# ACUTE AND SUB-LETHAL EFFECTS OF BISPHENOL-A ON SOME HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF *CLARIAS GARIEPINUS* FINGERLINGS

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**Abstract:** Bisphenol-A is a plastic constituent, known as an endocrine disruptor, has become ubiquitous in the environment due to its presence in various products such as food and beverage packaging. This study investigated both acute and sub-lethal effects of Bisphenol-A on some haematological and biochemical indices of *Clarias gariepinus* fingerlings (mean weight, 8.0 ± 0.9g and Standard length, 5.0 ± 0.3cm), exposed to varying concentrations (0, 3, 5, 8, 10, 12mg/l) of bisphenol-A exposed for 96 hours and 60 days in plastic aquaria of 20L capacity, to determine both acute and sublethal toxicity respectively in static bioassay. Haematologically, the acute and sub-lethal exposures of *C. gariepinus* to BPA showed significant changes in levels of anaemia and haemodilution with packed cell volume values (19.67±0.33 and 30.00±0.58), while biochemical indices also showed significant changes in levels of induced hyperproteinaemia with total protein values (1.70±0.10 and 1.83±0.09) in the highest concentrations of both acute and sub lethal toxicity. Further research should be carried out to investigate the effects and bioaccumulation of this chemical on the muscle, brain and gonads of the fish.

**Keywords:** *Clarias gariepinus*, fingerlings, bisphenol-A, toxicity, haematology, biochemical, indices

## Introduction

Bisphenol A, a constituent of plastic, is known to be an "endocrine-disrupting compound" that may affect embryonic development, gonadal formation, sex differentiation and growth, by either binding or blocking hormone receptors, thereby triggering or preventing hormonal responses (Hotchkiss *et al*., 2008). It has become ubiquitous in the environment within the past eighty years because of its presence in a multitude of products including food and beverage packaging, flame retardants, adhesives, building materials, electronic components, and paper coatings. As the demand for these products increased, so did BPA production (Staples *et al*., 1998). The sources of plastic waste vary by region. For example, shipping and fisheries are significant contributors in the East Asian Seas and the southern North Sea regions (Kershaw *et al*., 2011), whereas tourism is a major source in the Mediterranean. Other sources include industrial (primary), commercial and municipal wastes emanating from residential areas (domestic or household waste), streets, parks, collection depots and waste dumps (Nwachukwu, *et al.*, 2013).

Bisphenol A are plasticizers that when consumed, can introduce toxins into the chain, which can thereby biomagnify to higher trophic levels (Setala *et al*., 2014). Hu *et al.* (2009) reported that phthalates and bisphenol A affect reproduction and impair development in crustaceans and amphibians thereby resulting in low reproductive ability and decrease in their population. *Clarias gariepinus* is a fresh water fish that is widely distributed in Africa and is of high demand. It is the most cultured fish in Nigeria and has high tolerance to all kinds of environmental conditions (such as well and poor oxygenated waters), and is thus used as a biological indicator in ecotoxicology (Ayoola, 2008).

The acute toxicity test is the adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 96 hours, or an inhalation exposure of 4 hours while chronic toxicity involves a stimulus which is continuous for a long time. It is usually a long term study that often signifies periods of about one-tenth of the organism's life span (Sprague, 1973). Blood is pathophysiological reflector of the body because it is highly susceptible to internal and external fluctuations. Physio-morphological changes in blood indicate the alterations in the quality of the environment and in diagnosing the functional status of the animal exposed to toxicants, this blood parameter is measured (Seth and Saxena, 2003). The haematological and biochemical parameters of fish blood serves as a suitable indicator for environmental stress resulting from human activities (Celik, 2004). The aim of this study was to determine the acute and sub lethal toxicity of bisphenol-A on some haematological and biochemical parameters of *C. gariepinus.*

