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ZOOLOGY

# BP-3, a major component of sunscreen, alters water column use and feeding, but not aggression in Nile tilapias (*Oreochromis niloticus*)

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**ABSTRACT.** Tourism has increased the contact of tourists with the aquatic environment and is transporting harmful substances such as benzophenone (BP-3) into the water. This is an emerging contaminant present in sunscreens and other cosmetics, which can alter the behavior of fish by acting on the hypothalamus-pituitary-gonad axis. We hypothesized that the use of the water column, feeding, and aggressiveness would be affected by this contaminant. Thirty-six juvenile male Nile tilapia (*Oreochromis niloticus*) exposed to BP-3 for 1 to 97 hours were analyzed. We compared the time spent at the bottom of the tank, the latency to feed, and the latency to attack a mirror between control and three BP-3 concentrations using an Anova, both parametric and non-parametric followed by post-hoc tests. We observed that the fish spent less time at the bottom of the tank, a normal condition for tilapia, and ate faster, but showed no change in aggressiveness. Effects varied with concentration and duration of exposure. Previous studies have shown similar results with other fish species, suggesting the metabolic cost of BP-3 as a contaminant, but with no significant effect on aggressiveness. Ours is the first study to address the effect of BP-3 on aggressiveness and habitat use of a bottom-dweller and territorial fish also widely used in aquaculture. Protection against ultraviolet radiation is necessary, but the use of BP-3 for this purpose should be considered with caution.

**Keywords:** behavior; benzophenone; Cichlidae; ecotoxicology; fish; tourism.

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## Introduction

Sun and sand tourism transports people to a new environment (Rowe & Santos, 2016), but it also increases the interaction between fish and humans, with several detrimental effects on fish (Bessa, Silva, & Sabino, 2017). Human-fish interaction raises environmental issues that need to be reduced to prevent the ecological impacts generated by aquatic mass tourism. According to Denchak (2018), water contamination can generate toxic environments for the health of animals such as fish. Increased tourism in aquatic environments will affect fish behavior due to factors such as human presence (Samia et al., 2019), feeding (Silva, Ferreira Junior, Artoni, & Bessa, 2020), noise (Leduc et al., 2021), and contaminants (Carić & Mackelworth, 2014).

Due to the growing awareness of the risks of ultraviolet exposure, the use of solar cosmetics has also grown, increasing the presence of substances such as benzophenone 3 (BP-3) or oxybenzone in the marine environment (Tovar-Sánchez et al., 2013). According to Downs et al. (2015), BP-3 is considered a contaminant of great concern in marine environments, reaching these environments through bathers and residual discharges. Despite protecting against UV radiation (Agin, Anthony, & Hermansky, 1998), sunscreens can lead to abusive sun exposure, by overconfidence in the product and can reduce the synthesis of vitamin D (Lodén et al., 2011).

Approximately 16,000 to 25,000 tons of sunscreen have been used in tropical countries, resulting in approximately 4,000 to 6,000 tons of sunscreen are being released into the aquatic environment annually (Danovaro et al., 2008). BP-3 is present in several cosmetics, including sunscreens, and may pose a risk to aquatic life. BP-3 is 43 times more toxic than other sunscreens, such as octyl methoxycinnamate (EHMC), amyloxate (IAMC), para-aminobenzoic acid (OD-PABA), octocrylene (OC), and 4-methyl benzylidene (4-MBC), however, it is still widely used (Rodil, Moeder, Altenburger, & Schmitt-Jansen, 2009). This substance is widespread in water bodies around the world and is not removed by common sewage treatment processes (Schneider & Lim, 2019). It has even been found in drinking water in Brazil (Silva, Emídio, & Marchi, 2015).

