

Heavy Metals  
avoidance

CHEMOSENSORY BIOASSAY OF TOXICITY OF LAKE WATERS  
CONTAMINATED WITH HEAVY METALS FROM MINING EFFLUENTS

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ABSTRACT

The toxicity of natural lake waters contaminated with heavy metals from mining effluents was studied by perfusing water samples through the olfactory organs of Arctic char (*Salvelinus alpinus*) while recording the olfactory bulbar electrical responses to the standard stimulant, L-serine.

Of the five lake waters tested, only Ross Lake, the closest to the mining-smelting site, showed significant depressing effect on the olfactory response at levels as high as 100 times dilution. Chemical analysis of lake waters revealed that Ross Lake had the highest concentrations of heavy metals; 43 µg/l Cd, 98 µg/l Cu, <15 µg/l Ni and 7720 µg/l Zn. Single component experiments showed that Cu and Cd are probably responsible for most of the depressive effect.

The depressive effect of natural lake water was less than that of an artificial lake water composed of heavy metals based on the natural lake concentrations. Possible involvement of complex formation of heavy metals in the reduction of their toxicity is discussed.

INTRODUCTION

An electrophysiological method has been developed to demonstrate acute effects at various sublethal concentrations of heavy metals on the olfactory responses of rainbow trout (Hara et al. 1976). The method monitors changes in the olfactory bulbar electrical responses to a standard chemical stimulus while the nares are perfused with water containing toxicants. In contrast with some acute sublethal bioassay techniques reported (Sprague and Drury 1969; Kleerekoper et al. 1972; Drummond et al. 1973; MacLeod and Pessah 1973), this method is specific in that functional derangement of the olfactory system of fish caused by brief exposure to sublethal levels of pollutants can be directly measured.

Olfaction plays an important role in the survival of fish. It mediates such diverse phenomena as feeding, recognition of prey and predator, sexual and social behaviour, and migration (Hara 1975). The initial sensory process of olfactory reception probably takes place at

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olfactory receptor membranes. Since they are not protected by external barriers, man-made alterations in the water quality could easily interfere with their functioning, the consequences being a breakdown in communication among fish and between fish and environment.

In the present study the toxicity of natural lakewaters contaminated with heavy metals from mining-smelting activities was investigated electrophysiologically in Arctic char. Attempts were also made to compare the toxicity of natural lake waters with that of an artificial lake water made upon the basis of natural lake concentrations of heavy metals. Previous work done on the olfactory system of Arctic char (Døving et al. 1973; 1974; Höglund and Åstrand 1973; Höglund et al. 1975) make this salmonid species suitable for our studies and for possible future toxicology experiments.

#### MATERIALS AND METHODS

Arctic char (*Salvelinus alpinus*), ranging from 18 to 24 cm in body length, were used. They were hatched and reared at the Freshwater Institute from eggs collected at Kent Peninsula, Northwest Territories in April, 1975. Fish were held in large laboratory tanks with a continuous flow of thiosulfate dechlorinated water for an acclimatization period of more than 3 weeks. The fish were lightly anaesthetized with TMS (ethyl m-aminobenzoate methanesulfate, Kent Laboratories Ltd.) (1:8000 dilution), and then immobilized with an intramuscular injection of Flaxedil (gallimine triethiodide, Pulenc Ltd.) (4 mg/kg body weight). The fish were secured in a plastic trough, with the gills being perfused with dechlorinated water (1 l/min; temperature  $11 \pm 0.5^\circ\text{C}$ ) throughout the experiments. Total hardness of water was 90 mg/l as  $\text{CaCO}_3$  and the pH ranged from 7.5 to 7.8. Operative procedures on the fish, recording techniques of the electrical responses from the olfactory bulb and data acquisition procedures have been described previously (Hara 1973; Hara et al. 1976).

An amino acid, L-serine, an effective olfactory stimulant for many fish species (Hara 1975), was used as the standard stimulus at  $10^{-5}\text{M}$ . The stimulant solution was delivered to both nares at the rate of 0.1 ml/naris/sec for 10 sec, at intervals of 2 min, using an automatic stimulatory apparatus (Hara et al. 1973). Lake waters and heavy metal solutions were introduced to fish using essentially the same method described previously (Hara et al. 1976), except that only the nares were perfused and for short periods of time (10 to 30 min).