## Materials and methods

Fingerlings of *Clarias gariepinus* of fairly uniform size (mean weight, 8.0±0.9g; standard length, 5±0.3cm) were purchased from a fish farm in Area BZ located at Ahmadu Bello University staff quarters, Zaria, Nigeria. They were transported in a well aerated jerry can to the Garden of the Department of Biological Sciences, Ahmadu Bello University, Zaria. They were then introduced into a pond containing dechlorinated water to acclimatize for two weeks, under a natural daylight photoperiod. During the acclimatization period, they were fed twice daily, with Coppens commercial feed manufactured by Alltech coppens. Feeding was stopped 24hours prior to the commencement of the experimental run. Bisphenol A (>99% pure), registered under the Aldrich Chemical company was purchased from a chemical store in Kaduna,

Nigeria. The method described by Reish and Oshida (1987) was used for the preparation of different strengths of the test solution by diluting measured volumes with dechlorinated tap water. The control solutions were made up of only dechlorinated tap water.

***Pilot studies***

Series of range finding tests were conducted to determine the concentration of bisphenol-A used for the definitive test. The test concentrations and control were run in triplicates. For each triplicate, the test concentrations and control were prepared and labeled. Plastic tanks measuring 30cmx20cmx20cm were used in carrying out the experiment. No toxicant was introduced to the control.

***Acute toxicity test***

Acute toxicity test was carried out in order to determine the 96-hr lethal concentration $_{50}$. Acute toxicity test was carried out according to the methods of Sprague (1973) and America Public Health Association (2005). Five concentrations and a control were run in triplicates, which gave a total of 18 experimental tanks. The nominal concentrations of bisphenol-A were: 3mg/l, 5mg/l, 8mg/l, 10mg/l and 12mg/l dispensed into 20 liters of each tank. Ten fingerlings were randomly distributed into each test tank. The physicochemical parameters (temperature, dissolved oxygen, electric conductivity, pH) were monitored every 24 hours as reported by APHA (2005).

***Sub-lethal bioassay***

Based on the results of the acute toxicity test, fingerlings were exposed to a series of concentrations of bisphenol-A which were 1mg/l, 3mg/l and 5mg/l while the control was with no toxicant. Fingerlings were not fed 24hours prior to experimentation and the experiment was conducted as a completely randomized design with three treatments and a control in triplicates. Therefore, a total number of 12 experimental tanks were used. Fingerlings were fed 3% body-weight per day (morning and evening) by sprinkling their diet over the surface of each tank, within the period of experimentation (eight weeks)

***Haematological study for acute and sub-lethal bioassay***

Blood samples were collected according to the method of Blaxhall and Daisely (1973) by severance of the caudal peduncle. For the acute toxicity study, blood was collected at the end of 96 hour exposure into heparinized haematocrit tube. For sub lethal bioassay, blood was collected at the end of the eight week. The blood samples were taken to the Department of Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Parameters taken were total erythrocyte count, total leucocyte count, leucocyte differential count, haematocrit (packed cell volume), mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. The 'absolute values' made up of mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the results obtained from RBCC, Hb and PCV (Ht) as:

$$\text{MCV } (\mu M^3) = \frac{Ht\ \% \times 10}{RBCC\ (Cells\ mm3)}$$

$$\text{MCH (Pg cell)} = \frac{Hb\ (g/100ml) \times 10}{RBCC\ (Cells\ mm3)}$$

$$\text{MCHC (G/100ml)} = \frac{Hb\ (g/100ml) \times 100}{Ht\ \%}$$

Where:

MCV= mean corpuscular volume; Ht= Haematocrit; Hb= haemoglobin; RBCC= red blood cell counts; MCHC= mean corpuscular haemoglobin concentration; Pg= pigmented

***Biochemical determination for acute and sub-lethal bioassay***

For acute and sub lethal studies, some biochemical parameters were taken to determine the effects of toxicants exposed on the fingerlings. The caudal peduncles of fish from each of the experimental tank were severed and blood was collected into non-heparinzed tubes. The samples were taken to Mary Hallaway Teaching laboratory, Department of Biochemistry, ABU, Zaria for analysis.

