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*Full Length Research Paper*

# Toxic effects of endosulfan on haematological and biochemical indices of *Clarias gariepinus*

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**The effects of endosulfan pesticide were investigated on juvenile of *Clarias gariepinus*. Acute test was carried out using 0.04, 0.05, 0.06, 0.07 and 0.08 ppm of endosulfan pesticide with the 96 h $LC_{50}$ value determined by probit analysis. Chronic bioassays were evaluated on haematological and biochemical indices of the fish for a period of 60 days using four sub lethal concentrations (0.0005, 0.0010, 0.0025 and 0.0050 ppm). Blood sample was collected on days 15, 30, 45 and 60 for haematological and at day 60 only for biochemical analysis. The 96 h $LC_{50}$ value of endosulfan for *C. gareipinus* was 0.052 ppm. There was significant reduction ($P<0.05$) in red blood cell (RBC) at days 15, 30 and 45, haemoglobin (Hb) at days 15 and 45, and packed cell volume (PCV) at all days of the evaluation. White blood cell (WBC) values however showed significant increase ($P<0.05$) at days 45 and 60. There were variations in mean values of mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) with significant increase observed only at day 15 of MCHC. Significant reduction in values was obtained for total protein and globulin while significant increase was observed in cholesterol. Results obtained in this study suggest that exposure to low concentrations of endosulfan induced stress and altered the haematological and biochemical indices of treated fish.**

**Key words:** Chronic, acute, biochemical, haematological, endosulfan, cholesterol and glucose, *Clarias.*

## INTRODUCTION

The need to produce food in large quantities to cater for the ever increasing human population in the developing parts of the world has led to increase in the use of agrochemicals (fertilizer and pesticides). Pesticides are used to control pests of food crops, livestock and human health. Due to their injudicious and indiscriminate usage, water bodies like ponds, lakes and low lying water filled areas are continuously polluted (Kumar and Saradhamani, 2004). Exposure to low level of pesticides may have profound effects on non-target organisms. Most pesticides enter into the food chain and cause physiological damage (Abdul Naveed, 2003; Waliszewski et al., 2003) and may interfere with the endocrine system (Kira, 2000; Min, 2000). Silva and Gammon (2009) also reported that distruption of the endocrine system by endosulfan occurs only at doses that cause neurotoxicity.

Endosulfan (Figure 1), an organochlorine pesticide under the cyclodiene sub group, is commonly used in Nigeria against a broad spectrum of insects and mite in agriculture and other allied sectors. It was recognized as a persistently toxic substance (PTS) and considered as a potential organic pollutant (Anon, 2002). Because of its persistence in the environment, its usage has been banned in most developed countries. However, it is still being used in the developing countries because of its availability through illegal importation. Endosulfan residues had been reported to have reached alarming level in rivers and soil sediments and were also detected in animal samples from countries like Benin, Nigeria, Cote d'ivore, Madagascar, South Africa and Kenya (Anon. 2002).

Injudicious and indiscriminate use of agrochemicals have caused great concern among health and environmental scientists because records of field application of pesticides even in developed countries revealed that less than 0.1% of pesticides applied to crops reach target pest, thus over 99% moves into ecosystem to

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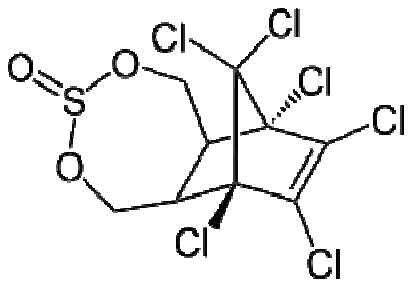

**Figure 1.** Structure of endosulfan ($C_9H_6Cl_6O_4S$).

contaminate the land, water and air (Pimentel and Levitan, 1986).