Water samples were obtained from 5 lakes in two different watersheds, at varying distances from the mining and smelting sites at Flin Flon, Manitoba during July, 1976. These samples were collected in Nalgene bottles, stored at  $4 \pm 0.5^\circ\text{C}$ . The pH of the samples ranged from

6.5 to 7.3. Water samples were analysed for heavy metals (Cd, Cu, Zn and Ni) within 48 hrs using flame atomic absorption spectrometry (Table 1). Stock solutions of artificial lake water and heavy metals were made up in distilled water and diluted as needed in the same water as perfused the gills and nares.

Table 1. Chemical analysis of lake waters for heavy metals

Sample	Heavy metals analysed ( $\mu\text{g/l}$ )			
	Cu	Cd	Ni	Zn
Ross Lake	81	42.0	<15	7720
Schist Lake	16	1.1	<15	709
Douglas Lake	13	0.4	<15	51
Phantom Lake	18	0.5	<15	89
Meridian Lake	8	0.2	<15	14

## RESULTS

Figure 1 illustrates typical responses recorded from the olfactory bulb of the same fish when the nares were stimulated with  $10^{-5}\text{M}$  L-serine before, during and after exposure of the nares to Ross Lake water. The patterns of response and depression of response caused by lake water were qualitatively similar regardless of types of stimulants, such as other kinds

of amino acids, food extract and hand rinse. No appreciable change in the background electrical activity was observed during exposure to lake waters under the present experimental conditions.

The bulbar response to the standard stimulus was depressed within the first 2 min after Ross Lake water replaced the dechlorinated water. The maximum depression was normally reached in 4 min, and maintained throughout the period of treatment. The response usually returned to the original level after rinsing for 6 min with dechlorinated water (Fig. 2). Longer exposure times did not further depress the olfactory response. Of 5 lake waters tested, only Ross Lake, located closest to the mining-smelting site, significantly depressed the olfactory response. All other lake waters that were tested produced little or no depression.

Chemical analysis showed that Ross Lake is heavily contaminated with high levels of Cd, Cu and Zn. This contamination is probably the prime factor for the depressive effect on the olfactory response. Other components of natural waters may account for a portion of the observed depression. To determine the involvement of these three heavy metal elements in depressive action of Ross Lake water, an artificial water containing heavy metals at the same concentrations as those found in Ross Lake was tested. The artificial lake water was found to be more depressive than natural Ross Lake water (Fig. 2). However, the recovery of the response was slower after treatment with Ross Lake water than with artificial water. At dilutions of 10 times, depressive effect of artificial lake water was only slight, while natural lake water retained a strong depressive action. At further dilution, natural Ross Lake water was still significantly depressive (Fig. 3).

