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# Acute Toxicity of Dragon (Paraquate Dichloride) Exposed to African Freshwater Fish, *Heterbranchus bidorsalis* (Geoffrey Saint-Hilaire, 1809)

*F.A. Elebe, U.N. Uka, C.A. Uhuo and C.N. Mgbabu*

Department of Applied Biology, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria

**Abstract:** 300 juveniles of Freshwater fish, *Heterobranchus bidorsalis* (Family: Clarridae) (25.6g± 23.4g) were purchased from Ajimmy fish farm, Yenagoa, Bayelsa State, Nigeria were acclimatized for a period of two weeks under laboratory conditions. A range finding test was carried out to ascertain the concentration of Dragon (Paraquate Dichloride) to be used in the assay. A set of ten fish were exposed to 44.16, 46.92, 49.68 and 52.44 $mgl^{-1}$ in triplicates. The exposure lasted for 96hrs. Abnormal behaviours such as erratic swimming, somersaulting and convulsion were observed in the exposed fish while the fish in the control maintained a normal behaviour. No death was recorded in the control tank throughout the exposure. There was a significant difference ($P<0.05$) in mortality at different concentrations. The Median Lethal Concentration $LC_{50}$, (95% confidence interval) values estimated by probit analysis were 5.94 (5.66-6.20), 51.41(49.49-56.88), 48.56(47.34-49.86) and 46.13(44.89-47.11) $mgl^{-1}$, at 24, 48, 72 and 96hrs respectively. A significant difference ($P<0.05$) in the lethal concentrations $LC_{10}$ to $^{LC}90$ was recorded. The safe levels estimated by different methods varied from $1.51x10mgl^{-1}$ to $4.6x10^{-4}mgl^{-1}$. Histopathology changes in the heart and stomach were analyzed at the end of the 96hours.Normal architecture of sections of the organs ( heart and stomach) were maintained in the control group. However there was increasing disorganization of the organs in the exposed groups as the concentration increased. Expansion of the heart chambers and loss of cardiac muscles were observed in higher concentrations. In the stomach, there was a loss of the epithelial lining of the mucosa, clumping of the sub-mucosa and the muscularis layer as the concentration increased. The present study shows that paraquate dichloride is toxic to juveniles of *Heterobranchus bidorsalis*. Therefore, this herbicide must be used with caution to avoid destruction of juveniles of fish to ensure sustainability of fish species.

**Key words:** *Heterbranchus bidorsalis* · Toxicity · Paraquate Dichloride · Mortality · Ebonyi State

## INTRODUCTION

The global agricultural output can't be achieved without weed management, "an estimated 70 percent of the world's crops might be lost weed control is not considered [1]. Herbicides, commonly known as weed killers are chemicals used to kill unwanted plants [2].It was observed that farmers deliberately apply herbicides to kill weeds in farms because herbicides as to play significant role in modern agriculture. Herbicides such as paraquate are important because weed compete vigorously with crops for water, light and other nutrients. As a result, if they are not suppressed they reduce crop yield up to 80% [1]. Despite the huge benefits of herbicides, careless handling, accidental discharge, water runoff and leaching can cause these herbicides to find their ways into aquatic habitats. Although, chemical herbicides has greatly improved yields in agriculture and forestry and their non-target impacts have caused considerable concern [3]. The benefits derived from the use of pesticides cannot be overemphasized without the side effects been considered. Research hasabout 99% of the pesticides use for agriculture go into atmosphere, water bodies and the earth (soil) and 0.1% reach the desired target as reported by [4].The application of herbicides in agriculture, aquaculture, animal husbandry and postharvest technology is a threat to aquatic biota (ecosystem), public health and welfare of mankind [3]. The presence of pesticides in water bodies most often result in alteration of the physicho-chemical properties of water [5].Fishes are sensitive to a wide variety of agrochemicals that may arise from not only deliberate discharge of chemicals into

**Correspondin Author:** F.A. Elebe, Department of Applied Biology, Ebonyi State University, PMB 053 Abakaliki, Nigeria.

waterways but also from approved agricultural practices which stands to cause damage to them. Therefore, the toxicity study of chemicals to early life stages is essential to understand the environmental impacts of herbicides [6].

