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RELATIVE TOXICITY OF DICHLOROVOS TECHNICAL GRADE AND 76% EC (NUVAN) TO FOUR DIFFERENT FRESHWATER FISH: LABEO ROHITA (HAMILTON), CATLA CATLA(HAMILTON), CIRRHINUS MRIGALA (HAMILTON) AND CTENOPHARYNGODON IDELLA (VALIENCIENNES)

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Abstract

Toxicity bioassay experiments determine LC_{50} values by employing static and continuous flow through system which are conducted for four different freshwater fish *Labeorohita*, *Catlacatla*, *Cirrhinusmrigila* and *Ctenopharyngodonidella* for the toxicants Dichlorovos technical grade and 76% EC(Nuvan). The toxic values are characterised by sensitivity and the values are discreet for 24h duration. The determined values of 24h employing static and continuous flow through system for technical grade and 76% EC Nuvan for the four fishes in mg/L⁻¹ are 19.969, 15.205, 20.016 and 17.25 for *Labeorohita*, 11.430, 11.149, 13.477 and 11.430 for *Catlacatla*, 48.288, 12.641, 19.753 and 14.589 for *Cirrhinusmrigala* and 15.317, 12.649, 17.723 and 11.909 for *Ctenopharyngodonidella*. During the experimentation, the fish behaved differently and such observations are noticed which accentuate as behavioural alterations which are due to toxic stress and strained.

Key words: Dichlorovos, Technical grade, 76% EC, Nuvan, Static tests, Continuous flow through system, Behavioral changes.

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Introduction:

Man, to lead a life and sustain food, forage, fiber are necessities, which when damaged, tries to use something that damage his necessities to restore normalcy. He need not be friendly or ecofriendly association with other fellow animals tries to live while combating any damage tries to use chemicals, to control the loss. In such, spoilage, sometimes have spillage either by ignorance or indiscriminateusage result in nearby aquatic environment a phenomenon of defilement (Figen*et al.*, 2013).

Resulting an effect not only ambient characteristics but also the inhibiting organism particularly, the nektonic, cold blooded poikilothermic fish which have a connecting link in the conduct of energy to the other sub diversion of the earth, terrestrial from hydrosphere owing to palatability of human beings.

Such, chemicals broadly classified under four main categories Organochlorines, Organophosphates (OP), Carbamates and Synthetic pyrethroids. Among the op-compounds due to their short persistence and quick in action are in use and they take lions share in its usage; Assis*et al.*, 2007, and Patar*et al* (2015). The usage is more in the world wild and also locally. Due to its presence in waters, effect the fish and such are observed in the ecotoxicology science as reviewed by reviewers, Sana Ullah and Jallil (2015), Shankaramurthy*et al.* (2013), Chandra SekharaRao*et al.* (2017), SuchismitaDas (2013), and also in many individual researchers studies which are well documented.

In such endeavor, the present study in an attempt to characterize the relative toxicity of dichlorvos(OP) 2,2 dichlorovinyldimethoiate phosphate and its formulation marketed as Nuvan 76% EC to the fresh water fish *Labeorohita*, *Catlacatla*, *Cirrhinusmrigila* and *Ctenopharyngodonidella*. While determining 24h lethal concentrations (LC_{50}) which is undertaken by employing static and continuous flow through systems (C.F.T.S) the fish is characterized by its sensitivity and also behaved abnormally. Such changes are also observed which are not normal.

MATERIAL AND METHOD

Test species; The fresh water fish were brought from the local fish farm where *Ctenopharyngodonidella* cultured along with the major carps. The fish are *Labeorohita* (length 5-6cm and weight 1.5-2gm), *Catlacatla*(length 4.5cm and weight 3-4gm), *Cirrhinusmrigala*(length 5.5cm and weight2-3gm) and *Ctenopharyngodonidella* (length 3.5cm and weight 4-5gm). The fish were acclimatized to the laboratory conditions in large plastic tanks with unchlorinated ground water per two weeks at a room temperature of $28\pm2^{\circ}$ c. During the period of acclimatization, the fish were fed with groundnut oil cake and ricebran. Feeding was stopped one day prior to the experimentation. All the precautions laid by committee on toxicity tests to aquatic organisms (APHA *et al.*, 1998,2005,2012)are followed.