BP-3 has great potential to become a harmful pollutant because it is very stable in the environment and deposits on surfaces (Lodén et al., 2011). In addition, it has been shown to be highly toxic (Li, 2012). It impacts the composition, cellular structure, growth, and biomass of green microalgae (Teoh, Sanusi, Wong, & Beardall, 2020). In reef-building corals, with filter feeding that enhances bioaccumulation, BP-3 in sublethal concentrations led to bleaching (Schneider & Lim, 2019), polyp retraction (Conway, Gonsior, Clark, Heyes, & Mitchelmore, 2021), altered symbiotic microbiota and reduced photosynthetic capacity of zooxanthellae (Wijgerde et al., 2020). In fish, BP-3 was lethal for zebrafish embryos exposed for 96 hours to a concentration of 4.74 mg $L^{-1}$, while delaying embryo hatching, reducing tail movements and heart rate at sub-lethal concentrations (Zhang et al., 2021). Clownfish exposed to BP-3 performed erratic swimming and stopped eating, resulting in the mortality of a quarter of the fish after 97 hours of exposure to a concentration of 100 mg $L^{-1}$ (Barone, Hayes, Kerr, Lee, & Flaherty, 2019). A marine flounder that received an intraperitoneal injection of BP-3 showed increased swimming, and altered enzymatic action in the liver and intestine histology (Carvalhais et al., 2021). BP-3 underwent bioaccumulation and biomagnification in several species of fish (reviewed by Schneider & Lim, 2019). Another previously reported sub-lethal effect in fish concerns feminization.

Exposure to BP-3 resulted in vitellogenin production and feminization in trout and medakas (Coronado et al., 2008). Kunz and Fent (2006) reported altered levels of estrogens in the water and in fish that have residues of sunscreens. These levels of vitellogenin and estrogen can result in the reduction of aggressive behavior in fish, as cited by Wilson, Boer, Arnott, and Grimmer (2011). Aggressiveness plays an important role in maintaining territory, competition for spawning sites, and reproduction in several fish species (Keenleyside, 2012). The feminization created by BP-3 can reduce aggressive behavior, affecting their entire biology, especially in known aggressive and territorial species such as Cichlids (Gonçalves-de-Freitas et al., 2019). However, research is still lacking on the sub-lethal effects of BP-3 on behavior, including aggressive behavior, in highly territorial fish. BP-3 can reduce aggressiveness, even without reducing locomotor activity in betta fish, acting through endocrine pathways (Chen, Wu, & Ding, 2016). In zebrafish, BP-3 prevents aggressive behavior, reduces sociality, and reduces risk perception (Moreira & Lucchiari, 2022). Taken together, these observations indicate that exposure to BP-3 may have important effects on habitat use, energy expenditure, and aggressiveness in territorial fish such as Cichlids, although there are still no empirical data supporting this proposal.

Our study aims to evaluate the effect of sublethal BP-3 on the locomotor, feeding, and aggressive behavior of the territorial Cichlid *Oreochromis niloticus* (Linnaeus, 1758), the Nile tilapia, up to 97 hours of exposure. Contamination with this pollutant is one of the factors boosted by sun and sand tourism, which especially affects aquatic environments. We hypothesize that BP-3 will affect behavior and predict that the fish modify their pattern of using the water column, spending more time away from the bottom; feed faster, indicating altered metabolism; and become less aggressive, implicating feminizing effects, when exposed to this sunscreen component.

## Material and methods

### Study animals and ethics

The species we used for the study was *Oreochromis niloticus*. We selected it because tilapias show intense aggressive behaviors, as a form of territoriality (Lowe-Mcconnell, 1987), in addition to being a bottom-dweller, very resistant in captivity, and used as an experimental model (Gonçalves-de-Freitas et al., 2019). This species is also widely used in aquaculture around the world, giving our study another level of application.

For our study, we used 36 juvenile males measuring 0.8 cm TL obtained from the Vale do Sol breeding Farm in Brasília, Brazil. In the fish farm, these fish were kept in the same aquaria with about 30,000 tilapias (density of 0.15 individuals $L^{-1}$), with constant oxygenation and daily feeding with commercial feed for tilapia juveniles. They were transported in a bag with a jet of oxygen inside that allowed for transportation with less stress to the fish. Transportation lasted less than 1 hour.

Upon arrival at the laboratory, at the University of Brasília, they were placed in individual 2 L tanks (density of 0.5 fish $L^{-1}$), visually isolated from the others, constantly aerated, and fed daily with industrial food for fish in general. Before the beginning of the experiments with BP-3, the experimental subjects were acclimatized for 3 days to the laboratory conditions with the temperature kept between 24-28°C, pH at 6.8, and exposed to a 12-12 hour light-dark regime.

