

## Evaluation of 8-hydroxyquinoline physiological effect and Genotoxicity on *Paramisgurnus dabryanus* using hepatase activity and comet assay

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**Abstract:** With economic development and industrialization, vast genotoxic chemicals were produced and distributed in the environment. As an important industrial raw material, 8-hydroxyquinoline (8-HOQ) has been used in a wide variety of industrial circle. Therefore, human beings and other organisms, especially aquatic organisms might be exposed to these drugs and would have health risks. This study was a preliminary step to evaluate the toxicity effect of 8-hydroxyquinoline (8-HOQ) on *Paramisgurnus dabryanus* through using the methods of acute toxicity test, hepatase activity and comet assay. The results indicated that 8-HOQ had obvious toxicity effect on *Paramisgurnus dabryanus*. With the increase of the treatment-concentration and -time of 8-HOQ, the hepatic GPT and GOT activity of *Paramisgurnus dabryanus* were decreased obviously. Meanwhile, three comet parameters of hepatocyte were increased significantly, and there was significant difference between control group and each treatment group ( $p < 0.05$ ). These results suggest that 8-HOQ may become toxic chemical contaminant in environment and threaten aquatic and other organism health.

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### 1. Introduction

With economic development and industrialization, vast genotoxic chemicals were produced and distributed in the environment. 8-Hydroxyquinoline is a white to off-white crystal or crystalline powder that is insoluble in water or ether and freely soluble in ethanol, acetone, chloroform, benzene, and aqueous mineral acids (Elena et al., 2009). As an important industrial raw material, 8-hydroxyquinoline (8-HOQ) has been used in a wide variety of fields such as preservative, antimicrobial, healing drugs (medicine), agricultural pesticides and dyestuffs (Ling et al., 2009). Compared with aliphatic series and aromatic series compounds, 8-HOQ which is a nitrogen heterocyclic compound has a very much lower performance of biodegradation (Gros et al., 2009). Therefore, 8-HOQ and its derivatives have huge potential of bioaccumulation and persistence in the environment and would lead a major worldwide (water) environmental contamination. In addition, some previous studies indicate that the quinoline of 8-HOQ parent compound and its derivatives have obvious toxicity effect on organisms (Shen et al., 1999; Volkova et al., 2007; Gary et al., 2008; Hsu et al., 2008; Carolina et al., 2010; Siddharth et al., 2011). However, there is limited information available on organisms exposure to 8-hydroxyquinoline.

Therefore, the toxicity study about 8-HOQ is needed.

The long-term accumulation of toxic substances in animal body would cause grievous injury in hepatic tissue, and then would cause animal hepatase activity changes. Among all hepatase, glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT) are important aminotransferase broadly existing in animal mitochondria. Under normal circumstances, the aminotransferases have higher activity in liver cells. However, when organism is in toxication, the activity of liver aminotransferases would decrease and the blood serum aminotransferases activation would increase (Saravanan et al., 2012). Therefore, the enzymological parameters of liver and blood serum in tested fish were assessed by GOT and GPT activity assays in this study. In addition, the comet assay is a simple visual technique to measure DNA damage in cells (Singh et al., 1988). Comet assay is a quick, simple, reliable, and sensitive technique to detect and measure genetic damage in almost any type of eukaryotic cell, by using a small number of cells (Eşref et al., 2010). Therefore, this technique has been widely used for various *in vitro* and *in vivo* studies to monitor the effects of DNA-damaging agents in several fishes and other organisms (Capriglione et al., 2011; Piperakis et al., 2006; Cheryl et al., 2012). Then, in this study, the genetic

toxicity effect of 8-HOQ on *Paramisgurnus dabryanus* was also assessed by comet assay.

*Paramisgurnus dabryanus*, which belongs to *Paramisgurnus*, *Cypriniformes*, and *Cobitidae*, is widely distributed freshwater fish and can be found in Chinese water bodies. Furthermore, with the advantages of short life cycles, easy to catch and quick responses to environmental disturbances in an integrated and continuous manner, *P. dabryanus* is used in toxicity tests of chemicals usually. Earlier studies also had confirmed that *P. dabryanus* was the ideal test organism for assessing the toxicological effects of pollutants (Zhang et al., 2008; Seok-Ki et al., 2010). However, to our knowledge, the information of 8-HOQ-induced biotoxicity in *P. dabryanus* is limited. Thus convenient and valid biomonitoring methodology is essential for evaluating possible health risks for fish and other organism health due to 8-HOQ contaminant. The aim of this study is to evaluate the water environmental risk of 8-HOQ contamination for fish populations, and propose the comet assay applied to fish as a biomonitoring methodology to evaluate 8-HOQ genotoxicity.