Histopathopalogy refers to the application of light or electron microscopy to the study of tissue defects [7]. The objective of histology is to understand the microanatomy of cells and to correlate structure with function [2].Water pollution induces pathological changes in fish and as an indicator of exposure to contaminants; histology represents a useful tool to assess the degree of pollution, particularly for sub-lethal and chronic effects [8], [9], [10], [11] and [12] have noted that when fish are exposed to various contaminants a number of leisions are likely to be induced in various organs. Many authors have used Gills, liver, kidney and skin as suitable organs for histopathological examinations to determine the level of pollution in aquatic environment; [13]; [14]; [15], [16], [17], [18]. [19], [20], [21], [22] and [23].

The present study employed the histopathological examination of the heart and stomach as a tool to assess the impact of paraquat dichloride on fish juveniles. These organs play vital roles in the metabolic processes of an organism.

Dragon with the active ingredient, Paraquat dichloride, a herbicidal pesticide, is one of the most widely used herbicides in the world. Paraquat dichloride is quick-acting and non-selective, killing green plant tissues on contact. Before paraquat was developed, farmers in hot, wet climates had a limited range of dealing with weeds [1]. Paraquat has several unique properties; it is fast acting, so it can be used even in wet condition, under most usage conditions, it does not damage or interfere with or its roots ( it is not systemic), it does not contaminate ground water [1], however, treatments within the range of l mg/L to 4 mg/L in water have resulted in 0.1 mg/L or less of paraquat being detectable in water from 6 to 14 days after application[24]. In some cases, cover crop management and their roots are not killed. In this case, paraquat will help keep the structure of the soil reducing soil erosion. These properties meant that, since its introduction in 1961, paraquat has become the herbicide of choice for millions of farmers around the world. Over 25million farmers in over 120 countries currently use paraquat [25]. Assessment of the toxicity of such widely used herbicide on non-target organisms is absolutely necessary.

The choice of *Heterobranchus bidorsalis* in this work was motivated by; its ability to survive adverse conditions, availability all year round and its high commercial value.

## MATERIALS AND METHODS

**Sources and Collection of Fish Samples:** The study was conducted with fish samples from Ajimmy fish farm at Azikoro community in Yenagoa, Bayelsa State, Nigeria while the analysis was done in Abakaliki Ebnyi State Nigeria. Dragon (Paraquat Dichloride) manufactured for West Africa cotton Company Limited Lagos, Nigeria, were bought from Abakpa market in Abakaliki and used in the research.

300 juveniles (20.3±25g of Fresh water fish *Heterobronchus bidorsalis* (Family: Clarridae) were acclimatized for two weeks under laboratory conditions in the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. The fish samples were fed on a commercial floating pellets diet (3% of the body weight per day). The water was changed daily to remove the fecal matter, ammonia content and other waste materials in water. Some of the fish (= 3%) of the specimen died during the acclimation period while the rest survived and were used in the range finding test and the definitive test.

The range finding tests were carried out prior to the definitive test to determine the concentration of the test solutions to be used in the bioassay. Five concentrations of the herbicide were prepared from the original solution using the formula described by [26].

**Definitive Test:** Acute toxicity assay was conducted to determine the 96hr $LC_{50}$ value of Dragon for *Heterobronchus bidorsalis* with definitive test in semi-static system in the laboratory according to the Organization for Economic Cooperation and Development [27], 1992 guide line. Based on the range finding test five concentrations of Dragon (44.16, 46.92, 49.68 and 52.44 $mgl^{-1}$) were prepared and tested on the *Heterobranchus bidorsalis* juveniles. A set of ten acclimated fish were randomly exposed to each static tank containing the different concentrations of the toxicant. The experiments were set in triplicates. A control experiment was set up simultaneously. The test media were changed every 48hours to maintain a more constant concentration of the test media to which the animals were exposed and to prevent excessive accumulation of toxic metabolites. The exposure was continued up to 96 hrs. During the exposure period changes in the behavioral pattern of the fish such as convulsions, equilibrium status, fin movement, hyperactivity, somersaulting activity, swimming rate and operculum movement were observed in exposed as well as the control group and mortality rate