All procedures are in accordance with the best animal welfare guidelines of species and sample-size choice; transportation, housing, and handling; and disposition of the animals after the tests (ASAB Ethical Committee/ABS Animal Care Committee, 2023).

## Exposure to BP-3

To carry out the tests, we used individual circular tanks with 2 L of water kept in the same conditions mentioned above. The concentration treatments were 0 + ethanol solvent (control), 2.5, 5, and 10 μg (N = 9 fish). These concentrations are in line with previous studies such as Chen, Wu, and Ding (2016) used dosages from 10 to 1,000 μg $L^{-1}$ for 28 days; Blüthgen, Zucchi, and Fent, (2012) exposed the animals for 5 to 14 days to concentrations of 10, 200, and 600 μg $L^{-1}$. Moreira and Lucchiari (2021) used concentrations of 10, 100, and 1,000 μg $L^{-1}$ for 15 days in zebrafish. We chose the dosages used because we observed high fish mortality at higher concentrations tested in a pilot study (50-100 μg $L^{-1}$), however, it was less concentrated and exposed for a shorter time than in other studies that recorded a more conclusive effect about the fish. And, considering the values found in real outdoor situations in Parque Nacional de Brasília, with 250 thousand bathers/year (Brasil, 2020), we estimate that bathers release less BP-3 in the water than the concentrations we used.

We dissolved the crystallized benzophenone with 0.1% ethanol before introduction into the tanks. This same amount was included in the control tank. We collected water column use and feeding data after 1, 25, 37, 49, 73, and 97 hours of exposure to BP-3 (Figure 1). Most ecotoxicological studies on fish evaluate the pollutant's effect after 96 hours of exposition, we chose to measure the effects on the timeframe presented above due to a technical recommendation from the Organization for the Economic Cooperation and Development (Wilhelm & Maibach, 2012). It will also avoid a significant loss by evaporation of the contaminant into the atmosphere. At each hour we recorded each tank for 5 minutes with a camera positioned in front of the tank.

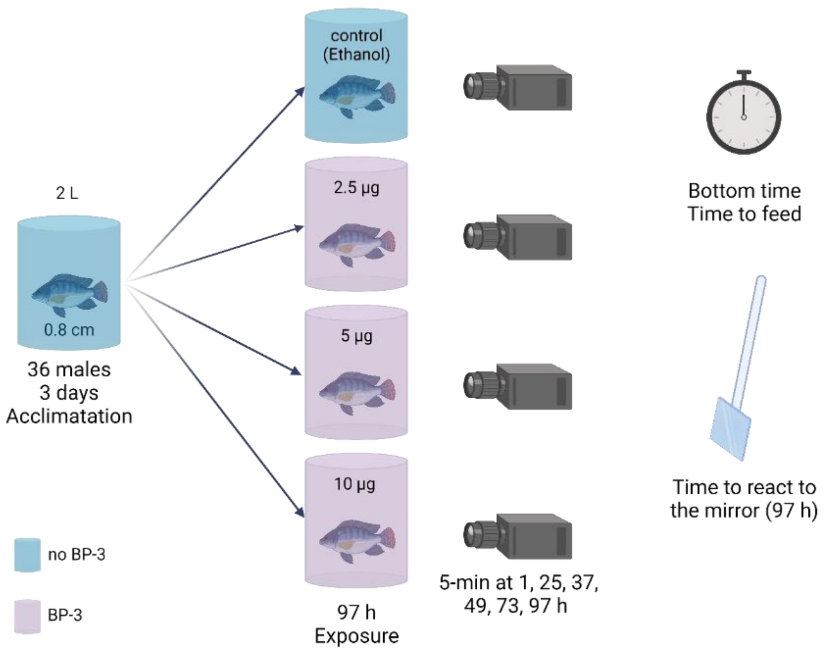


**Figure 1.** Experimental design. Created with BioRender.com.

## Experimental measurements

Tilapias are primarily bottom-dwelling animals (Gonçalves-de-Freitas et al., 2019), but they can approach the surface under intoxication, such as under copper (Ezeonyejiaku, Obiakor, & Ezenwelu, 2011) or charcoal (Shrivastava, Thakur, & Shrivastava, 2011) pollution. Thus, first, we measured the time that the experimental subjects spent near the bottom, expecting it to decrease with dosage and exposure time. Second, Volkoff and