## 2. Materials and methods

### 2.1. Tested fish and major chemicals

Adult fish (*P. dabryanus*) ( $16.4 \pm 3.2$  g) were collected from wetlands in the old course of Yellow River, Yanjin (Henan, China), and were breed in the exposure of tap water under laboratory conditions. The 8-hydroxylquinoline was purchased from the Nanjing Jiancheng Bioengineering Institute (China), and its purity was more than 99%. All other chemicals used were of analytical grade.

### 2.2. Acute toxicity on *P. dabryanus*

Acute toxicity test was carried out by using the Spearman-Kärber method with some modification to obtain a 50% lethal concentration ( $LC_{50}$ ) of 8-HOQ in *P. dabryanus* after 24, 48 and 96 h of exposure (Kärber, 1931; Li et al., 2012). Sixty adult fish were randomly divided into 6 groups (10 fish in each group), out of which 5 groups served as HOQ-treatment group and another as a control. The treatment groups were exposed to the 8-HOQ solution at the concentration of 14.37, 21.48, 32.23, 48.34 and 72.58  $mg\ l^{-1}$  for 96 h, respectively. And the control group was placed in aerated tap water for the same period. Each test-group was all placed in plastic aquaria (6 L in volume), exchanged with its same concentration 8-HOQ solution after exposure of 24 h and no food was provided during the treatment-time. Each test was conducted in duplicate. The dead and surviving fish were recorded in each group during the exposure period. The  $LC_{50}$  of 8-HOQ on *P. dabryanus* after 24 h, 48 h and 96 h of exposure were calculated using SPSS version 13 with the amended Spearman-Kärber method.

### 2.3. Hepatic transaminase activity assays

By the result of acute toxicity, ninety adult *P.*

*dabryanus* were randomly divided into five groups (3 parallels in each group, 6 fish in each parallel), out of which four groups served as 8-HOQ-treatment group and another as a control. The treatment groups were exposed to the 8-HOQ solution at the concentration of 3.629, 7.258, 10.887 and 14.516  $mg\ l^{-1}$  for 6 days. Its treatment process was the same as the acute toxicity test. After 2, 4, and 6 d of exposure, three fish of each group (1 fish in each parallel) were all anaesthetized with 100  $mg\ l^{-1}$  MS-222 (Tricaine) and then dissected. Samples (blood serum and liver) were collected for the hepatic amino acid transaminase (GPT/GOT) activity assays. GPT/ GOT were determined using the Diagnostic Reagent Kits, purchased from the Nanjing Jiancheng Bioengineering Institute (China), according to the manufacturer's instructions. The results of these enzymatic assays were given in units of enzymatic activity per milligram of protein ( $U/mg\ prot$ ), whereas 1 U of GOT/GPT was defined as a change of 0.001 in the absorbance of NADH/min (Yin et al., 2011).

### 2.4. Comet assay

The experimental design of comet assay was similar to the enzymes activity assay test. Three fish of each group (1 fish in each parallel) were anaesthetized with 100  $mg\ l^{-1}$  MS-222 (Tricaine) and followed by the severing of the spinal cord of tail stern after 6, 24, 48 and 96 h of exposure, respectively. Then, the livers were dissected from the tested fish quickly, placed in PBS (pH 7.5), kept on ice and gently cut up with scissors. After isolated hepatocytes were released as a suspension, the cell number and cell viability were measured with the performance as described by Mitchelmore et al. (Mitchelmore et al., 1998), with some modifications. Cells were typically > 80% viable with  $1 \times 10^6 \sim 1 \times 10^8$  cells isolated per liver.