One of the ways to detect changes in the physiology of organisms is through analysis of the blood samples. Haematological profile of an animal gives a clear insight into the effect of environmental stressors and blood being a medium of intercellular and intracellular transport which comes in direct contact with various organs and tissues of the body. Alteration in erythrocytic indices had been reported in fishes subjected to environmental stressors like pesticides and heavy metals (Gill and Pant, 1987; Bhatia et al., 2004; Johal and Grewal, 2004). It is with this view that this study was carried out to investigate the potential toxic effects of endosulfan on the haematological and biochemical indices of juvenile *Clarias gariepinus.*

## MATERIALS AND METHODS

### Test chemical

The organochlorine pesticide, endosulfan (Thionex 35 EC, 350 g/L) used was manufactured by Makhteshim Limited, Beer-sheva, Israel and procured from pesticides retail office in Dugbe, Oyo State,. Nigeria. The test material is a light yellow coloured liquid and stored at ambient temperature.

### Fish and aquaria

Juvenile *C. gariepinus* were obtained from Oyo State Fishery Farm, Apitipiti in Oyo town. The average body weight and length were 65.10 (±4.24) g and 19.60 (±3.27) cm respectively. They were acclimatized for duration of 14 days and fed with commercially available fish meal. Physicochemical properties of the borehole water used were evaluated using standard method (APHA, 2005). The fish were acclimatized in 80 L capacity glass aquaria (90 × 32 × 28 cm) with each containing 30 fish. Healthy fish (very active and without mark) were selected from the pool for each of the acute and chronic tests.

### Range finding test

Renewal bioassay was used with the solution changed every 24 h. The range finding test was conducted in line with recommendation of Reish and Oshida (1987). Ten fish per concentration were exposed to 20 L of each of these concentrations: 0.001, 0.01, 1.0 and 10 ppm. The solutions were aerated and the behavioural responses of the fish were monitored while number of dead fish was recorded.

### Acute test

Acute toxicity test was carried out using five different concentrations, namely, 0.04. 0.05. 0.06, 0.07 and 0.08 ppm within the range obtained. Ten fish were exposed to each of the concentrations prepared in 20 L of water and the control in two replicates. Renewal bioassay was used where each of the test concentration was changed at every 24 h with freshly prepared solution. Behavioural and death responses per concentration were monitored and cumulative mortality response from both replicates was recorded over a period of 96 h. Lethal concentrations (LC) at 96 h were extrapolated from pooled mortality response from the replicates using probit analysis.

### Chronic toxicity test

Chronic toxicity test was carried out by exposing 30 fish to 40 L each of 0.0005, 0.0010, 0.0025 and 0.0050 ppm of endosulfan. Control was set up alongside with other concentrations using borehole water. Each concentration was renewed every 72 h using freshly prepared endosufan solution. Blood samples collected through cardiac puncturing with needle and syringes from four fish (n=4) per endosulfan concentration and control on days 15, 30, 45 and 60 of the exposure period were used to estimate haematological indices.

Blood samples used for biochemical analysis were collected separately on the day 60 of evaluation but without anticoagulant and then centrifuged.

### Haematological indices evaluation

The blood collected was used for the estimation of PCV using the method of ICSH (1980). RBC count, Hb concentration (%) and WBC were estimated using the method of Sander and Skerry (1961), Drubkin (1946) and Hurch et al. (1977) respectively with EDTA (1%) used as the anticoagulant.

### Derived parameters

Haematological parameters were further used to evaluate the effects of the pesticides on the fish blood. The calculation for each of the derived parameters used is as shown below:

**MCV:** The average volume of a single cell expressed in femtolitre (fl) or $\mu m^3$;

$$MCV = \frac{PCV\ (\%)}{RBC\ (10^6)} \times 10$$

**MCH:** This expresses the average Hb content in picorgramme of a single RBC;

$$MCH = \frac{Hb}{RBC\ (10^6)} \times 10$$

**Table 1.** Physicochemical analysis of water sample used for the fish experiment.

| Parameter | Values obtained | Standard for aquaculture |
|---|---|---|
| Temperature | 24.33 ±0.19 | - |
| pH | 6.97±0.07 | 6.7-8.5 |
| DO | 6.8±0.43 | 5 - 10 |
| BOD | 2.67±0.04 | <10 |
| Alkalinity | 67.58±1.02 | 50-300 |
| Total hardness | 66.67±0.77 | 30-180 |
| Total dissolved solid | 108±6.93 | < 500 |
| Total suspended solid | 74±8.08 | - |
| Total solid | 182±9.24 | |
| Lead (Pb) | ND | |
| Cobalt (Co) | ND | |
| Copper (Cu) | ND | 3 |
| Chromium (Cr) | ND | |
| Zinc (Zn) | ND | 5 |

All parameters were measured in mg/l except pH and temperature (℃).
Values are mean ± SEM.
Standard for aqua culture was adapted from Chaudhary et al. (2004).