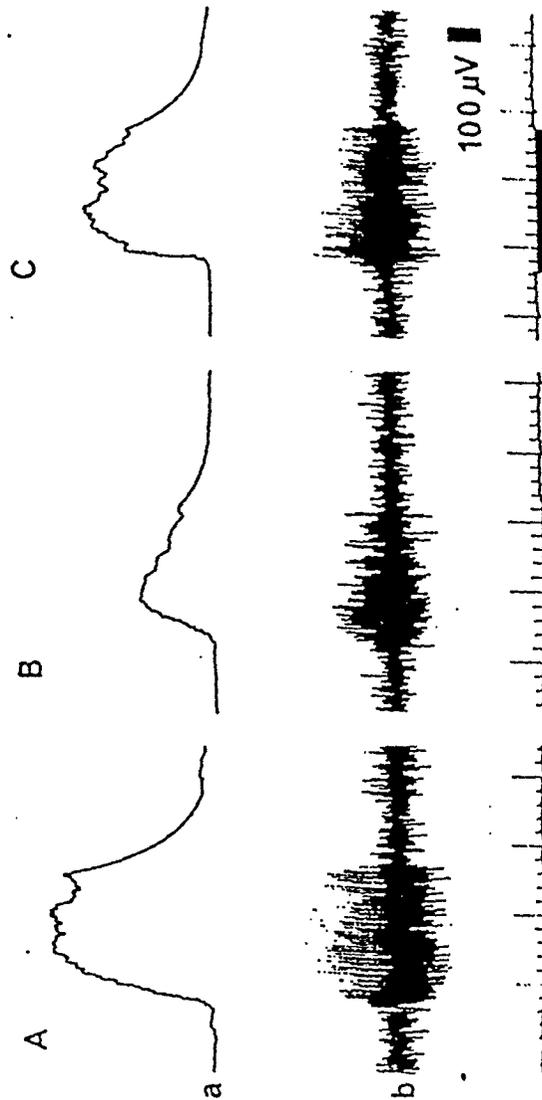


Fig. 1. Electrical responses recorded from the olfactory bulb of the same fish when the nares were stimulated with  $10^{-5}M$  L-serine before (A), during exposure to diluted Ross Lake water (B), and after 10 min rinsing (C). The upper tracing (a) of each pair is the integrated responses of the lower (b). Duration of stimulation is indicated by heavy lines below each record. Time scale, each division = 1 sec.