Procurement of technical grade and commercial grade:

The technical grade was supplied by the Syngenta India Ltd, Mumbai-400 020.

The pesticide Nuvan76%EC is locally purchased manufactured by Hikal Limited 629/630 GIDC Industrial estate Panoli Bharuch Gujarat marked by Syngenta India., 14-J, Tata Road, Mumbai-400 020.

Preparation of stock solution:-

The stock solutions were made with acetone and concentrations were taken in mgL^{-1} . Controls were maintained for each experiment and they were with the quantity of acetone equal to the highest concentration used in the test and also a precaution is taken to minimize the acetone as solvent.

Test conditions:

The water used for acclimatization and conducting experiments was clearunchlorinated ground water and the hydrographical conditions as physical and chemical properties of water are: Turbidity-8 silica units, Electrical conductivity at 28° C -816Micro ohms/cm, pH at 28° C-8.1, Alkalinity: Phenolphthalein-Nil mg/l, Alkalinity: Methyl orange-172mg/l, Total Hardness (as CaCO₃-232mg/l, Carbonate Hardness (as CaCO₃)-232mg/l, Non-Carbonate Hardness (as CaCO₃)-Nil mg/l, Calcium Hardness (as CaCO₃)-52mg/l, Magnesium Hardness-40mg/l, Nitrite Nitrogen (as N) - Nil mg/l,Sulphate (as SO4₂⁻)-Trace mg/l, Chloride (as Cl⁻)-40mg/l, Fluoride (as F⁻)-1.8mg/l, Iron (as Fe)-Nil mg/l, Dissolved Oxygen – 8–10ppm, Temperature -28±2°C. The fish are tested in both static and continuous flow through systems, as the two methods recommended by APHA (1998, 2005, and 2012). Pilot experiments were conducted to choose the concentrations to be taken for each test and 10 fish were introduced in toxicant glass chambers with a capacity of 10 liters. For continuous flowthrough system, reservoirs of 60 liters capacity were used and the test water was let into test containers at a rate of 4 liters per hour usingpolyethylene drip sets regulators and for every 6 hours fresh test toxicant solutions were prepared in reservoirs.

Experiments were conducted to determine the toxicity of Dichlorovos and Nuvan in various concentration with technical grade and commercial grade formulation in the types of both tests along with taking a precaution of removingdead fish immediately. The toxic tests were conducted to choose the mortality range from 10%t to 90% for 24h in static and continuous flowthrough system in both are used for analysis to determine LC_{50} value for 24 hrs.

Finney's probit analysis (Finney,1971) as recorded by Roberts and Boyce (1972) was followed to calculate the LC_{50} values. The respective probit values were taken from Table IX of Fisher and Yates (1938). For the determination of the 95% confidence limits, LC_{50} values and a normal variety of 1.96 were taken into consideration.

The data was further also analysed by probit analysis of Finney (1952), to obtain regression lines of probit against logarithmic transformations of concentrations. Slope function (S) and confidential limits (Upper and Lower) of the regression line are calculated by windows excel 2010software package as output.

www.ijcrt.org Result and discussion:

The toxicity values along with regression equations for 24h to the four different fishes are given in table 1 and as figures 1–4 as computer drawn sheets. In the present study, a comparison has been made to find out the relative sensitivity of the fresh water fish *Labeorohita*(Hamilton), *Catlacatla*(Hamilton), *Cirrhinusmrigala*(Hamilton) and *Ctenopharyngodonidella*(Valenciennes) when the fish are exposed to technical grade Dichlorovos and 76% EC Nuvan in both steps employed.

Labe or ohita > Cirrhinus mrigala > Ctenopharyng od onidella > Catlacatla

In the present study for 96hrs dichlorovos LC_{50} is for *Labeorohita* 15.009 mg/L, 8.561mg/L, for *Catlacatla*, 9.333mg/L, 9.7.061mg/L, for *Cirrhinusmrigala*. 11.370mg/L, 6.758mg/L, for *Ctenopharyngodonidella* 9.841mg/L, 9.363mg/Lin Static and CFT respectively. The acute toxicity values depend on size, weight, and sensitivity shown by the fish. The sensitivity is in the order of the present experiment as

Labe or ohita > Cirrhinus mrigala > Ctenopharyng od onidella > Catlacatla

Not only in agricultural but also even aquaculture practices during disease management OP compounds are sprayed. Being transported either directly or indirectly if it exceeds the permissible limits, effect the culturable fish wherein all the four of the experimental are candidate species for this venture and will be at loss.