**MCHC:** This refers to the percentage haemoglobin in 1dl of packed RBC.

$$MCHC = \frac{Hb}{PCV} \times 100$$

#### Biochemical indices evaluation

The serum glucose, total protein, albumin, globulin and cholesterol from the control and endosulfan treated fish were evaluated spectrophotometrically based on the method of Roe (1955), Lowery et al. (1951), Reinhold (1953), Coles (1974) and Scheuler et al. (1975) respectively.

#### Statistical analysis

SPSS 17 ® statistical package was used for the analysis. Probit analysis as described by Finney (1985) was used to evaluate the LC values. Results obtained for haematological and biochemical indices were evaluated using one way ANOVA at 0.05 level of probability. Further evaluation, where significant difference was observed, was done using Duncan's test.

## RESULTS

The physicochemical properties of the water used for the experiment conforms with the standard for aquaculture (Table 1). In acute toxicity test, the least and highest mortality response were observed at 0.04 and 0.08 ppm respectively. Lethal concentrations at different level of percentages were obtained using probit analysis with 0.052 ppm obtained for the 96 h $LC_{50}$ of the endosulfan (Table 2).

Haematological parameters showed reduction in the treated *C. gariepinus* (Table 3) compared to the control. The PCV and RBC were significantly reduced at 0.0050 ppm throughout the experimental periods. However, the value of RBC obtained for 0.0005 ppm at day 45 was not significantly different from the control ($P>0.05$). Although no significant difference was observed at day 60, there was dose dependent reduction in the RBC values.

Haemoglobin values at days 15 and 30 followed no specific pattern except that the value obtained at 0.0005 ppm on the 15th day of the treatment was significant. At day 45, Hb values at the tested concentrations except 0.0005 ppm were significantly different from the control. While no significant difference was observed in Hb at day 60, dose dependent reduction in values was obtained. Similarly, no specific pattern of variation was shown by WBC at days 15 and 45 but there was an increase at days 30 and 60 of which only that of day 60 showed significant difference.

There was reduction in values of MCV obtained at 0.0005 ppm throughout the period of evaluation compared to the control. The mean values of MCH and MCHC obtained for exposed *C. gariepinus* showed no specific pattern. There was no significant difference in value of MCH while MCHC showed significant difference ($P<0.05$) in values obtained at day 15 with the highest value obtained at 0.0050 ppm.

Biochemical parameters (Table 4) showed an increase in glucose levels with the highest value recorded at 0.0050 ppm but none was significantly different. Total

**Table 2.** The 96 h lethal concentrations obtained for *Clarias gariepinus* treated with different concentrations of endosulfan.

| | 95% confidence limit | | |
|---|---|---|---|
| **Probit** | **Concentration** | **Lower** | **Upper** |
| $LC_5$ | 0.033 | 0.020 | 0.041 |
| $LC_{10}$ | 0.037 | 0.024 | 0.044 |
| $LC_{20}$ | 0.042 | 0.030 | 0.048 |
| $LC_{30}$ | 0.045 | 0.035 | 0.051 |
| $LC_{40}$ | 0.049 | 0.040 | 0.054 |
| $LC_{50}$ | 0.052 | 0.045 | 0.058 |
| $LC_{60}$ | 0.056 | 0.049 | 0.063 |
| $LC_{70}$ | 0.060 | 0.054 | 0.071 |
| $LC_{80}$ | 0.065 | 0.058 | 0.081 |
| $LC_{90}$ | 0.073 | 0.064 | 0.101 |
| $LC_{95}$ | 0.081 | 0.069 | 0.122 |

Number of fish per concentration 'n' =20. LC, lethal concentration; probability level = 0.05.