The single cell gel electrophoresis was performed as described by Singh et al. and Piperakis et al. (Singh et al., 1988; Piperakis et al., 2006) with minor modification. After cell lysis (2.5M NaCl, 10 mM Tris, 100 mM  $Na_2EDTA$ , 1% Triton X-100, 10% DMSO, 1% Na sarcosinate, pH 10.0,  $\geq 1$  h), DNA was placed in an alkaline electrophoresis buffer (300 mM NaOH, 1 mM  $Na_2EDTA$ ; pH13, 4°C) for 10 min to allow the DNA to unwind, followed by electrophoresis in the same buffer for 20 min at 25 V, 300 mA at 4°C. Samples were stained with ethidium bromide (10  $\mu g/ml$ , 10 min) and examined under fluorescent microscope (OLYMPUS BX60) or placed in a humid, dark box at 4°C until analysis (within 48 h). All slides were coded and the whole slide was scanned randomly. Two slides per specimen were prepared and fifty random cells per slide were analyzed and scored by using an image analysis package. Cells with damaged DNA appeared as comets (Fig 3), whose tail length, tail DNA% and tail moment ( $TM = \text{tail DNA}\% \times \text{tail length}$ ) were assessed by using the CytoVision NT automatic image

analysis system. Then tested cells were categorised for four grade of damage (using Tail Moment) based on the criteria reported by Anderson et al. (Anderson et al., 1994) with minor modifications (grade of damage: zero or minimal 1-5, low damage 5–20, mid damage 20-60, and high damage > 60).

### 2.5. Statistical analyses

All data were expressed as mean  $\pm$  SD and analyzed by one-way analysis of variance. And the statistical significance was evaluated by SPSS version 17.0 (P values less than 0.05 were considered statistically significant) and the individual comparisons were obtained by Duncans' multiple range test.

## 3. Results

### 3.1. Acute toxicity of 8-HOQ on *Paramisgurnus dabryanus*

The acute toxicity data of 8-HOQ on adult *P. dabryanus* was described in table1. As was shown by the table1, the mortality (%) were increased with the increase of treatment-concentration and treatment-time of 8-HOQ. Additionally, the LC50 of 8-HOQ to *P. dabryanus* was decreased with the increase of treatment-time of 8-HOQ. The 24, 48 and 96 h LC<sub>50</sub> of 8-HOQ on adult loach were 69.70 $\pm$ 3.29,

48.35 $\pm$ 4.23 and 28.53 $\pm$ 2.84 mg l<sup>-1</sup>, which indicated that 8-HOQ had obvious toxicity to *P. dabryanus*.

**Table 1.** Mortality rate of adult *P. dabryanus* induced by 8-HOQ

8-HOQ (mg l <sup>-1</sup> )	Mortality (%)		
	24 h	48 h	96 h
14.37	0 $\pm$ 0	0 $\pm$ 0	10 $\pm$ 0
21.48	0 $\pm$ 0	10 $\pm$ 0	20 $\pm$ 0
32.23	10 $\pm$ 0	25 $\pm$ 5	35 $\pm$ 5
48.34	25 $\pm$ 5	45 $\pm$ 5	85 $\pm$ 5
72.58	45 $\pm$ 5	70 $\pm$ 0	100 $\pm$ 0

### 3.2. GOT and GPT activity assay in hepatocyte and serum of adult fish

The Data of enzyme activity assays of 8-HOQ to adult *P. dabryanus* were described in Table 2. Subacute exposure to 8-HOQ decreased the liver GOT and GPT activity in tested fish, while the blood serum GOT and GPT activity increased compared with their control (Table 2). And there was significant difference (p<0.05) between each treatment group and its control group, except that the hepatic GPT and GOT activity of treatment-group 3.629 mg L<sup>-1</sup> of 2 d exposure (p > 0.05).