Chandra Sekhara Rao *et al* (2017) in their review article, mentioned that 96 hrs LC_{50} values to different fishes by different researchers and such data is presented in the Table II, the present study is also in the same range but *Ctenopharyngodonidella* is not tested and methodology is different in other fishes as given in the Table II. The toxicity varies not only the fish but also depends on the hydrographical condition of experimentation hence variation. The fish such showed toxic effect due to the inhibition of the enzyme acetylcholine (AChE) esterase rendering the fish to be effected (Patar*et al* 2015, Assis*et al* 2010 & 2007, Koul*et al* 2007, and Varo *et al* 2008.

Table 1. The calculated LC_{50} (in mg/l) values with lower and upper limits of Dichloryos Technical and 76% EC Nuvan to freshwater fishes; *Labeorohita, Catlacatla, Cirrhinusmrigala* and *Ctenopharyngodonidella* in Static and Continuous Flow Through methods for 24h of exposure with regression values, slope, intercept and R^z values.

S No	Name of the fish	Technical	Static	24h	Regression for Technical	Regression for Technical	Regression for 76%EC Static	Regression for 76%EC_CET
5.110.	I value of the fish	76%EC	C.F.T	2711	Static 24h	C.F.T 24h	24h	24h
1	Labeorohita	Technical 76%EC	Static C.F.T Static C.F.T	19.969 15.245 21.016	Y=12.218x- 10.888 Slope: 12.039 Intercept: - 10.655 R ² : 0.960	Y= 10.747x- 7.7129 Slope: 10.704 Intercept: - 7.665 R ² : 0.990	Y= 8.3839x- 6.0884 Slope: 8.378 Intercept: - 6.080 R ² : 0.988	Y= 9.8961x- 7.2398 Slope: 9.847 Intercept: - 7.178 R ² : 0.987
2	Catlacatla	Technical 76%EC	Static C.F.T Static C.F.T	11.430 11.149 13.477 11.430	Y= 6.9435x- 2.3427 Slope: 6.880 Intercept: - 2.280 R ² : 0.972	Y= 4.9002x- 0.1297 Slope: 4.877 Intercept: - 0.107 R ² : 0.964	Y= 8.1403x- 4.1914 Slope: 8.074 Intercept: - 4.121 R ² : 0.974	Y=6.9435x- 2.3427 Slope: 6.880 Intercept: - 2.280 R ² : 0.972
3	Cirrhinusmrigala	Technical 76%EC	Static C.F.T Static C.F.T	18.288 12.641 19.753 14.689	Y=12.926x- 11.312 Slope: 12.883 Intercept: - 11.260 R ² : 0.991	Y= 8.3593x- 4.2145 Slope: 8.258 Intercept: - 4.098 R ² : 0.951	Y=11.624x- 10.056 Slope:11.512 Intercept: - 9.915 R ² : 0.962	Y= 8.6771x- 5.1218 Slope: 8.579 Intercept: - 5.011 R ² : 0.957
4	Ctenopharyngodonidella	Technical 76% EC	Static C.F.T Static C.F.T	15.317 12.649 17.732	Y=9.9455x- 6.7871 Slope: 9.941 Intercept: - 6.782 R ² : 0.997	Y=7.4864x- 3.2457 Slope: 7.393 Intercept: - 3.147 R ² : 0.953	Y= 10.449x- 8.0441 Slope: 10.342 Intercept: - 7.915 R ² : 0.960	Y= 5.763x- 1.2011 Slope: 5.762 Intercept: - 1.199 R ² : 0.988



Figure 1

Graphical representation of *Labeorohita*(Dichlorvos Technical grade and 76% EC Nuvan) in Static tests and Continuous Flow Through System (C.F.T) for 24h along with % Mortality, Log concentration and Probit analysis





Graphical representation of *Catlacatla*(Dichlorvos Technical grade and 76% EC Nuvan) in Static tests and Continuous Flow Through System (C.F.T) for 24h along with % Mortality, Log concentration and Probit analysis.