Peter (2006) homeostatic and environmental factors regulate feeding behavior, since central and peripheral hormonal factors, often affected by pollution, influence fish appetite due to the metabolic costs of decontamination. Thus, we expect a reduction in the latency to feed with dosage and duration of exposure to BP-3. In the so-called mirror test (Gerlai, Lahav, Guo, & Rosenthal, 2000) we place a mirror at the rear of the aquarium, away from the experimental animal. Third, the mirror test was performed once, after 97h of exposure to benzophenone. The mirror test was performed a single time because there is evidence that repeated aggressive contests change the results due to fish's perception of status (Franck & Ribowski, 1987; Chang, Li, Earley, & Hsu, 2012). With the mirror test, one can see the occurrence of aggressive behavior, we measured the latency to attack its image. As aggressive behavior is frequent and natural in this very territorial species (Gonçalves-de-Freitas et al., 2019), we expect a reduction in aggressiveness in response to benzophenone, as it may have a feminizing effect.

After the recordings were made, an observer blinded to the treatments watched the footage and recorded the following variables: time spent at the bottom (use of the water column), feeding latency, and latency to attack the mirror.

### Statistical analysis

To analyze the bottom time data and feeding latency, a two-factor analysis of variance was performed for concentrations and exposure duration as independent variables. Pairwise comparisons were done using Tukey's *post hoc* tests, especially considering the control group. Our data for Time in the bottom and latency to feed attended the premises of this parametric test for normal distribution (Shapiro-Wilks; $p > 0.05$). The mirror test data did not have a normal distribution and thus were submitted to a non-parametric test of Kruskal-Wallis. All tests used the statistical package Bioestat 5.3.

## Results

Contamination altered the use of the water column, reducing the bottom time (Figure 2a). Longer exposure to BP-3 reduced bottom time ($F = 4.414$; $DF = 5$; $p = 0.0009$), especially between 37 and 73 hours according to Tukey's post hoc test (p between 0.02 and 0.003). Increasing the BP-3 dosage also seems to have slightly affected the use of the water column according to the two-way ANOVA ($F = 2.819$; $DF = 4$; $p = 0.0409$), but the effect of each particular dosage could not be detected in the *post hoc* test. As expressed by the blue line in Figure 2a, the control group seems to be the one that responded more to the decrease in bottom time. Though, this difference is not statistically supported by the post-hoc test ($Q = 2.94$; $p = 0.16$).

The contaminant also resulted in a shorter latency to feed (Figure 2b), both with the duration of exposure to BP-3 ($F = 5.222$; $DF = 5$; $p = 0.00021$) and with the BP-3 concentration ($F = 2.82$; $DF = 3$; $p = 0.0416$). This difference occurred after 37 hours of exposure (p between 0.03 and 0.0002), but no specific dose effect was found between the control and the other concentrations of the contaminant in the *post hoc* test.

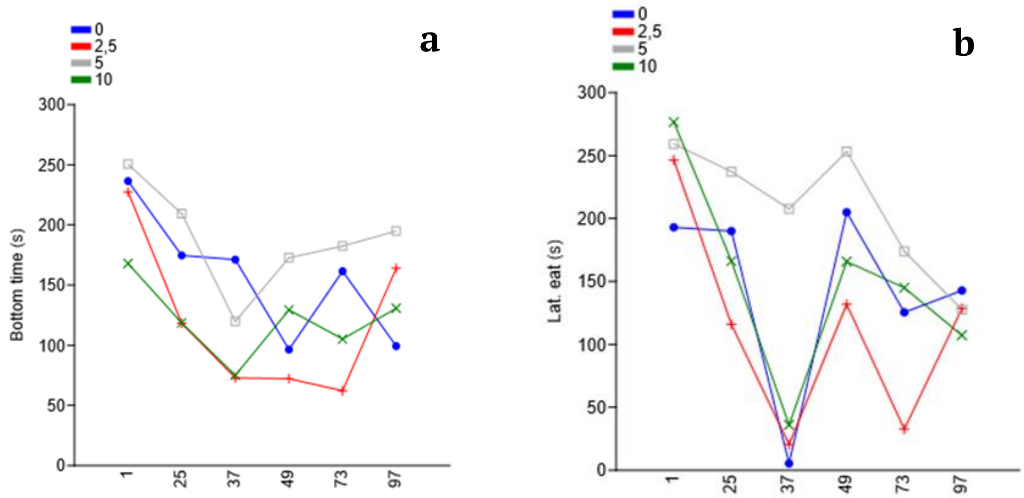


**Figure 2.** Effect of BP-3 Concentration (colored lines) and duration of exposure (x-axis) on a) water column use (Bottom Time) and b)

latency to feed.