**Table 2.** GOT and GPT activity of hepatocyte and serum induced by 8-HOQ in *P. dabryanus*

8-HOQ ( $\mu$ g l <sup>-1</sup> )		2d		4d		6d	
		GPT (U mg <sup>-1</sup> prot <sup>-1</sup> )	GOT (U mg <sup>-1</sup> prot <sup>-1</sup> )	GPT (U mg <sup>-1</sup> prot <sup>-1</sup> )	GOT (U mg <sup>-1</sup> prot <sup>-1</sup> )	GPT (U mg <sup>-1</sup> prot <sup>-1</sup> )	GOT (U mg <sup>-1</sup> prot <sup>-1</sup> )
0	H	233.00 $\pm$ 24.98	196.47 $\pm$ 13.29	228.00 $\pm$ 13.95	188.26 $\pm$ 15.55	218.22 $\pm$ 17.56	188.02 $\pm$ 15.77
	S	19.79 $\pm$ 3.37	18.46 $\pm$ 2.24	20.19 $\pm$ 3.34	19.24 $\pm$ 3.06	21.26 $\pm$ 3.44	20.97 $\pm$ 2.09
3.62	H	210.76 $\pm$ 10.59	170.61 $\pm$ 11.36	175.01 $\pm$ 10.82*	117.13 $\pm$ 6.17*	172.24 $\pm$ 13.75*	115.37 $\pm$ 12.10*
	S	31.23 $\pm$ 3.27*	29.91 $\pm$ 2.59*	43.50 $\pm$ 3.75*	35.79 $\pm$ 4.81*	48.89 $\pm$ 5.28**	47.46 $\pm$ 4.69**
7.25	H	193.51 $\pm$ 14.47*	144.05 $\pm$ 12.93*	161.34 $\pm$ 11.43*	101.76 $\pm$ 6.22*	144.03 $\pm$ 10.13*	97.45 $\pm$ 8.27*
	S	53.65 $\pm$ 6.47**	41.34 $\pm$ 3.53*	62.27 $\pm$ 7.86**	52.5 $\pm$ 3.71**	70.68 $\pm$ 6.24**	63.12 $\pm$ 3.97**
10.8	H	181.52 $\pm$ 10.64*	108.91 $\pm$ 4.49	162.53 $\pm$ 10.79*	95.54 $\pm$ 7.69*	134.25 $\pm$ 12.24*	84.89 $\pm$ 3.97*
	S	66.28 $\pm$ 6.42**	64.24 $\pm$ 4.39**	79.56 $\pm$ 8.26**	62.71 $\pm$ 7.43**	88.91 $\pm$ 6.42**	72.43 $\pm$ 5.34**
14.5	H	157.22 $\pm$ 10.70*	106.77 $\pm$ 12.49*	145.39 $\pm$ 11.74*	97.96 $\pm$ 8.97*	159.22 $\pm$ 12.98*	87.47 $\pm$ 10.04*
	S	84.61 $\pm$ 12.16**	80.22 $\pm$ 9.01**	95.24 $\pm$ 7.92**	86.69 $\pm$ 5.36**	101.31 $\pm$ 8.75**	91.62 $\pm$ 10.32**

Note : H: the GOT and GPT activity induced by 8-HOQ in the hepatocyte of *P. dabryanus*; S: the GOT and GPT activity induced by HOQ in the serum of *P. dabryanus*. Values followed by "\*" and "\*\*" mean significant difference at 0.5 and 0.01 level, respectively. ( )

### 3.3. Comet assay and data analysis

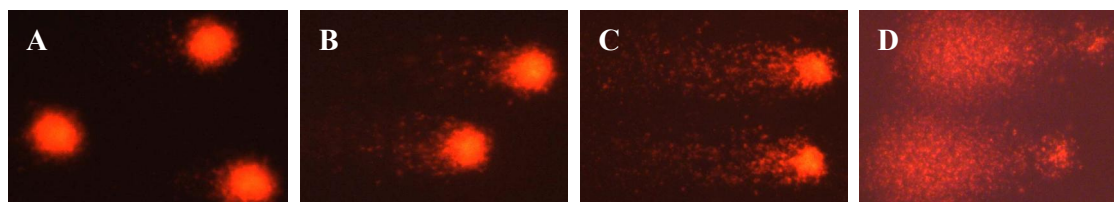
Comet scores of control and exposed groups were shown in Figure 1. Figure 2 was made on the basis of three comet parameters of Trailing rate (%), tail length ( $\mu$ m) and tail moment by SPSS version 17.0, which summarises the results for Comet assay of liver cells in tested fish. The SCGE assay showed an obvious increase in three comet parameters in exposed fish, where the genome damage was significantly different (P<0.05) from control fish (Fig. 2). The DNA damage documented by the comet assay test, i.e. increased tail length paralleled by a reduction in head size (Figs. 1, 2), increasing with

