	0.39 ± 0.01^{b}	0.50 ± 0.04^{d}	0.20 ± 0.00^{a}	0.40 ± 0.00^{a}	9.68 ± 0.12^{e}	11.58 ± 0.14^{e}	0.68 ± 0.00^{a}	0.60 ± 0.00^{a}
0.20	0.05 ± 0.00^{b}	0.13 ± 0.00^{b}	0.19 ± 0.01^{a}	0.27 ± 0.00^{b}	0.39 ± 0.07^{b}	0.49 ± 0.06^{c}	0.19 ± 0.01^{a}	0.70 ± 0.07^{b}	8.59 ± 0.08^{d}	9.19 ± 0.14^{d}	1.12 ± 0.08^{b}	1.20 ± 0.07^{b}
0.25	0.06 ± 0.00^{c}	0.15 ± 0.02^{b}	0.38 ± 0.07^{c}	0.39 ± 0.07^{c}	0.38 ± 0.08^{b}	0.49 ± 0.07^{c}	0.38 ± 0.08^{b}	0.89 ± 0.05^{c}	7.68 ± 0.21^{c}	8.87 ± 0.07^{c}	1.22 ± 0.21^{c}	1.51 ± 0.21^{c}
0.30	0.06 ± 0.21^{c}	0.22 ± 0.01^{c}	0.39 ± 0.07^{d}	0.42 ± 0.00^{d}	0.30 ± 0.00^{a}	0.44 ± 0.02^{b}	0.39 ± 0.06^{b}	1.10 ± 0.01^{d}	5.01 ± 0.07^{b}	8.25 ± 0.25^{b}	1.22 ± 0.07^{c}	1.59 ± 0.14^{d}
0..35	0.20 ± 0.00^{d}	0.25 ± 0.01^{d}	0.56 ± 0.07^{e}	0.57 ± 0.01^{e}	0.29 ± 0.07^{a}	0.33 ± 0.00^{a}	0.56 ± 0.07^{c}	1.27 ± 0.06^{e}	4.80 ± 0.02^{a}	7.26 ± 0.01^{a}	1.40 ± 0.07^{d}	1.81 ± 0.14^{e}

Values are means ± SEM of three determinations. Values along the same column with different superscripts are significantly different ($P < 0.05$).
MCP = Monocrotophos ; ALT = Alanine aminotransferase; AST= Aspartate aminotransferase; LDH = Lactate dehydrogenase; and SOD = Superoxide dismutase
* Expressed in nm/min/mg protein

Table 5: Growth parameters of *H. longifilis* exposed to Monocrotophos

Concentration of MCP (µg/l)	Growth Parameters				
	W_1 (g)	W_2 (g)	W_3 (g)	W_4 (%)	SGR (g/day)
0 (Control)	9.98 ± 0.94^{a}	18.87 ± 0.86^{e}	8.89 ± 0.77^{e}	89.08	1.04 ± 0.06^{e}
0.20	9.93 ± 0.98^{a}	16.79 ± 0.81^{c}	6.86 ± 0.71^{d}	69.08	0.85 ± 0.03^{d}
0.25	9.86 ± 0.89^{a}	14.21 ± 0.69^{c}	4.35 ± 0.58^{c}	44.12	0.57 ± 0.01^{c}
0.30	9.80 ± 0.99^{a}	12.51 ± 0.75^{b}	2.74 ± 0.46^{b}	27.96	0.36 ± 0.03^{b}
0.35	9.73 ± 0.78^{a}	10.69 ± 0.72^{a}	0.96 ± 0.39^{a}	9.87	0.18 ± 0.05^{a}

Values are means ± SEM of three replicates. Means along the same column with different superscripts are significantly different ($P < 0.05$).
W_1 = Initial weight, W_2 = Final weight, W_3 = Weight gain, W_4 = Percentage weight gain, SGR = Specific growth rate.

tion of MCP increased compared with the lowest value recorded at the highest concentration of MCP. Similarly, SGR followed the same pattern of significant ($P < 0.05$) reduction in fish exposed to different concentrations of the MCP when compared with the control group.