*Determination of serum total protein*

$$\text{Serum Total Protein} = \frac{Abs.\ of\ test \times standard\ concentration}{Abs.\ of\ standard}$$

***Lipid Peroxidation***

$$\text{MDA conc.} = \frac{Absorbance\ of\ sample}{1.56\times10-5\ cm-1\ M-1}$$

TBARS conc. Nmols/mg protein =

$$\frac{Absorbance\ of\ sample}{1.56\times10-5\times protein\ conc.(mg)}$$

*Superoxide Dimutase (SOD)*

Increase in absorbance per minute = (A5 – A1)/ 2.5

$$\%\ \text{inhibition} = 100 - \left(\frac{increase\ in\ absorbance\ for\ substrate}{increase\ in\ absorbance\ for\ blank} \times 100\right)$$

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of adrenaline to adrenochrome in 1 minute.

***Catalase (CAT) Activity***

$$\text{Catalase conc.} = \frac{Absorbance\ of\ sample}{molar\ extinction\ coefficient} \times \text{protein concentration (mg/ml)}$$

1 unit is the amount of catalase that decomposes 1Nmol of hydrogen peroxide per minute at pH 7.0.

***Statistical analyses***

Data was subjected to one-way analysis of variance (ANOVA) to test for the significant differences between means at $P \leq 0.05$. Duncan's Multiple Range Test (DMRT) was used to separate the significantly different means. Regression coefficient between the probit value and log of concentration of the toxicant was determined after the acute toxicity bioassay.

**Results**

***Haematological parameters of Clarias gariepinus exposed to acute and sub lethal doses of bisphenol-A***

Table 1 shows the effects of different concentrations of BPA on the blood parameters of *C. gariepinus* at 96 hours. There was a significant ($P \leq 0.05$) decrease in PCV, HGB, RBC and a significant ($P \leq 0.05$) increase in WBC with increase in concentrations as compared to the control. The mean values for PCV, RBC and HGB were 34.33 ± 0.33, 8.07 ± 0.67 and 11.33 ± 0.89 for control, which were higher than that of the exposed fish. Highest mean value for PCV, RBC and HGB

in the exposed group was recorded in the lowest concentration (3mg/l) with 32.83 ± 0.17, 5.60 ± 0.13 and 11.03 ± 0.03 respectively and with lowest WBC mean value of 6.60 ± 0.10. There was a significant (P≤0.05) difference in MCV and MCH, with the highest value of MCV as 60.80 ± 0.15 and lowest as 56.57 ± 0.09, while highest value of MCH as 20.00 ± 0.12 and lowest as 18.80 ± 0.06. MCHC was insignificantly different with almost indifferent values. Table 2 showed the effects of different concentrations of BPA on the blood parameters of *C. gariepinus* for 8 weeks. PCV, HGb and RBC of the control were higher than those of the exposed group with values 38.00 ± 1.15, 12.53 ± 0.18 and 6.20 ± 0.06 respectively while WBC of the control of the were lower than those of the exposed with 8.00± 0.50. There was a significant difference (P< 0.05) between the control and the exposed fingerlings. PCV, Hb and RBC values of the exposed fingerlings decreased with increase in concentration while WBC increased with increase in concentrations of the exposed fingerlings. MCV, MCH and MCHC of the control were higher than those of the treated groups.