**Table 3.** Mean values of haematological parameters obtained from *C. gariepinus* treated with different concentrations of endosulfan.

| **Treatment** | **Day** | | | |
|---|---|---|---|---|
| | **15** | **30** | **45** | **60** |
| **PCV** | | | | |
| Control | $24.50^{a}$±1.85 | $22.50^{a}$±1.44 | $21.50^{a}$±1.50 | $18.50^{a}$±1.55 |
| 0.0005 | $19.50^{b}$±1.19 | $18.00^{b}$±0.91 | $22.00^{a}$±1.47 | $17.00^{a}$ ±1.29 |
| 0.0010 | $23.00^{ab}$±1.68 | $21.00^{ab}$±1.29 | $14.00^{b}$±0.91 | $17.00^{a}$±2.86 |
| 0.0025 | $23.00^{ab}$±1.08 | $20.50^{ab}$±0.96 | $10.50^{b}$±1.04 | $10.00^{b}$±0.91 |
| 0.0050 | $10.00^{c}$±0.91 | $12.50^{c}$±1.71 | $10.00^{b}$±2.20 | $8.00^{b}$±1.58 |
| **RBC($10^6$)** | | | | |
| Control | $1.745^{a}$±0.08 | $1.682^{bc}$±0.10 | $1.655^{a}$±0.03 | 1.583±0.05 |
| 0.0005 | $1.695^{a}$ ±0.10 | $1.450^{c}$±0.04 | $1.634^{a}$±0.10 | 1.580±1.22 |
| 0.0010 | $1.878^{a}$±0.05 | $2.490^{a}$±0.06 | $1.150^{b}$±0.02 | 1.230±0.03 |
| 0.0025 | $2.081^{a}$±0.26 | $1.855^{b}$±0.08 | $0.860^{b}$±0.07 | 0.892±0.37 |
| 0.0050 | $1.010^{b}$±0.08 | $1.600^{c}$±0.09 | $0.844^{b}$±0.17 | 0.7820.07 |
| **Hb** | | | | |
| Control | $7.00^{ab}$±0.25 | 6.70±0.26 | $6.50^{a}$±0.29 | 6.48±0.29 |
| 0.0005 | $6.45^{ab}$±0.60 | 5.40±0.27 | $6.95^{a}$±0.44 | 5.28±0.35 |
| 0.0010 | $6.00^{bc}$±0.18 | 6.20±0.61 | $4.65^{b}$±0.25 | 4.95±0.52 |
| 0.0025 | $8.25^{a}$±0.77 | 6.50±0.12 | $3.75^{b}$±0.35 | 4.40±1.35 |
| 0.0050 | $4.80^{c}$±0.90 | 5.20±0.73 | $3.80^{b}$±0.78 | 3.40±0.37 |
| **WBC ($10^3$)** | | | | |
| Control | 0.852±0.01 | 0.817±0.02 | 0.872±0.02 | $0.807^{c}$±0.02 |
| 0.0005 | 0.640±0.02 | 0.861±0.08 | 0.814±0.05 | $0.718^{c}$±0.17 |
| 0.0010 | 0.720±0.01 | 1.318±0.37 | 1.026±0.09 | $0.686^{c}$±0.07 |
| 0.0025 | 0.920±0.07 | 1.298±0.26 | 0.958±0.04 | $1.078^{ab}$±0.06 |
| 0.0050 | 0.760±0.09 | 1.161±0.13 | 0.895±0.07 | $1.149^{a}$±0.085 |
| **MCV ($10^{-5}$)** | | | | |
| Control | 1.399±0.05 | 1.404±0.04 | 1.420±0.04 | 1.462±0.02 |