Graphical representation of *Cirrhinusmrigala* (Dichlorvos Technical grade and 76%EC Nuvan) in Static tests and Continuous Flow Through System (C.F.T) for 24h along with % Mortality, Log concentration and Probit analysis.



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Graphical representation of *Ctenopharyngodonidella* (Dichlorvos Technical grade and 76% EC Nuvan) in Static tests and Continuous Flow Through System (C.F.T) for 24h along with % Mortality, Log concentration and Probit analysis.







Mallum*et al* (2016) reported acute toxicity of dichlorovos to *Oreocromisniloticus* and its haematological effects. They reported the fish having a length of 6.0 ± 21 cm and weight of 9.20 ± 13 g of body weight - and the study estimated the LC₅₀ values for 24, 48, 72 and 96hrs, as 0.50μ L⁻¹, 1.00μ L⁻¹, 1.50μ L⁻¹ and 2.00μ L⁻¹ respectively. They further opined that the toxic action of the toxicants appeared combined effects of precipitation of mucus on the gills, and osmo-regulatory stress with death resulting from suffocation of the gill that have suffered blood haemorrhage. The present investigation too, has same way of fish causing the death.

Sahaet al (2016) reported that dichlorovos toxicity to fresh water fish *Oreochromismossambicus* the 24, 48, 72 and 96 hrs- LC_{50} values are 3.84 mg/l, 3.50 mg/l, 3.12 mg/l **2.90** respectively in static test were in the present study the same result. A comparison is made in the two types of tests, which is varied higher in the static and lower in the CFT. Whereas in both tests the duration of the exposure decreases the toxicity concentration value. A sense of response to adjust the stress conditions to **overcome** it-a sort of ethological response externally and internally physiological alteration to achieve homeostasis. Suneel Kumar (2016) reported the toxic impact on *Channapunctatus* (Bloch) too.

Farid and Mehana (2015) reported on the pesticide toxicity in fish with particular reference to insecticides. According to their review articleorganophosphate like Dichlorvos - first cause inhibition of enzyme AChE and accumulation of the muscle twisting and paralysis. The difference in production and inactivation, and no production (inhibition) as we know are totally different. The former in the toxic action of organochlorines and later is the OP Compound to which the present study of toxicant belongs. The study opined, the toxicity is species specific and varies with the chemicals nature of the toxicant.

The study opined that the pesticide effect to fish is depended upon seven items like, type of pesticides product, use rates, weather conditions, aquatic species involved, extent of the problem (number of fish killed), location and size of pond or lake affected (Farid and Mehana, 2015).

Mishra and Poddar (2014) reported the 96 hrs LC_{50} values to *Channapunctatus* ranged from 0.17 to 11.1 mg/L in flow through system, whereas in static the lowest was 0.340 mg/L. They opined that neurotoxic action is the cause of the toxicity and increased mucus secretion may be considered a protective method to counter the irritating effect of the toxicant.

Gautam*et al* (2014) reported the toxic effects of Nuvan the freshwater fish *Clariasbatrachus* - where in 96h LC_{50} value to the fish was determined as 0.07 mg/L. They opined that due to hepatocellular destruction, renal damage and caused by toxicant lead to the liver dysfunction. The result indicated marked neurotoxic effect of Nuvan which may be true even in the present study.

Lakshmananet al (2013) reported effects of Dichlorovos on freshwater fingerlings *Oreocromismossambicus*(Peters) whereas they reported lower, middle and higher sublethal concentrations as 0.0037, 0.0075 and 0.015 ppm of dichlorovos to the fish.

Omoniyai*et al* (2013) reported the 96 h LC_{50} for fingerlings and Juveniles were 275.2 and 492.0 mg/L⁻¹ respectively of *Clariesgariepinus* (Burchell, 1822). The study revealed size sensitivity is the factor of toxic action. The present study of the *Ctenopharyngodonidella* of the present experiment can be assumed as fingerlings. They concluded that mucus accumulation in the gills is responsible for toxic effect.