**Table 1:** Effects of different concentrations of BPA on the Blood parameters of *C. gariepinus* at 96 hours

| Concentrations (mg/l) | PCV (%) | HGB (g/dL) | WBCC ($\times10^9$/L) | RBCC ($\times10^{12}$/L) | MCV ($\mu M^3$) | MCH (Pgcell) | MCHC (g/100ml) |
|---|---|---|---|---|---|---|---|
| 0 | 34.33±0.33$^a$ | 11.33±0.89$^a$ | 6.07±0.07$^e$ | 8.07±0.67$^a$ | 56.57±0.09$^c$ | 18.80±0.06$^c$ | 33.20±0.00$^a$ |
| 3 | 32.83±0.17$^b$ | 11.03±0.03$^a$ | 6.60±0.10$^d$ | 5.60±0.13$^b$ | 58.83±0.03$^b$ | 19.47±0.07$^b$ | 33.23±0.07$^a$ |
| 5 | 31.67±0.33$^c$ | 10.37±0.19$^b$ | 7.13±0.09$^c$ | 5.30±0.15$^b$ | 59.13±0.09$^b$ | 19.47±0.13$^b$ | 32.37±0.68b$^a$ |
| 8 | 29.33±0.33$^d$ | 9.17±0.89$^c$ | 8.07±0.07$^b$ | 4.60±0.20$^c$ | 60.43±0.03$^b$ | 19.33±0.09$^b$ | 32.17±0.17$^a$ |
| 10 | 24.17±0.60$^e$ | 8.13±0.09$^d$ | 8.37±0.09$^b$ | 3.97±0.09$^d$ | 60.80±0.15$^a$ | 20.00±0.12$^b$ | 33.23±0.03$^a$ |
| 12 | 19.67±0.33$^f$ | 6.30±0.17$^e$ | 9.57±0.29$^a$ | 3.23±0.12$^e$ | 59.27±0.37$^a$ | 19.43±0.09$^a$ | 33.00±0.00b$^a$ |
| **Mean** | **28.67±0.35** | **9.39±0.38** | **7.64±0.12** | **5.13±0.23** | **59.17±0.13** | **19.42±0.09** | **32.87±0.16** |
| **P Value** | **0.000** | **0.000** | **0.036** | **0.000** | **0.003** | **0.001** | **0.072** |

Mean±SE with the different superscript along the column were significantly different (P≤0.05)

**Table 2:** Effects of sub lethal exposures of BPA on the blood parameters of *C. gariepinus* fingerlings

| Concentrations (mg/l) | PCV (%) | HGB (g/dL) | WBCC ($\times10^9$/L) | RBCC ($\times10^{12}$/L) | MCV ($\mu M^3$) | MCH (pgcell) | MCHC (g/100ml) |
|---|---|---|---|---|---|---|---|
| 0 | 38.00±1.15$^a$ | 12.53±0.18$^a$ | 8.00±0.50$^d$ | 6.20±0.06$^a$ | 60.20±0.06$^a$ | 20.00±0.00$^a$ | 33.07±0.03$^a$ |
| 1 | 35.60±0.33$^a$ | 12.00±0.00$^b$ | 10.53±0.27$^c$ | 6.03±0.03$^a$ | 59.67±0.33$^a$ | 20.00±0.00$^a$ | 33.20±0.06$^a$ |
| 3 | 33.00±0.58$^b$ | 11.27±0.15$^c$ | 12.77±0.62$^b$ | 5.50±0.71$^b$ | 57.67±0.88$^b$ | 19.17±0.17$^a$ | 33.13±0.07$^a$ |
| 5 | 30.00±0.58$^c$ | 10.23±0.14$^d$ | 15.7±0.09$^a$ | 4.97±0.09$^c$ | 59.67±0.33$^a$ | 19.17±0.60$^a$ | 33.13±0.09$^a$ |
| **Mean** | **34.15±0.66** | **11.51±0.12** | **11.75±0.37** | **5.67±0.22** | **59.30±0.40** | **19.58±0.19** | **33.13±0.06** |
| **P Value** | **0.000** | **0.000** | **0.030** | **0.000** | **0.003** | **0.572** | **0.145** |

Means ±SE with different superscript along the column were significantly different (P≤0.05)