at different durations in the different concentrations were observed and recorded. Dead fish were promptly removed to reduce further fowling of the water. The effects of the herbicide on some physico-chemical parameters of the test water were analyzed using the standard methods (APHA, AWWA, WPCE, 2005). The $LC_{50}$values of the herbicide were calculated following the probit analysis method as described by [28] for estimating Median Lethal Concentrations in toxicity Bioassays. Safe level were estimated based on [29], [30], Committee on Water Quality Criteria [31], National Academy of Science/National Academy of Engineering (NAS/NAE, 1973), Canadian Council of Resources and Environmental Ministry [32] and the International Joint Commission [28] methods At 96hours, the surviving fish from the different concentrations and the control were sacrificed and the heart and stomach removed and fixed in 10% formal-saline in specimen tubes with air tight fitting lids for 1 week and prepared for histopathological assessment. This was done to prevent autolysis, improve staining quality and to aid optical differentiation of cells. The organs were then washed with 70% ethanol and dehydrated through a graded series of ethanol [33], [34]. They were embedded in paraffin, sectioned at 4-5µm thickness, cut using a microtome, stained with hematoxylin and eosin and examined using light microscope and photomicrography [35]

**Statistical Analysis:** The data obtained were analyzed by statistical package SPSS (version 20). The data were subjected to one way analysis of variance (ANOVA) and Duncan`s multiple range test to determine the significance difference at 5% probability..

## RESULTS

**Physicho-Chemical Parameters of the Test Water:** The temperature varied from 27 to 28.8(℃), the pH ranged from 7.5 to 8.3, the dissolved oxygen varied from 1.6 to 2.6(mg/L), the conductivity ranged from 118 to 594(µS/cm) while the total dissolved solutes ranged from 108 to 208 (mg/L). The summary of the physicho-properties of the test water is presented in Table 1.

Table 1: Summary of the physicho-chemical parameters of the test water

| Parameters | Mean | Range |
|---|---|---|
| Water temperature (°C) | 27.82 | 27-28.8 |
| pH | 7.72 | 7.5-8.3 |
| Dissoved oxygen (mg/L) | 2.06 | 1.6-2.6 |
| Conductivity (µS/cm) | 343 | 118-594 |
| Total dissoved solutes (mg/L) | 133 | 108-208 |

**Behavioural Changes:** Abnormal behaviours such as erratic swimming, somersaulting and convulsion and increased opercula movement were observed in the exposed fish while the fish in the control maintained a normal behavior.

**Mortality Rate:** No death was recorded in the control tank throughout the exposure. There was a significant difference ($P<0.05$) in the mortality rate of the organisms in the different concentrations. Summary of the mortality rate is presented in Table 2.

Table 2: Mortality rate at different test concentration and time interval in *Heterobranchus bidorsalis*

| Conc... | Exposure time (h) Mortality | | | | |
|---|---|---|---|---|---|
| ($mgl^{-1}$) | 24 | 48 | 72 | 96 | Total (%) |
| | 0.00 | 0 | 000 | 00% | |
| 44.16 | 2 | 2 | 2 | 6 | 1240% |
| 46.92 | 2 | 4 | 38 | 15 | 50% |
| 49.68 | 4 | 6 | 68 | 23 | 76.67% |
| 52.44 | 6 | 8 | 7 | 30 | 100% |

Table 3: Lethal concentration of Dragon (mg/l) (95% confidence interval) depending on exposure time for *Heterobranchus bidorsalis* fish