Satyavardhan (2013) evaluated comparative toxicity values for the fish species *Cyprinouscarpio*, *Puntiussophore*, *Ctenopharyngodonidella*, *Channapunctatus*, and *Anabas testudineus*exposed to fenvalerate. The observed lethal concentration values are species specific, *Ctenopharyngodonidella* is more resistance than *Cyprinuscarpio* and *Puntiussophore* and is not as resistant as *Channapunctatus* and *Anabas testudineus*. In his study the static LC_{50} values are higher than continuous flow through systems. The commercial formulation is more toxic than technical grade.

Suchismita Das (2013) reviewed dichlorvos toxicity in fish. According to their study that the acute toxicity has been previously determined by a number of researchers, its toxicity for fresh water and estuarine fish is moderate to high, and it does not bioaccumulate in fish. For fresh water and estuarine fish, 96h – LC_{50} values range from 0.2 to 12 mg/L (WHO, 1989). For marine fish the toxicity was estimated to be more than 4 mg/L for adults and pre-adults of Atlantic salmon *Salmosalar*. The 96h LC_{50} value of Dichlorvos obtained for fingerlings of European sea bass (*Dicentrarchuslabrax*) was 3.5 mg/L (Varo *et al.*, 2003). A comparison of the 96h - LC_{50} values published for several teleost fish species (WHO, 1989) indicates that fingerlings of the European sea bass are more resistant to dichlorvos exposure than the most part of the other species of estuarine and fresh water fish studied. However, the comparison with fathead minnow (*Pimephalespromelas*) or *with mosquito fish* (*Gambusiaaffinis*) of similar size indicates that sea bass fingerlings are more sensitive to dichlorvos. The 96h LC_{50} values have been reported (WHO, 1989) for both species as 12.0 and 5.3 mg/L of Dichlorvos obtained for *Labeorohita* was 16.71ppm. The fish in the same study exhibited erratic swimming, copious mucus secretion, loss of equilibrium and hitting to the walls of test tank prior to mortality in acute toxicity tests (Bhat*et al.*, 2012).

The 96h LC_{50} values of dichlorvos has been reported in *Cirrhinusmrigala* as 9.1ppm (Velmurugan*et al.*, 2009), in Zebra fish, the 24h post fertilization LC_{50} value of dichlorvos in the semi static test was 39.75 mg/L (Sisman, 2010).

During experimentation the control fish behaved in a natural manner whereas as the toxicant exposed fish showed erratic swimming movements and they appeared to be in distress. During the first four days opercular movements were quicker and the fish surfaced more frequently gasping for air but subsequently this breathing difficulty seemed to have subsidized. Hyper excitation loss of equilibrium, increase cough rate, flaring of gills, increase in production of mucus from the gills, darting movements and hitting against the walls of test tanks were noticed in all the species tested. A film of mucus was also observed all over the body and also on the gills. Such observed changes are in agreement, Mallum*et al* 2016, Saha*et al* 2016, Mishra and Podar*et al* 2014, Satyavardhan 2013, Yogithadevi and Abha Mishra, 2013, Omoniyi, 2013, Elif and Yerli 2012 and Anoop Kumar Srivatsava*et al* 2010.

Thus, the summary of the results of toxicity is as follows:

- (1) The toxicity is characterized by species specific.
- (2) The formulations are more toxic than technical grade Dichlorovos.
- (3) The static values are higher to continuous flow through system.
- (4) Due to inhibitory act of AChE the fish showed the effect of mortality.
- (5) And, of all the fish *Catlacatla* is more sensitive a profitable species in aquaculture and more resistant one is *Ctenopharyngodonidella*.

Conclusion:

Thus it can be concluded that Dichlorvos technical grade as well as 76% EC Nuvan characterized differential toxicity showing certain behavioural alterations. The commercial formulations have to be viewed seriously before giving its representation in the environmental use for abatement of pollution in a monitoring concept.