***Biochemical indices of Clarias gariepinus exposed to acute and sublethal doses of bisphenol-A***

The results of some biochemical parameters of *C. gariepinus* exposed to acute concentration of BPA for 96 hours are presented in Table 3. There was a decrease in the values of TP of the exposed fingerlings with increase in concentrations compared to the control. There was also increase in MDA and CAT with increasing concentrations of BPA, while SOD values of the exposed fingerlings increased initially, followed by a reduction in value with increase in concentrations and thus, dose dependent. Thus, there was a significant difference ($P \leq 0.05$) in control and exposed fingerlings. Table 4 shows the result at sub lethal concentrations, with the values of TP of control fingerlings higher than that of the exposed groups while the values of MDA, CAT and SOD were lower than the exposed group. Thus, there was a significant difference ($P \leq 0.05$) between the control and exposed fingerlings.

**Table 3:** Effects of different concentrations of BPA on the biochemical indices of *C. gariepinus* at 96 hours

| Concentrations(mg/l) | TP | CAT | SOD | MDA |
|---|---|---|---|---|
| 0 | $3.13\pm0.13^{a}$ | $40.63\pm0.18^{f}$ | $56.34\pm0.75^{f}$ | $361.27\pm3.79^{e}$ |
| 3 | $2.73\pm0.06^{b}$ | $43.88\pm0.90^{e}$ | $67.68\pm0.93^{e}$ | $556.73\pm16.29^{d}$ |
| 5 | $2.70\pm0.15^{b}$ | $56.66\pm0.60^{d}$ | $50.79\pm0.32^{d}$ | $561.83\pm5.63^{d}$ |
| 8 | $2.03\pm0.03^{c}$ | $88.87\pm0.30^{c}$ | $47.54\pm1.80^{c}$ | $635.73\pm7.59^{c}$ |
| 10 | $2.00\pm0.00^{c}$ | $123.34\pm1.70^{b}$ | $43.19\pm0.35^{b}$ | $704.67\pm2.95^{b}$ |
| 12 | $1.70\pm0.10^{d}$ | $134.99\pm1.06^{a}$ | $23.19\pm0.72^{a}$ | $799.70\pm1.48^{a}$ |
| **Mean** | **2.38±0.06** | **81.39±0.79** | **48.12±0.81** | **603.32±6.29** |
| **P Value** | **0.01** | **0.034** | **0.000** | **0.004** |

Mean±SE with the different superscript along the column were significantly different ($P \leq 0.05$)

**Table 4:** Effects of sub lethal exposures of BPA on some biochemical indices of *C. gariepinus*

| Concentrations (mg/l) | TP (g/dL) | CAT (U/mg protein) | SOD (U/Ml) | MDA (nmols/mg protein) |
|---|---|---|---|---|
| 0 | $3.16\pm0.17^{a}$ | $47.09\pm2.43^{c}$ | $23.54\pm1.39^{c}$ | $120.43\pm0.81^{d}$ |
| 1 | $2.16\pm0.03^{b}$ | $115.45\pm3.73^{b}$ | $29.37\pm1.27^{b}$ | $136.90\pm2.71^{c}$ |
| 3 | $2.03\pm0.03^{bc}$ | $120.64\pm0.81^{b}$ | $41.31\pm0.35^{a}$ | $172.83\pm1.09^{b}$ |
| 5 | $1.83\pm0.09^{c}$ | $132.65\pm1.55^{a}$ | $43.36\pm0.45^{a}$ | $211.07\pm1.13^{a}$ |
| **Mean** | **2.29±0.08** | **104.46±2.13** | **34.39±0.87** | **160.31±1.44** |

Means ±SE with different superscript along the column were significantly different ($P \leq 0.05$)

TP= Total protein, CAT= Catalase activity, SOD= Superoxide dismutase, MDA= Lipid peroxidation