| Lethal | Exposure time (h) | | | |
|---|---|---|---|---|
| Conc | 24 | 48 | 72 | 96 |
| $LC_{10}$ | 5.06 | 43.84 | 44.02 | 42.65 |
| | (4.45-5.39) | (36.52-46.14) | (40.97-45.56) | (39.78-44.07) |
| $LC_{20}$ | 5.35 | 46.30 | 45.53 | 43.82 |
| | (4.85-5.63) | (41.76-48.12) | (43.23.46.80) | (41.55-44.99) |
| $LC_{30}$ | 5.57 | 48.17 | 46.65 | 44.67 |
| | (5.16-5.82) | (45.47-50.18) | (44.86-48.28) | (42.84-45.72) |
| $LC_{40}$ | 5.76 | 49.82 | 47.63 | 45.42 |
| | (5.42-5.99) | (47.92-53.09) | (46.20-48.78) | (43.92-46.40) |
| $LC_{50}$ | 5.94 | 51.41 | 48.56 | 46.13 |
| | (5.66-6.20) | (49.49-56.88) | (47.34-49.86) | (44.89-47.11) |
| $LC_{60}$ | 6.14 | 53.06 | 49.51 | 46.85 |
| | (5.88-6.43) | (50.76-61.38) | (48.35-51.14) | (45.79-47.94) |
| $LC_{70}$ | 6.35 | 54.88 | 50.55 | 47.64 |
| | (6.10-6.72) | (52.00-66.78) | (49.31-52.69) | (46.64-48.96) |
| $LC_{80}$ | 6.61 | 57.09 | 51.79 | 48.57 |
| | (6.32-7.12) | (53.41-73.84) | (50.34-54.70) | (47.53-50.33) |
| $LC_{90}$ | 6.98 | 60.30 | 53.57 | 49.89 |
| | (6.62-7.74) | (55.35-78.57) | (51.70-57.73) | (48.63-52.47) |

Table 4: Estimate of the Safe Level of Dragon from Different Methods

| Chemical | 96 h (mg/l) | Method | AF | Safe level (mg/l) |
|---|---|---|---|---|
| Dragon | 46.13 | Hart *et al*. (1948)* | 1.51 | |
| Sprague | (1971) | 0.1 | 4.61 | |
| CWQC | (1972) | 0.01 | $4.6 \times 10^{-1}$ | |
| NAS/NAE | (1973) | 0.01-0.00001 | $4.6 \times 10^{-1}$-$4.6 \times 10^{-4}$ | |
| CCREM | (1991) | 0.05 2.31 | | |
| IJC | (1977) | 5% 96 h $LC_{50}$ | 2.31 | |

*C=48 h $LC_{50}{}^{\times 0.03/S}$, Where C is the presumable harmless concentration and S=24 h $LC_{50}{}^{/48\ h\ LC_{50}}$

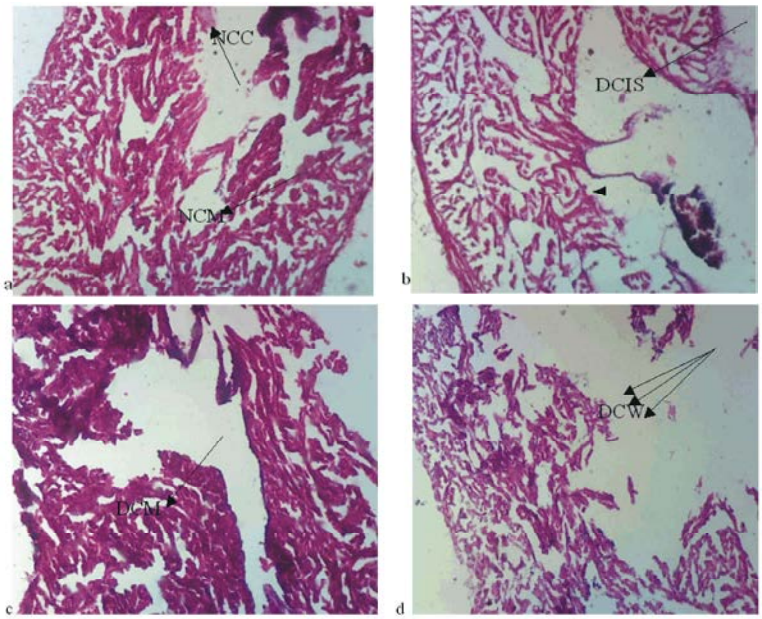


Plate 1: (a)Normal cardiac muscle (NCM) and normal cardiac chambers. (NCC) the overall architecture is normal (Control group, stained, H/E(X 60) (b) Disorganization of cardiac internal structures (DCIS) from the 46.92 mg/l stained in H/E(X 60). (c )Disorganization and loss of cardiac muscles (DCM) with expansion of cardiac chamber in 49.68 mg/l stained in H/E(X 60).(d) Distortions of cardiac wall (DCW) with a loss of cardiac muscles in some areas in the heart of *heterobranchus bidorsalis* exposed to 52.44mgl$^{-1}$ mg/l stained in H/E(X 60).