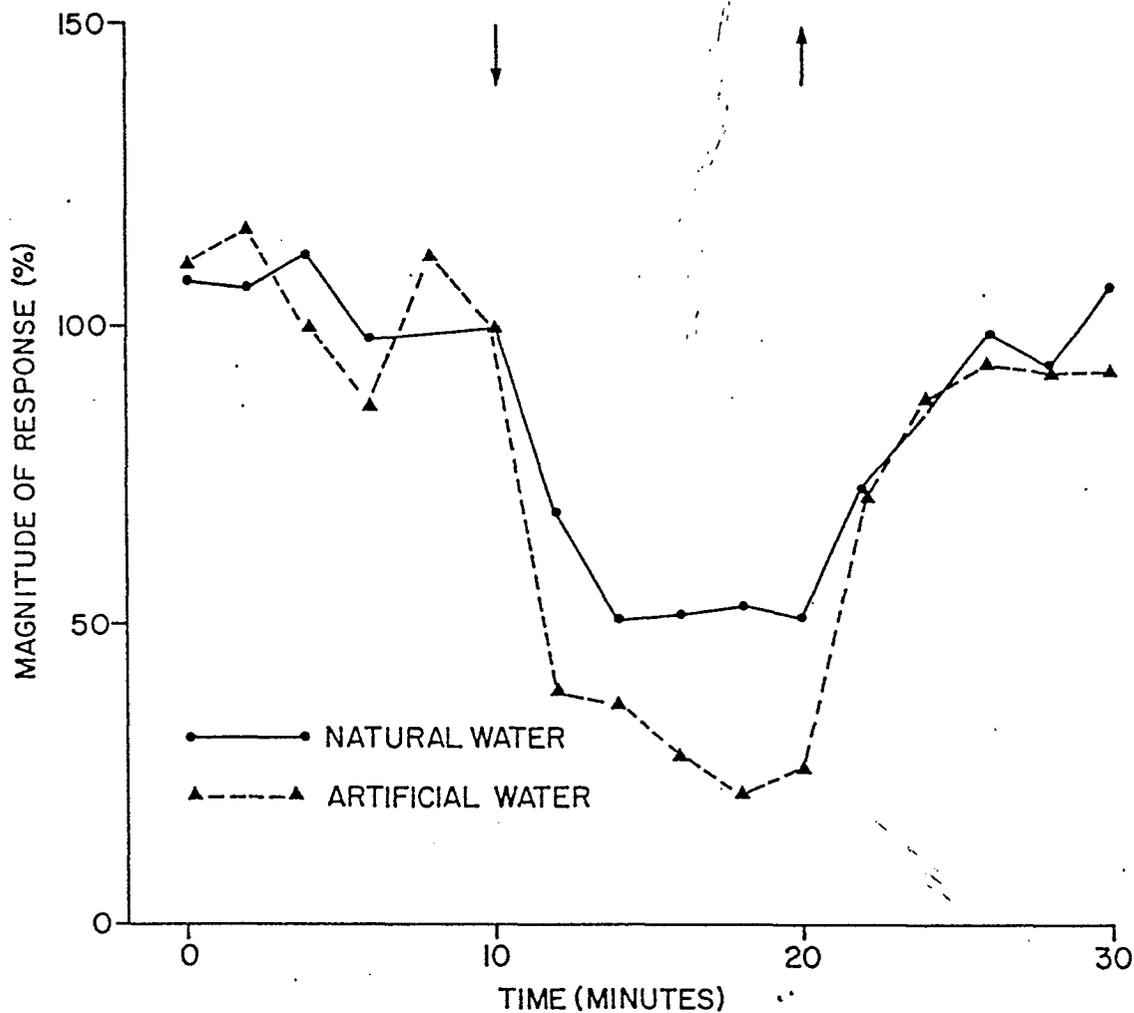


Fig. 2. Effects of natural lake water contaminated with mining effluents and artificial water containing heavy metals at the same concentrations as found in the natural lake water. † indicates onset of treatment and ‡ commencement of rinsing. The magnitude of the responses in this and the following figures is represented as a percentage of that of the control response before treatment

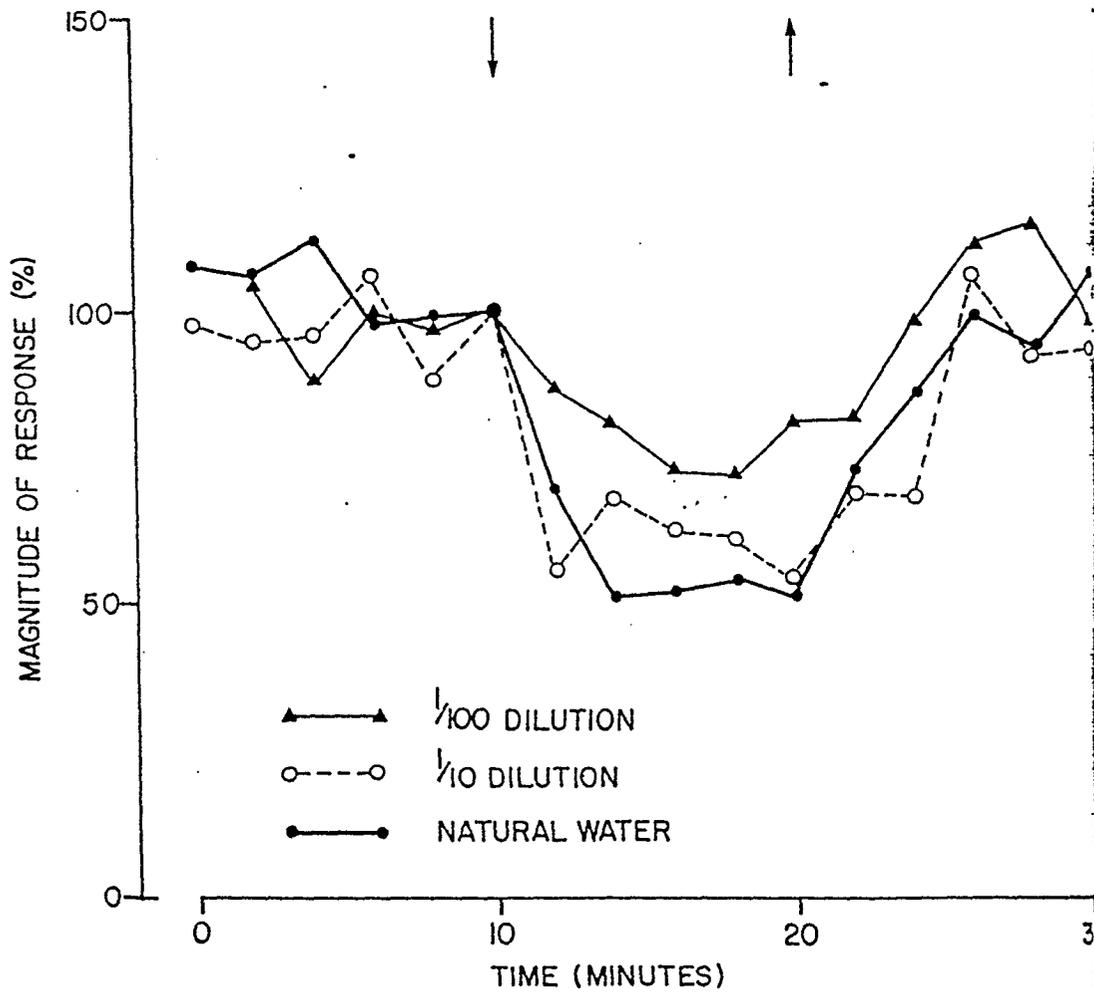


Fig. 3. Olfactory bulbar responses to standard stimulant L-serine when the nares were exposed to 1/100, 1/10 and full strength Ross Lake waters for 10 min. Arrows delimit period of treatment.