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 Table II. Toxicity values of dichlorvos in different fish (Chandra SekharaRao, 2017)

 According to Chandra sekharaRao et al (2017) review article, the toxicity data for dichlorvos to different fishes which can be summarized as follows:

S.No.	Test Species	96h/ LC ₅₀ ,Value/ Range	References	
1	Labeorohita	16.71ppm	Bhat and Bhat (2016)	
2	Oreochromismossambicus	3.84mg/L, 3.51mg/L, 3.12mg/L, 2.9mg/L for 24, 48.72 and 96 hrs	Sahaet al., (2016)	
3	Anabas testudineus	2.35mg/L	Patar (2015)	
4	Heteropneustesfossilis	19ppm	Deka&Mahanta (2015)	
5	Labeorohita	0.11mg/L	Giridharet al (2015a) (2015b)	
6	Channapunctatus	1 mg/l -48h	Mishra &Poddar (2014)	
7	Cyprinuscarpio	0.95mg/L	Taket al.,(2014)	
8	Channapunctatus	0.024mg/L	Kumar S (2014)	
9	Channapunctatus	0.024mg/L	Kumar &Gautam (2014)	
10	Cirrhinusmrigala	20mg/L	Srivastava et al. (2014)	
11	Clariasbatrachus	0.07ml/L	Gautamet al., (2014)	
12	Heteropneustesfossilis	6.4mg/L	Ahmad &Gautam (2014)	
13	Clariasgariepinus	275.2µg/L (Fingerlings) 492µg/L (Juveniles)	Omoniyiet al., (2013)	
14	Cyprinuscarpio	2.51mg/L	Gunde and Yerli (2012)	
15	Poecilia reticulate	1.84 mg/L	$\mathbf{P}_{\mathbf{r}}$	
13	Labeoronita	10./1ppm 2	Al Lemeri (2011)	
16	Gambusiaaffinis (Mosquito fish)	2μg/L-48n	Al-Jowan (2011)	
17	Daniorerio	39.75mg/L 24h (post fertilization)	Sisman (2010)	
18	Daniorerio	51.3mg/L -24h 13mg/L -96h	Zhang <i>et al</i> (2010)	
19	Ctenopharyngodonidella	6.5mg/L 7.5mg/L	Tilak&SwarnaKumari (2009)	
20	Cirrhinusmrigala	9.1ppm	Velmuruganet al.,(2009)	
21	Etroplussuratensis	0.09mg/L	Sobhanaet al., (2006)	
22	Cyprinuscarpio	46.24 mg/L	Ural and Koprochu (2006)	
23	Cyprinuscarpio	9410ppm	Ural &Calta, (2005)	
24	Heterobranchuslongifilis	1.32 mg/L	Ekpo and Okorie (2004)	
25	Heterobranchuslongifolis	132 mg/L	Ekpo and Okrie (2004)	
26	Dicentrarchuslabrax	3.5mg/L	Varo <i>et al.</i> , (2003)	
27	Clariasgariepinus	0.184ml/L	Ashade <i>et al.</i> , (2011)	
28	Abramisbrama	16.66 mg/L	Chinko and Slymo (1995)	
29	Abramisbrama	16.66 mg/L	Chinko and Slymo (1995)	
30	Cyprinodonvariagatus	7.5 ppm	Jones and Davis (1994)	
31	Cyprinodonvariagatus	7.5 ppm	Jones and Davis (1994)	
32	Liziaparsia	0.482 mg/L	Mohapatra and Noble (1991)	
33	Clupiaherengus(Larvae)	0.12 mg/L	McHenry (1991)	
34	Liza pursia	0.482 mg/L	Mohapatra and Noble (1991)	
35	Clupiaharengus(Larvae)	0.12 mg/L	Mc Henry (1991)	
36	Clariasbatracus	8.8 ml/L (48 h)	Benerjee and Rajendranath (1990)	
37	Labeorohita, Catlacatla, CirrhinusmrigalaandCtenopharyngodonidella	mg/L ⁻¹ /24 hrs 19.969*, 21.016 ¹ 15.245**, 17.251 ² 11.430*, 13.477 ¹ 11.149**, 11.430 ² 16.288*, 19.753 ¹ 12.641*, 14.689 ² 15.317*, 17.732 ¹	Present work: (R.SwarnaKumari) 2018 *Static system TG **CFTS-TG ¹ Static 76% EC ² CFTS 76% EC	
		$12.649^{**}, 11.909^2$		

It seems there is no report on the fish Catlacatla, Ctenopharyngodonidella (Sl.No.38 - it is also the author's work).

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