**Table.3** cont.

| | | | | |
|---|---|---|---|---|
| 0.0005 | 1.152±0.04 | 1.240±0.05 | 1.361±0.13 | 1.077±0.03 |
| 0.0010 | 1.221±0.06 | 0.844±0.06 | 1.220±0.08 | 1.313±0.24 |
| 0.0025 | 1.189±0.11 | 1.108±0.05 | 1.235±0.12 | 1.611±0.43 |
| 0.0050 | 0.995±0.07 | 0.806±0.16 | 0.983±0.15 | 1.100±0.33 |
| **MCH ($10^{-5}$)** | | | | |
| Control | 3.186±0.94 | 3.803±0.33 | 3.929±0.18 | 4.046±0.17 |
| 0.0005 | 3.810±0.31 | 3.719±0.12 | 4.250±0.04 | 3.629±0.47 |
| 0.0010 | 3.195±0.06 | 2.895±0.25 | 4.062±0.30 | 5.098±1.57 |
| 0.0025 | 4.266±0.38 | 3.531±0.13 | 3.396±0.99 | 4.684±0.73 |
| 0.0050 | 3.315±0.97 | 3.240±0.35 | 4.460±0.44 | 4.600±1.02 |
| **MCHC** | | | | |
| Control | $28.83^{b}$±1.32 | 31.77±1.70 | 31.32±1.18 | 33.05±3.47 |
| 0.0005 | $30.48^{b}$±1.44 | 30.02±0.63 | 31.99±2.77 | 30.86±1.96 |
| 0.0010 | $26.37^{b}$±1.33 | 34.39±2.55 | 33.58±2.57 | 41.70±13.63 |
| 0.0025 | $34.76^{ab}$±3.75 | 31.91±1.57 | 35.71±3.68 | 40.20±15.92 |
| 0.0050 | $40.86^{a}$±5.09 | 45.55±12.13 | 47.42±16.96 | 45.93±7.08 |

Unit of measurement: PCV (%), RBC and WBC ($mm^{-3}$), Hb (g/dl), MCV (fl), MCH (pg), MCHC (g/dl).
Values are mean ± SEM. Mean with different alphabet show significant difference ($p< 0.05$) while those without alphabets show no significant difference.

**Table 4.** Mean values of biochemical parameters of *C. gariepinus* after 60 days exposure to different concentrations of endosulfan.

| Concentration (ppm) | Glucose | Total protein | Albumin | Globulin | Cholesterol |
|---|---|---|---|---|---|
| Control | 93.33±7.86 | $6.20^{a}$±0.64 | 4.10±0.70 | $2.10^{a}$±0.25 | $120.4^{c}$± 5.77 |
| 0.0005 | 98.67±11.22 | $5.60^{a}$±0.46 | 3.50±0.38 | $2.10^{a}$±0.001 | $163.0^{ab}$±10.82 |
| 0.0010 | 119.33± 8.74 | $4.77^{ab}$±0.78 | 2.77±0.44 | $2.00^{a}$±0.35 | $139.2^{bc}$±15.95 |
| 0.0025 | 118.33±13.86 | $4.47^{ab}$±0.47 | 2.23±0.19 | $1.83^{a}$±0.08 | $184.33^{a}$± 8.69 |
| 0.0050 | 123.67±6.49 | $3.23^{b}$±0.35 | 2.63±0.38 | $1.33^{b}$±0.24 | $184.00^{a}$±13.11 |

Unit of measurement: g/dl. Values are mean ± SEM. Mean with different alphabet show significant difference ($p< 0.05$) while those without alphabets show no significant difference.

protein showed dose dependent reduction with only the value obtained at 0.0050 ppm significantly different from the control ($P<0.05$). Albumin values were generally low at the tested concentrations whereas globulin showed significant reduction only at 0.0050 ppm compared with the control. However, there was significant increase in the cholesterol level at all concentrations except 0.0010 ppm..

## DISCUSSION

There were behavioural changes in the activities of *C. gariepinus* treated with different acute concentrations of the endosulfan compared to the control. These changes included hyperactivity, air gulping, and surface erratic swimming to sudden death as the concentration increases. Bhatia et al. (2004) reported similar behavioural changes in *Heteroneustes fossilis* treated with endosulfan. Reports had shown endosulfan to be very toxic to non-target organisms. Low $LC_{50}$ values of 4.7 to 5.0 mg/lL was reported for *H. fossilis* (Singh and Narain, 1982), 0.14 mg/L (Thiotox) and 0.023 mg/L (Thiodan) for *Saccrobranchus fossilis* and 0.02 ppm was obtained as 48 h $LC_{50}$ for *Clarias batrachus* (Bhatia et al*.,* 2004). The 96 h $LC_{50}$ value of 0.052 ppm obtained for endosulfan treated *C. gariepinus* in this study further confirms its high level of toxicity. This high value of $LC_{50}$ obtained in this study compared to the value reported for *C. batrachus* (Bhatia et al*.,* 2004) might be due to differences in species of test organisms used, exposure period as well as geographical locations. Corroborating this is the report of Rao and Murty (1982) which showed that the slope of endosulfan toxicity curves were different for three