4.0 Discussion

Fish species are useful indicators of aquatic pollution because of their close linkage to man in the food chain. The wide use of MCP provides many routes of entry into aquatic ecosystems, thereby exposing fish and other aquatic biota to pollutants that may affect them; thus threatening the conservation of this important resource on which man depend for food protein and livelihood. The absence of changes in water quality parameters reported in this study is an indication that the various concentrations of monocrotophos did not significantly affect the water quality to the levels that can enhance fish survival. The dissolved oxygen concentration obtained in this study (6.30-7.50 µg/l) was higher than the NESREA (2011) minimum acceptable limit (4.00µg/l), but conversely fall within Alabaster and Lloyd's (1980) recommended range of dissolved oxygen of 2-7mg/l; an evidence that the range of dissolved oxygen in the experimental aquaria was within the desirable range of fish culture. Although the levels of all physicochemical parameters investigated were within the NESREA (2011) standard (Table 1), the likely accumulation of MCP in the tissues of *H. longifilis* might have been sufficient enough to produce a stress response as reflected in the impairment of some of the physiological parameters of the fish. The 96 h LC_{50} value of $0.69 \mu gl^{-1}$ recorded in this study (Table 2) showed that *H. longifilis* is highly sensitive to MCP, suggesting a low resistance and appears not to be a potential candidate that could be considered for culture in the rice-cum-fish culture system where the pesticide is commonly used in Nigeria against birds. The high sensitivity to MCP and mortality of the fish during acute test could be explained by the fact that MCP might have acted as neurotoxicant by inducing oxidative stress in the fish. OPs have been implicated in causing serious damage to animals by inhibiting acetylcholinestarase (AChE) in cholinergic synapses and at neuromuscular junction. This occurs when their active derivatives (oxons) are activated forming an enzyme inhibitor complex (Barbieri and Ferreira, 2011). Although the present study did not investigate the effect of MCP on AChE activity, the observed behavioural changes were typical of neurotoxin toxicity. Similar behavioural changes have been reported in other fish species exposed to varying concentrations of MCP and other OPs (Rao, 2004; Kavitha and Rao, 2007; Maniyar *et al*., 2011). The mortality of this fish could have far reaching ecological implications as contribution to the food chain, through potentially viable recruits into the fishery, will be seriously impaired.

Very few comparative results of acute toxicity, 96 h LC_{50}, of MCP to tropical cultivable fish species are found in literature. Acute toxicity of MCP to *O. niloticus* for 96 h was found to be 4.9 mg/L (Thangnipon *et al*., 1995), while that of *O. mossambicus* was 11.51 mg/L (Rao, 2004), *Channa punctatus*, 18.56 ppm and *C. carpio* was 8.64 µg/L (Maniyar *et al*., 2011). However, the 0.69 µg/L recorded as 96 h LC_{50} for *H. longifilis* in this study is very low and not in agreement with previously reported values; indicating that *H. longifilis* may likely have a low tolerance to MCP toxicity in comparison to the values reported by the aforementioned authors. This differential acute toxicity may be attributed to size (i.e. length or weight), age, diet, environmental factors and differences in experimental protocols (Pickering *et al*., 1962; Pandey *et al*., 2008; Barbieri and Ferreira, 2011). Therefore, comparison with other species could be difficult due to the wide range of acute toxicity levels for OPs (Maniyar *et al*., 2011).

Fish live closely with their environment and are easily susceptible to xenobiotics, which can affect the biochemical and physiological functions of their blood. The decrease in the haematological parameters relating to oxygen transport (RBC, HB and PCV) (Table 3) with increasing concentration of the MCP, might have resulted from the haemolysing effect of the MCP on the red cells; indicating the likelihood of anaemic condition as a result of reduction in oxygen carrying capacity of the blood. Many agrochemicals have been reported to cause anaemia in fish (Pereira *et al*., 2013). According to Bloom and Brandt (2005), the anaemia may be due to reactive oxygen species (ROS)-induced oxidative deterioration of haemoglobin to methaemoglobin or the release of oxygen radicals triggered by the toxic stress of MCP. Decrease in haemoglobin content of blood due to methaemoglobin formation or release of oxygen radicals. The exposure of fish to MCP might have also triggered the secretion and accumulation of mucus over the gill surface as concentration of MCP increased. This could have caused the inhibition of red blood cells production in haematopoietic organ with concomitant accelerated disintegration of the RBC membranes, causing haemolysis and impairment in haemoglobin synthesis; hence, the reduction in HB and PCV values. The blockage of gill surface by mucus could annihilate nitrogen excretion and blood circulation with ensuing impairment of fish homeostasis processes. Gill blockage or damage by mucus has been observed in fish exposed to MCP (Maniyar *et al*., 2011) and methylparathion (Barbieri and Ferreira, 2011). The decrease in MCV, MCH and MCHC levels in the present study is similar to the findings of Yaji and Auta (2007) on *Clarias gariepinus* and Saravanan *et al* (2011) on *Cyprinus carpio* following exposure to MCP and lindane respectively. Increase in the volume of RBC or the release of abundant immature RBC into the blood circulation are likely reasons for the decrease in MCV and MCH values of fish under hypoxic condition as a result of chemical stress (Saravanan *et al*., 2011). The release of immature cells could be an adaptation to the hypoxic condition aimed at ameliorating the oxygen transporting system. These reasons might likely explain the decreases in the levels of MCV and MCH in this study. In addition, the decrease in the levels of MCHC might have been due to inhibition of haemosynthetic and erythropoietic processes. The disproportionate decrease in MCH over MCV along with the reduced MCHC is suggestive of microcytic hypochromic anaemic condition in *H. longifilis* in contrast to macrocytic normochromic type reported in *C. carpio* (Saravanan *et al*., 2011). Increased WBC count in stressed animals is indicative of protective response to stress. The concentration dependent increase in WBC count in the present study might have resulted from the hypersensitivity of leucocytes of *H. longifilis* to MCP-induced toxicity which provoked immunological reactions to produce antibodies as a coping and survival strategies to the MCP-induced stress. Yaji and Auta (2007) reported significant increase in WBC count in *C. gariepinus* exposed to MCP and attributed it to increase in lymphocytes.