Each heavy metal component was further tested separately at various levels in the same manner as were the lake waters. At the same concentration,  $\text{CuSO}_4$  solution was the most depressive of the three heavy metals, followed by  $\text{CdCl}_2$ ,  $\text{NiCl}_2$  and  $\text{ZnSO}_4$  solutions (Fig. 4). An increase in the Cu concentration resulted in increases in both the depressing effects and length of time for recovery after rinsing. An increase in the length of treatment time (up to 30 min) did not increase the effective depression, but did increase the time for recovery. The magnitude of depression for both Cd and Zn increased with length of treatment. Generally, following Cd-treatment, the response did not return to the original levels even after long rinsing.

#### DISCUSSION

The results indicate that the depressive effect of Ross Lake water, collected near mining-smelting activities, is probably due to the heavy metal content. Experiments with individual heavy metals suggest that copper and cadmium play the major role in the depressing action.

Zinc is not likely to be greatly involved in depressing the olfactory responses, as evidenced by the lower effectiveness of Schist Lake water contaminated with a relatively high Zn concentration. Although individual Ni solutions of 0.1 mg/l were effective depressants, as strong as Cu and Cd, it does not seem to take a significant part in the depressing action of lake water as a whole, since similar low concentrations of Ni were found in all lakes analysed.

One of the most interesting features of the present study is the result that natural lake water was less depressive than the artificial lake water at full strength. Natural water retained its effectiveness at dilutions as high as 100 times. The artificial water became ineffective at the same concentration. Most of the heavy metals in natural waters are believed to exist in complexed or chelated forms with the miscellaneous organic ligands which regulate the availability of these metals in the system (Prakash and Rashid 1968; Kunkel and Manahan 1973), especially in waters containing a high organic content (640 mg/l of total dissolved solids) as do Ross Lake and Schist Lake. Such a mechanism maintains a reservoir of metals ready for biological use. Carbonate complexes seem involved in  $\text{Cu}^{++}$  and  $\text{Cd}^{++}$  binding in water samples from certain English rivers (Stiff 1971; Gardiner 1974). Amino acids and polypeptides also play a significant role as carbonate in the complex formation with copper (Stiff 1971). In the Ottawa River, organic compounds such as fulvic acid are mainly responsible for binding with heavy metals (Ramamoorthy and Kushner 1975). It has been reported that copper is less toxic in river waters than laboratory tests would indicate (Ministry of Technology 1966). Organic ligands reduce  $\text{Cd}^{++}$  toxicity to fish and other animals and bound  $\text{Cd}^{++}$  is biologically