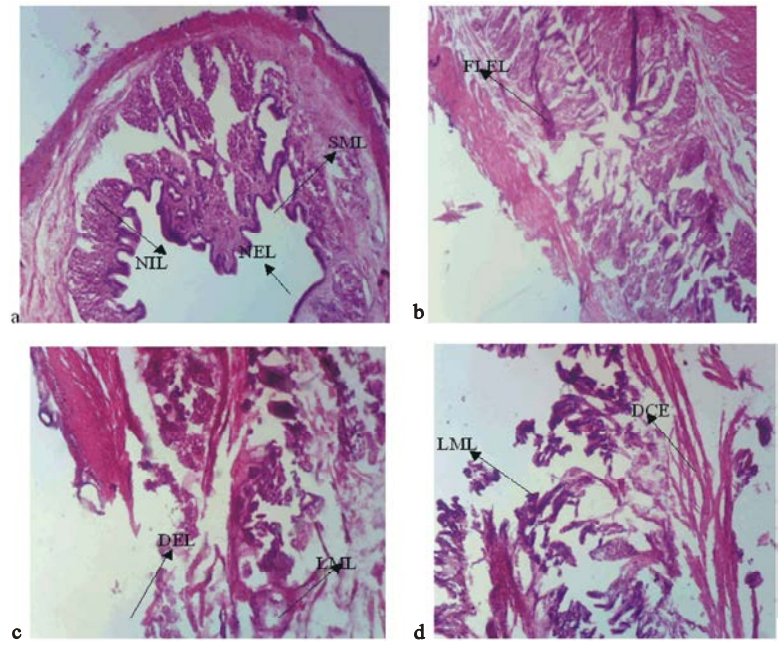


Plate 2: (a)Normal intestinal lumen (NIL), Normal epithelial lining with sub-mucosa and muscularis layer (SML) in the stomach of the control group stained in H/E(X 60).(b) Focal loss of epithelia lining (FLEL), the mucosa with mild distortion of the stomach tissue (MDST) in 44.16, mg/l stained in H/E(X 150) (c) distortion of epithelia lining (DEL) and loss of muscularis layers (LML) in (46.92, mg/l stomach stained in H/E (X 150). (d) Distortion and clumping of the epithelium (DCE), mucosa, sub-mucosa and loss of muscularis layers (LML) in 52.44mg/l stomach stained in H/E(X 150)

Median lethal concentration ($LC_{50}$). The median lethal concentration values (with 95% confidence limits) of Dragon (*Paraquat dichloride*) in *Heterobranchus bidorsalis* were 5.94 (5.66-6.20) at 24h, 51.41 (49.49-56.88) at 48h, 48.56 (47.34-49.86) at 72h and 46.13 (44.89-47.11) at 96h. These are shown in Table 3.

**Safe Level:** The summary of the estimated Safe levels by the different methods are presented in Table 4.

**Histopathological Analysis:** Normal architecture of sections of the organs (heart and stomach) were maintained in the control groups. However there were increasing disorganizations of the tissues in the exposed groups as the concentration increased. The results of the histopathological studies are presented in Plates 1 and 2.

The photomicrograph of the histopathological changes in the heart (Plate 1. A, b, c and d).

The photomicrograph of the histopathological changes in the stomach (Plate 2. A, b, c and d)

## DISCUSSION

The toxicity study of chemicals to early life stages is essential to understanding the environmental impacts of herbicides and behavioral changes are the most sensitive indicators of potential toxic effects in fishes [35], [36].Dragon with the active ingredient, Paraquat dichloride, a herbicidal pesticide, is one of the most widely used herbicides in the world. The normal behaviour of fish in the control group and abnormal behaviours such as erratic swimming, somersaulting and convulsion and increased opercula movement observed from the exposed groups in the present study were in agreement with the report of [13], [31], [37], [14], who reported normal behaviour in control groups but stress signs such as hyperactivity, vigorous jerks of the body and erratic swimming in exposed groups. [38], attributed such changes to the extraordinary need for oxygen which due to thick coating of the gills with profuse mucus together with congestion and hyper-plastic epithelium of the secondary lamella. [7], has reported that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration which will impair respiration leading to asphyxiation.