Unlike the previous variables, BP-3 showed no effect on mirror attack latency (Figure 3; H = 4.423; p = 0.2004), an indicator of aggressiveness in our study. The mirror test was performed a single time in our experiment.

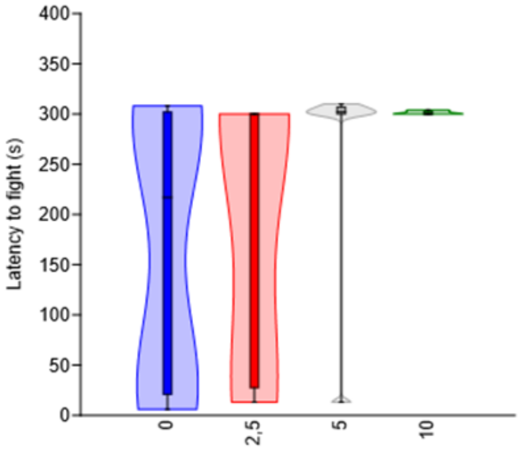


**Figure 3.** Latency to attack in the mirror test at concentrations of 0 (control), 2.5, 5, and 10 μg.

## Discussion

We found a reduction in the time that Tilapias spent on the bottom and in the latency to feed. Time of exposure was the main variable to affect these changes, but dosage was also significant regarding these two initial variables. On the contrary, we did not observe any change in the latency to attack the mirror, suggesting no effect on aggressive behavior.

Tilapias spent less time in the bottom, their normal behavior, as the time of exposure to the contaminant passed. Fish spending most of their time at the surface indicates possible hypoxia (Kramer, 1987; Xu, Liu, Cui, & Miao, 2006). Feeling uncomfortable with the increased metabolic demand and lack of oxygen caused by the processing of different types of pollutants, many species rise to the surface to breathe (Jacquin, Petitjean, Côte, Laffaille, & Jean, 2020). Srinonate et al. (2015), who conducted a study with tilapia exposed to nanosilver particles at concentrations of 1 ppm, did not notice any changes, but when other groups of tilapias were exposed to 10 and 100 ppm, they showed respiratory discomfort when swimming to the surface of the water. Another study carried out with zebrafish by Moreira and Lucchiari (2021) indicated that fish that were exposed to 10 μg $L^{-1}$ of BP-3 spent more time in the upper part of the aquarium. These authors associated this behavior with increased risk-taking and impaired cognition. Although the behavioral response is expected to vary in response to contaminant concentration (Coronado et al., 2008), sometimes the most significant changes occur at the lowest concentrations due to a rebound effect by activation of decontamination mechanisms at higher dosages that are not activated in lower ones. As we observed at our lowest concentrations, Blüthgen et al. (2012) observed greater behavioral stability at higher concentrations of BP-3 compared to lower doses. This could be a reason for the greater change between the 2.5 and 5 μg $L^{-1}$ concentrations in our study. Behavior is the first resource an animal can use to return its physiology to a state of homeostasis (Schreck, Olla, & Davis, 1997), perhaps with a higher dose of pollutant, behavioral changes no longer allow the resumption of this homeostatic balance, justifying changes only at lower concentrations.

The latency to feed decreased over time, mainly at 37 hours. Control fish also fed faster, maybe due to some chronobiological effect. The diet of some species can be affected by contaminants that pass through the sewage system, such as antidepressants, psychiatric drugs, and antihistamines (Brodin et al., 2014). Barone et al. (2019) report that when clownfish were exposed to 100 mg $L^{-1}$ of BP-3, they did not feed in the first 49h. By exposing tilapia to biological zinc nanoparticles (BIO-ZnONPs), El-Saadony et al. (2021) noted that the feeding behavior of fish had increased, but when using the same amount of chemical zinc nanoparticles (CH-ZnONPs) the opposite of biological zinc occurred, with a reduction in feeding. Another reason for increased feeding behavior and reduced latency in seeking food is the metabolic cost caused by the need to process contaminants (Jacquin et al., 2020). This metabolic cost may explain our results.