The increased blood glucose level of MCP-exposed fish may be due to increased gluconeogenesis so as to enhance the conversion of glycogen to glucose in order to meet the increased demand for energy; thereby reducing drastically the plethora of detrimental consequences that could likely be imposed by

MCP-induced hypoxia. It could also be an adaptive response to 'fight' hypoglycaemia and such elevation in glucose level could be useful in the bio-monitoring of MCP pollution in waters where the fish occurs.

The decline in protein level may be due to the increased catabolism of protein via the utilization of amino acids. In this study, the destruction of hepatocytes or necrosis and/or excessive loss of protein due to kidney damage with subsequent impairment of protein metabolic pathway might have also accounted for the depletion of protein levels in the tissues of *H. longifilis*. Such hypoproteinaemic condition due to the aforementioned factors has also been reported in fin and shell fishes exposed to OPs (Ahmad, 2012; Maharajan *et al*., 2012).

The reduction in the activities of aminotransferases (ALT and AST) in the gills and liver of fish exposed to MCP (Table 4) is suggestive of their leakage into blood, thus raising their levels in the blood. Similar alterations in the activities of aminotransferases in fish tissues due to MCP stress have been reported (Rao, 2006; Agrahari *et al*., 2007). An elevated level of these enzymes in the blood is an indication of tissue damage or injury (Ramaiah, 2007). In this study, therefore, the seemingly linearity in correlation indicated by the inhibition of these enzymes in the gills and liver and their elevation in the blood with increased MCP concentration may depend on severity of gill and liver damage. During short term exposure, response of LDH to MCP toxicity in this study appears to be biphasic, with low and high activities in the liver and gill respectively. In contrast, increased activity of the enzyme was observed in all the tissues during the long term exposure. This finding is in consonance with that of Rao (2006) and Agrahari *et al*. (2007). LDH is an important glycolytic enzyme that catalyses the reversible oxidation of lactate to pyruvate and it is usually induced by oxygen stress. The increase in blood glucose level (Table 3) due to MCP toxicity and related oxidative stress in the fish might have enhanced the activities of LDH (Table 4) to meet energy demands via the oxidation of lactate to pyruvate through glycolysis or conversion of lactate to glycogen through gluconeogenesis. The reduction in LDH activities in the blood and liver is an indication of decreased glycolytic activities in these tissues. LDH activity has been reported to increase during anaerobic condition to meet energy demand when aerobic respiration is lowered (Murray *et al*., 1995). The increase in the activity of SOD in this study could be an attempt by the fish to neutralize the impact of ROS which might have occurred as a result of the disruption in the balance between pro-oxidants and antioxidants leading to loss of functional integrity of the tissues.

The reduction in the weight and the growth of MCP-exposed fish (Table 5) may be due to the toxic effect exerted by the MCP; hence the increased growth rate of fish in the control, which was free of the MCP. The diversion of energy from food assimilation, otherwise meant for growth, tissue repair and homeostasis maintenance, to detoxify the MCP and counteract its generated oxidative stress might have been responsible for growth impairment in MCP-exposed fish. This corroborates the changes in protein metabolism in order to make energy available as well as that of ALT and AST due to oxidative stress. Induction of oxidative stress have been reported to have an inhibitory effect on fish growth as animals inhabiting chronically polluted environments can develop some adaptations or compensatory mechanisms (Pavlovic *et al*., 2010; Adeogun *et al*., 2012). Since growth depend on the interplay of factors such as feeding, food assimilation and energy trade-off (Cao *et al*., 2012), the feeding reluctance of MCP-exposed fish coupled with a decrease in SGR (Table 5) are suggestive of low food intake due to loss of appetite, hence, the growth reduction in the present study. The proposed loss of appetite may also be responsible for the anaemic condition earlier mentioned.

Although the water quality parameters investigated conformed with the standard for fish survival, the accumulation of MCP in the tissues of *H. longifilis* might have induced significant oxidative stress as reflected in the haematological, biochemical, growth and behavioural alterations in the fish. The sensitivities of the blood, enzyme and growth profiles of the fish upon exposure to varying concentrations of MCP are suggestive of their

potentials as biomarkers in the evaluation of MCP toxicity in *H. longifilis*.

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