The dose dependent increase in the mortality rate observed in the study is in agreement with the mortality pattern recorded by [39].This result is also in agreement with the report of [40], who reported that the mortality rate of the organism was dose dependent. He also reported a significant difference ($P<0.05$) in mortality of the organism in the different concentrations. [31] have also noted that heavy contamination of water by pesticides lead to oxygen depletion; poisoning and resultant mass mortality of fishes. The toxicity of a chemical is totally dependent on the concentration of the chemical in organisms or even the concentration at the target receptor in the organism [41].

Median lethal concentration ($LC_{50}$) is the concentration of a test chemical, which kills 50% of the test organism in a particular length of exposure usually 96h [37]. The $LC_{50}$ values (with 95% confidence limits) of the different concentrations of Dragon in *Heterobranchus bidorsalis* in the present study were, 5.94 (5.66-6.20) at 24h, 51.41 (49.49-56.88) at 48h, 48.56 (47.34-49.86) at 72h and 46.13$mgl^{-1}$ (44.89-47.11) at 96h. These were not in consonance with the results of [38], who reported $LC_{50}$ of 7.16±0.69, 4.46±0.43, 2.19±0.27 and 1.41±0.17mg/l of paraquat within 24, 48, 72 and 96 hours respectively of gourami fish (*Trichogaster trichopterus*). This is also different from the $LC_{50}$ of 31.55mg/l at 96h reported by [39] for European eel (*Anguilla anguilla*) exposed to dragon. The variations in $LC_{50}$ may due to species difference. [40], also reported that toxicity of chemicals to aquatic organisms has been shown to be affective due age, size and health of the species applied to.

There were variations in the safe levels estimated by different methods with lots of controversy over the acceptability of the variation in the safe levels estimated by different methods as reported by [36]. The question is, how safe is the safe level in the field? [37] argued that the extrapolation of laboratory data to the field is not always meaningful and hence it is difficult to decide an acceptable concentration based on the laboratory experiments that may be considered safe in the field.

The histopathological examination of the heart of *Heterobranchus bidorsalis* in the present study showed that in the control group, the overall architecture of the heart was normal. Normal cardiac muscle and normal cardiac chambers were observed. Disorganization of cardiac internal structures (DCIS) were observed from the 46.92 mg/l group. It also revealed disorganization and loss of cardiac muscles with expansion of cardiac chambers in fish exposed to 49.68 mg/l. The present study further reveal distortions of cardiac wall with a loss of cardiac muscles in some areas in heart of the fish exposed to 52.44$mg/l^{-1}$ of paraquat dichloride. [42], have reported cardiac haemohhrage and loss of tissue in the histopathological examination of the heart of *Clarias gariepinus* exposed to 0.36mg/L, 0.57mg/L and 0.72mg/L

of 2, 4-D for 96hours. The literature on the histopathological effect of the heart is sparse despite the vital role of the heart in any vertebrate.

The present study revealed normal intestinal lumen, normal epithelial lining with sub-mucosa and muscularis layer in the stomach of the control group. Focal loss of epithelia lining with mild distortion of the mucosa and the intestinal tissue were observed in the stomach of the fish exposed to44.16mg/l. Distortion of epithelia lining and loss of muscularis layers were revealed in the stomach of the group exposed to46.92mg/l. Distortion and clumping of the epithelium, mucosa, sub-mucosa and loss of muscularis layers in 18.00mg/l stomach. [43], have observed fused microvilli, vacuolation in the mucosa as well as sub-mucosa in the stomach of*Channa punctatus* exposed to 0.0002 PPM of endosulfan for 96 Hrs. They also observed severe damage of microvilli, narrower mucosa layer, in the stomach of *Channa punctatus* exposed to 0.0004 PPM of endosulfan for 96hrs. [44] have observed hyperchromasia, disintegration of epithelium duct and desquamation of gastric mucosa.

## CONCLUSION

The study reveals that dragon (*paraquat dichloride*) has toxic effect to fish as shown in the different behavioral parameters exhibited by the fish to different concentrations of the herbicide. The mortality rate was both time and dose dependent while the disorganizations of the organs of the fish became more severe as the dose increased. Therefore paraquat dichloride must be used with caution so that the health of aquatic organisms especially juveniles are protected to ensure the sustainability of different fish species.

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