We did not find differences between the aggressive behavior between the control and the treatments, probably due to the low concentrations we used compared to other studies. According to Gonçalves-de-Freitas et al. (2019), Nile tilapia has a pronounced aggressive behavior, necessary for sociality and reproduction, but aggressiveness will depend on variables in the external and internal environment. In our study, we did not observe any changes in aggressiveness. In other species, BP-3 resulted in decreased aggression, such as in the Siamese fighting fish *Betta splendens* (Chen et al., 2016). Likewise, the cichlid *Crenicichla lepidota* also reduced its aggressive response to territory invaders when exposed to human presence (Bessa & Gonçalves de Freitas, 2014), although it is more likely this was due to the perception of these humans as potential predators than to the sunscreen they were wearing. The mirror test simulates a territory invader, generating several aggressive responses such as body swaying, operculum, and fin dilation (Verbeek, Iwamoto, & Murakami, 2007). Thus, other variables could be measured to evaluate the effects of BP-3 on aggressiveness.

We especially expected an effect of BP-3 on the aggressive behavior of tilapia because this pollutant has been shown to stimulate the production of vitellogenin (Conrado et al., 2018), a feminizing agent in fish (Hansen et al., 1998). Exposure to Diuron herbicide metabolites resulted in reduced testosterone production in tilapia, resulting in the inhibition of aggressive behavior (Boscolo et al., 2018). The dosages applied in our study, however, did not trigger noticeable reductions in aggressiveness.

In our research, we found detrimental impacts of BP-3 on tilapia habitat use and feeding behavior. Our results were more markedly influenced by exposure time than by concentration. This may be due to a masking effect of the short-term exposures (1 to 25 hours) when the BP-3 effects had not yet manifested in any concentration (Moreira & Luchiari, 2021), zebrafish exposed to BP-3 reduced anxiety and aggressive responses. Chen et al. (2016), in their study using BP-3 and fighting fish, realized that the substance can promote aggressive behavior without affecting swimming. When using benzophenone 3 and 1 in zebrafish, metabolic residues were found after 14 days (Blüthgen et al., 2012). Another possibility is that our study uses comparatively low BP-3 concentrations, making our results similar between treatments and control.

We conclude that the use of sunscreen and other cosmetics containing BP-3 is affecting tilapia habitat use and feeding, although it appears to not affect aggressiveness. This occurs both in terms of dosage and duration of exposure. It is estimated that a person wearing sunscreen releases about 2.13 grams of BP-3 in 2 hours. This amount multiplied by the number of bathers diluted in the water of a highly frequented waterfall or a beach can cause changes to water column use and feeding of fish such as Nile tilapia. The evaluation of other behavioral indicators, especially those related to aggressiveness, should bring interesting results in future studies. We also recommend using experimental species that are common in tourism areas, such as the Piraputangas (*Brycon hilarii*) in snorkeling sites near the Pantanal in Brazil (Balduíno, Marques, & Bessa, 2017) or the Sargent major (*Abudefduf saxatilis*) in highly visited marine reefs (Medeiros, Grempel, Souza, Ilarri, & Sampaio, 2007). The use of BP-3 as a component of cosmetics must be further evaluated and, preferably, replaced by other UV-protection products or strategies, such as clothing. Sunscreen is yet another source of tourism environmental impact, acting as an emerging contaminant with behavioral effects on fish and other aquatic beings.

## Conclusion

Fish spent less time at the bottom of the tank, a normal condition for tilapia, and ate faster, but showed no change in aggressiveness. Effects varied with concentration and duration of exposure. Previous studies have shown similar results with other fish species, suggesting the metabolic cost of BP-3 as a contaminant, but with no significant effect on aggressiveness. Ours is the first study to address the effect of BP-3 on aggressiveness and habitat use of a bottom-dweller and territorial fish also widely used in aquaculture. Protection against ultraviolet radiation is necessary, but the use of BP-3 for this purpose should be considered with caution.

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