freshwater catfishes: *H. fossilis, Mystus cavasius* and *Mystus vittatus.*

The decrease in haematological variables (PCV, Hb and RBC) of the exposed fish may be due to haemolysis of red blood cells by endosulfan leading to significant decrease in haematocrit value which results in fish anaemia. Similar observations were reported for juvenile *C. gariepinus* separately treated with Lambdacyhalothrin, Cypermethrin and Deltamethrin pesticides (Yekeen, 2009). This may also be attributed to haemodilution resulting from impaired osmoregulation across the gill epithelium (Wedemeyer et al., 1984). Reduction in haematological indices may also be due to an appreciable decline in the haematopoiesis. Similar reduction in RBC was reported for Cypermethrin treated *Labeo rhoita* (Das and Mukherjee, 2003), fresh water common carp (*Cyprinus carpio* L.) treated with diazinone (Svoboda et al., 2001) and African cat fish (*C. gariepinus)* treated with diazinone (Adedeji et al., 2009). Other toxicants (effluent and heavy metals) had also been reported to have similar reduction effects on RBC of fishes (Goel et al., 1981; Kumari and Banerjee, 1993; Deoi et al., 2004). Reduction in Hb content of treated *C. gariepinus* may be an indication of decline in haemoglobin synthesis as well as reduction in oxygen carrying capacity which may perhaps be as a result of interference of endosulfan with haem or globin synthesis pathway. Significant reduction in Hb content and erthyrocyte count in the blood of a fresh water fish, *Sarotherodon mossambicus,* on exposure to an organophosphate (dimecron) and carbamate (cumin L.) pesticides had been reported (Ramaswamy et al., 1996). Significant decrease ($p < 0.05$) in values of erythrocyte count, haematocrit and haemoglobin content compared to the control groups had been reported for catfish (cultured and wild) on acute exposure to diazinon based organophosphorous pesticide (Adedeji et al., 2009). A 4-week exposure to sublethal levels of endosulfan (6.72 ppb) was reported to induce blood dyscrasia in the freshwater fish, *Barbus conchonius* (rosy barb) and clinical findings included erythropenia, anaemia, lymphocytosis, thrombocytosis, monocytosis and neutropenia (Gill et al., 1991). The reduction in values obtained for haematological parameters of treated fish in this study showed that the physiological activities of the treated fish were affected.

The increase in value of glucose observed compared to the control indicated that *C. gariepinus* generated more glucose to produce the energy used in combating the stress induced on the fish by endosulfan. Increase in glucose level that was observed might have resulted from increase in glucogenesis and glycogenolysis as well as inhibition of glucogenolysis and glycogenesis during stress (Metelev et al., 1981; Amudha, 1986; Yekeen, 2009). As the respiratory metabolism is being depressed, stored intracellular glycogen is utilized under such condition; the hyperglycaemic hormone is released for the degradation of glycogen and glucose thus leaked into the blood causing hyperglycaemia (Bhattacharya et al., 1975). This might have being the cause of low level of total protein, globulin and albumin observed in this study. Similar observation was reported for *Cyprinus carpio* treated with Monocrotophos (Maruthanayagam and Sharmila, 2004). The general reduction observed in the protein of the *C. gariepinus* may also be attributed to leakage and oedema, an indirect effect of diseases (Mulchay, 1975). A reduction in albumin is probably a non - specific effect which was noted as a feature of the reaction of fish to severe stress (Cardwell et al., 1971). The reduction that was observed in the protein most especially in globulin and total protein may be due to diversification of energy to meet the energy demand caused by the stress. Toxic substances like pesticides or heavy metals are known to depress blood protein in fish (Jana and Bandoyopadhyaya, 1987). The increased value of cholesterol indicated increased lipid content in the blood and retardation of fat metabolism. This may be due to heavy stress imposed on the exposed *C. gariepinus* by the pesticide.

## Conclusion

It can be deduced from this study that endosulfan has the potential to impair the physiological activities of the organism which led to changes observed in haematological indices. Persistent exposure to endosulfan may thus lead to mortality. Endosulfan usage in Nigeria should therefore be controlled in order to reduce its potential risk to human health.

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