treating time. For example, there was no apparent difference (P > 0.05) in the liver trailing rates between control and the lowest concentration exposed groups (3.629 mg l<sup>-1</sup>) after for 6 and 24 h exposure to 8-HOQ. However, the hepatocytes trailing rates between control and the lower concentration exposed groups (7.258 mg l<sup>-1</sup>) after for 6 h exposures to 8-HOQ had a significant difference (P < 0.05), and had a extreme significant difference (P < 0.01) between the higher exposed groups (14.516 and 29.032 mg l<sup>-1</sup>) and their control after for other all treatment-time (Fig. 2A). The results indicated that the trailing rates of *P. dabryanus* liver

cells were increasing with the increase of exposed concentration and exposed time, which showed an obvious dose-effect and time-effect relationships (Fig. 2A). In the meantime, the tail length and tail moment showed similar results (Fig. 2B and 2C).

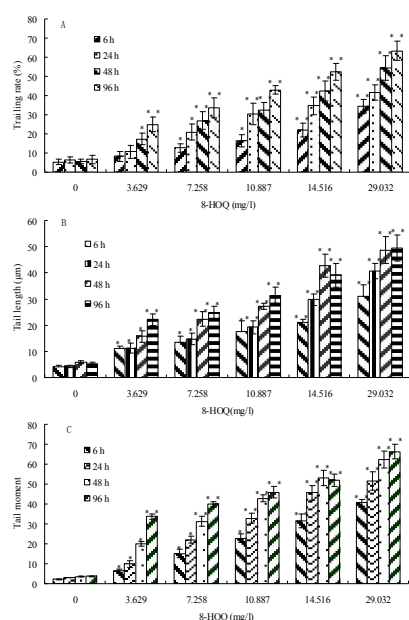
According to comet classifications, the DNA damage of control fish liver cells all belonged to class 1 (Fig. 1A, TM < 5). However, there was different class DNA damage of exposed fish liver cells at four treatment-times. For example, there were low damage cells in the lower concentration

treatment-groups ( $3.629, 7.258 \text{ mg l}^{-1}$ ) by exposed 6 h (Fig. 1B, class 2, TM: 5-20). The mid damage cells (class 3, Fig. 1C, TM: 20-60) appeared in all treatment-groups by exposed 24 and 48 h for 8-HOQ. And the high damage cells (class 4, Tail moment > 60, Fig. 1D) were noted in the highest concentration treatment-groups ( $29.032 \text{ mg l}^{-1}$ ) by exposed 48 h and longer than it for 8-HOQ. The comet assay results showed that 8-HOQ can induce obvious genotoxicity to *P. dabryanus* liver, which also showed dose-effect and time-effect.



**Figure 1.** The Comet scale of *P. dabryanus* liver cells induced by 8-HOQ ( $\times 400$ ), a four-class classification based on tail moment (TM) adopted from Anderson et al (Anderson et al. 1994)

Note: A: control cells (CC, TM < 5); B: low damage cells (LDC, TM 5-20); C: mid damage cells (MDC, TM 20-60); D: high damage cells (HDC, TM > 60)



**Figure 2.** DNA damage effect of 8-HOQ on liver cells of *P. dabryanus*: A. Effect of 8-HOQ on the % trailing rate of tested fish liver cells; B. Effect of 8-HOQ on the tail length of tested fish liver cells; C. Effect of 8-HOQ on the Tail Moment of tested fish liver cells

Note: Values followed by “\*” and “\*\*” mean significant difference at 0.5 and 0.01 level, respectively.

#### 4. Discussion

This study contributes significantly to the knowledge of 8-HOQ ecological consequences in the