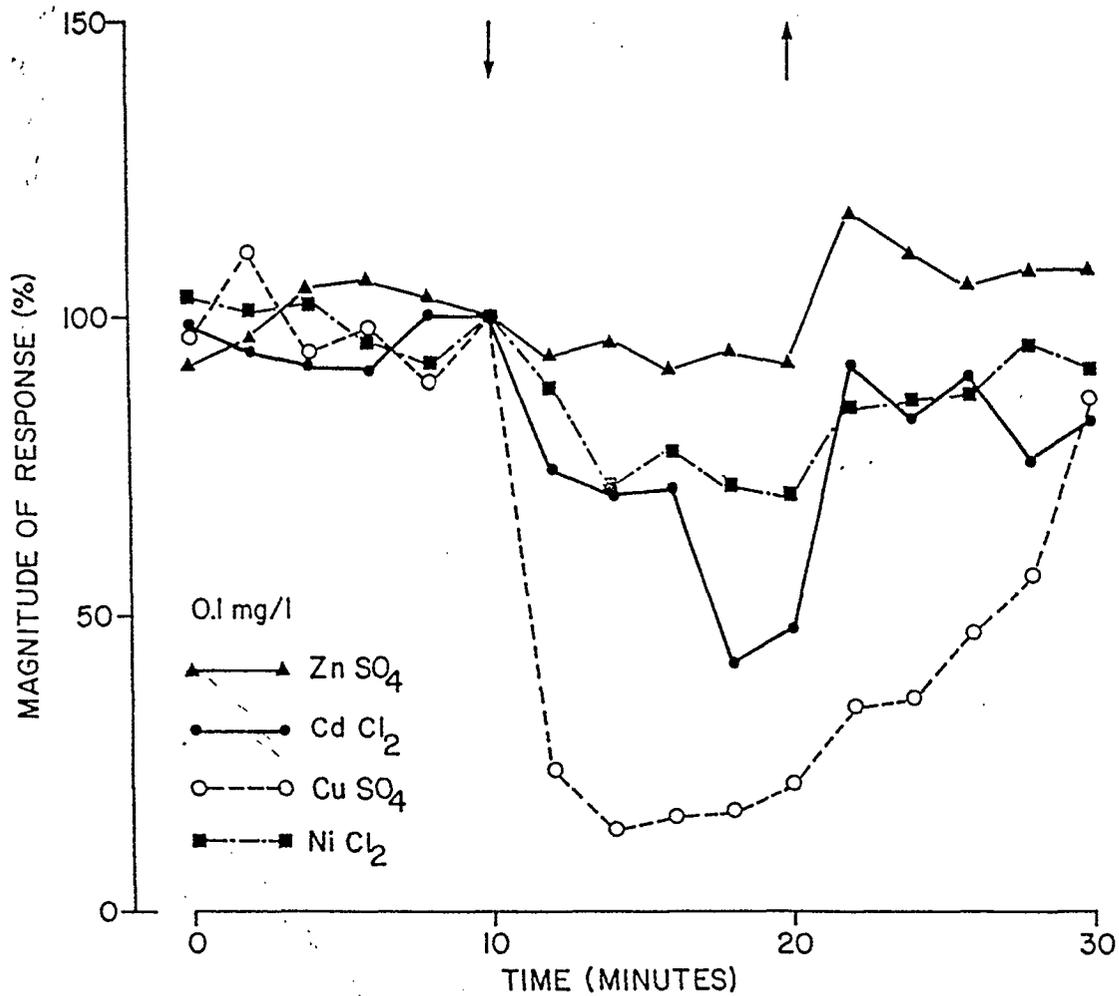


Fig. 4. Effects of individual heavy metals on the olfactory responses to  $10^{-5}M$  L-serine. The nares were exposed to 0.1 mg/l heavy metal for a 10 min period.

unavailable (Friberg et al. 1974). Reduced toxicity of the lake water observed in the present study is probably due to such complex formations. Bound heavy metals are unavailable to the olfactory membrane receptor system. In a partially complexed solution (artificial water) the number of ligands, e.g. thiosulfate, present upon dilution is increased, effectively binding more of the free ions, which results in a reduced effectiveness.

These heavy metals may interact with the olfactory receptors in several ways, causing a reduction in sensitivity for detection of odorous chemicals in the water. Cell impairment due to membrane dysfunction caused by binding of heavy metal ions to the receptors is the most probable mode of interaction. The effects of heavy metals as blocking agents have been demonstrated in other chemosensitive systems (Yur'eva 1957; Hidaka and Yokota 1967; Hidaka 1970; Sutterlin and Sutterlin 1970; 1971; Hara 1972). Heavy metals are known to bind with sulfhydryl and amino groups of proteins. These reactive groups seem to be the probable binding sites of receptor molecules for chemical stimuli. In fact, olfactory bulbar responses of fish to amino acids are inhibited or reduced by sulfhydryl agents such as N-ethylmaleimide, p-chloro-mercuribenzoate, and o-iodosobenzoate (T.J. Hara and S.B. Brown unpublished data).

The electrophysiological bioassay method described here was restricted to the chemosensory system which is highly sensitive to changes in the environment. The investigation of acute effects of sub-lethal levels of heavy metals by this method allows rapid and specific toxicities to be measured. However, it could be even more useful in conjunction with behavioural, histochemical and biochemical approaches by pinpointing the sites of interaction at the cellular and molecular levels.

#### ACKNOWLEDGEMENTS

We thank Dr. W.G. Franzin for critical reading of the manuscript and Mr. A. Lutz for chemical analysis.

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