**Discussions**

RBCC, Hb and PVC values of fish recorded in the control group were significantly higher ($P<0.05$) than those fish exposed to various concentrations of toxicant in both acute and sub lethal toxicity test. The inhibition of RBCC in *C. gariepinus* exposed to toxicant could be due to appreciable

decline in haematopoiesis, leading to various types of anaemia or possibly due to haemodilution resulting from impaired osmoregulation across the gill epithelium. Similar findings were reported by Jee and Kang (2004), after exposure of flounders to 1.0 to 2.0 µm phenanthrene that resulted in progressive decrease in RBCC, Hb and PCV levels indicating significant physiological stress. Present study revealed fluctuations in MCH, MCV and MCHC in acute and sub lethal exposures of BPA. MCV has been reported to provide information to the size and status of erythrocytes (Nussey *et al*., 1995). Giron-perez *et al.* (2006) reported that chloropyrifos had no effects on MCH and MCHC of Nile Tilapia (*O. niloticus*).

Values of WBCC recorded in both acute and sub lethal exposures were significantly elevated at higher concentrations compared to the controls. This suggests their ability to combat foreign substances, which resulted in the production of antibodies to cope with the stress exerted by the toxicant on the fingerlings. This is in line with the work of Gbem *et al*. (2003), who worked with tannery effluent effects on *C. gariepinus*, and had a steady increase in the TLC. Leucocytosis was evidence by increase in WBCC during fenvalleratee intoxication (Seth and Saxena, 2003). The authors opined that increase in WBCC correlated with an increase in antibody production which helps survival and recovery of the fish.

The decrease in serum total protein reported in this study was supported by the study of Das and Mukherjee (2003) on *Labeo rohita* exposed to sub lethal concentrations of cypermethrin. It would be logical therefore, to suggest that BPA in the present study caused a stress-induced effect on protein synthesis which must have led to the depletion in the serum protein. The decrease in serum total protein level suggesting high protein hydrolytic activity due to the elevation of protease activity as reported by Tiwari and Singh (2003) who recorded decreased serum total protein levels in snake head fish (*Channa punctatus*) exposed to sub lethal concentrations of lattices of *Euphorbia royleana.*

Superoxide Dismutase is one of the key enzymes that provide the first line defense against the pro-oxidants and catalyses the transformation of superoxide radicals to hydrogen peroxide and oxygen. Toxic stress is known to alter the activity of SOD in the vital tissues of fish. In the present study, the acute concentrations of BPA exposed to the fingerlings showed an initial increase for up to 24hours, followed by a drop in activity till the end of the 96 hour experimentation period while the sub lethal concentrations showed an increase of SOD activity with an increase in concentration. This is in line with the work of Sreejai and Jaya (2010), who studied the changes in lipid peroxidation and antioxidants in fishes exposed to hydrogen sulphide. These authors concluded that the initial increase in SOD activity indicated the generation of superoxide radical anion, and the inhibition at the end might be due to the higher amount of oxyradical formation and that could be neutralized by the enzyme.

Catalase activity belongs to the cellular antioxidant system that counteracts the toxicity of reactive oxygen species (ROS). They are the heme-containing enzymes that facilitate the removal of $H_2O_2$, which is metabolized to water and oxygen. In the present investigation, CAT activity increased with increase in the toxicant's concentration. This could be due to the high rate of activity of this antioxidant enzyme during exposure which may stem from a pro-oxidant condition induced by the toxicant, likely serving as an adaptive reaction (Sreejai and Jaya, 2010).

In conclusion, changes in haematological parameters in both bioassays significantly ($P<0.05$) decreases with increase in concentrations as compared to the control while the enzymatic activity estimation in *C. gariepinus* exposure to BPA indicates that the exposed fish are faced with metabolic crisis with a decrease in total protein and increase in catalase and lipid peroxidation with increase in concentration, with a significant difference ($P\leq0.05$) as when compared to the control. Further research should be carried out to investigate the effects and bioaccumulation of this chemical on the muscle, brain and gonads of the fish.

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