aquatic environment.  $LC_{50}$  can generally represent the degree of toxicity of toxicants, and thus it is the most important index that must be determined in an acute toxicity test (Li et al., 2012). In the present study, the acute toxicity data of 8-HOQ on adult *P. dabryanus* (Table 1) indicated that 8-HOQ had marked acute toxicity to loach. The 24 h, 48 h, and 96 h  $LC_{50}$  of 8-HOQ on *P. dabryanus* were  $69.7 \pm 3.29$ ,  $48.35 \pm 4.23$  and  $28.53 \pm 2.84 \text{ mg l}^{-1}$  respectively (table 1), which were lower compared with other environmental pollutants (Zhang et al., 2008; Seok-Ki et al., 2010). And tested fish (especially in the highest concentration 8-HOQ-treatment group,  $72.58 \text{ mg l}^{-1}$ ) had showed typical toxic symptoms as follows, moving about and writhing quickly at the initial phase of test, slowing in reacting and losing balance gradually after several hours, then died finally. The bodies of dead fish were forniciform frequently and their liver were swelling and aterrimus, which is similar to the research results about the fish toxic symptom induced by heavy metal and others poisonous substances (Palanivelu et al., 2005; Nurullah et al., 2010; Vijaya et al., 2011; Li et al., 2012). The results of above suggested that 8-HOQ had strong toxicity effect to organisms, especial in aquatic organisms. Subsequent GPT/GOT activity assay and comet assay also showed that 8-HOQ could induce obvious physiological effect and genotoxicity in tested fish liver.

The liver or hepatopancreas, is the target of toxic chemicals usually. The results of our physiology toxicity test also indicate that liver is the major target organ of 8-HOQ to fish. In the process of participating in organism metabolic activity on poisonous substances, liver could be damaged in



varying degrees (Palanivelu et al., 2005). And the enzymatic activity changes of liver major enzymes also reflect the damage degree of animal liver. Under normal circumstances, aminotransferase is mainly in hepatic cell cytoplasm, only a few GPT and GOT are released to blood serum. When pathological changes or damage induced by foreign toxic substances take place in liver, the cytomembrane permeability of hepatic cell would change, a lot of GPT and GOT in liver would seep into blood plasma. Therefore, the activation of liver aminotransferases would decrease when organism is in toxication, meanwhile, the blood serum aminotransferases activation would increase (Yin et al., 2011). In this study, the enzyme content in loach liver was remarkably declined and showed concentration-effect and time-effect (Table 3). However, the enzyme level in blood serum was significantly promoted when compared with the control and had showed concentration-effect and time-effect also (Table 3). Those results suggested that the physiology toxicity induced by 8-HOQ maybe occur in the adult loach. This result was coincide with the results of others studies (Storelli et al., 2005; Tepe et al., 2008; Tigano et al., 2009; Yin et al., 2011; Saravanan et al., 2012).

The comet assay or single cell gel electrophoresis (SCGE) assay is a rapid, sensitive and relative simple method for detecting microscopically DNA damage at the level of individual cells. The main advantages of the Comet Assay include: (a) the collection of data at the level of the individual cell, (b) a small number of cells per sample (<10,000) is needed, (c) sensitivity for detecting DNA damage and (d) use of any eukaryote single cell population both in vitro and in vivo. Therefore, comet assay is one of the very widely used assays to toxic test, such as eco-genotoxicological studies and environmental monitoring, regulatory genotoxicological studies, and this assay has also been used to show protective effects of different dietary factors in chemo-preventive studies (Bichler et al., 2007; Cheryl et al., 2012; Eşref et al., 2010; Capriglione et al., 2011). In our present study, three parameters of trailing rate (%), tail length (µm) and tail moment were adopted for detecting the DNA damage effect of 8-HOQ on loach. The results indicated that 8-HOQ had obvious DNA damage effect to loach liver cells, and showed a clear dose-effect. Those results were also in accordance with that of enzyme activity assay, which illustrated that 8-HOQ could not only damage DNA, but also affect the normal physiological metabolic activity of liver in organism. This research result indicated the potential of 8-HOQ to damage fish cells, which was in accord with the similar findings in mammalian cells (Chang et al., 1991).

In conclusion, this study revealed that 8-HOQ could have obvious biotoxicity effect on *p. dabryanus*. And the liver of tested fish was a visceral target of 8-HOQ. 8-HOQ exposure could induce

significant physiology effect (change in hepatic enzyme activity) and genetic toxicity (DNA damage) in liver, which might be one of the possible mechanisms of 8-HOQ toxicity to aquatic animals. Therefore, this study provided some theoretical knowledge and guidance for safety application of 8-HOQ, and the prevention and control of 8-HOQ to environment pollution. In addition, the comet assay of DNA damage in loach liver cells adopted in this study offered a fast and sensitive method for monitoring water contamination.

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