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The Effects of Residual Chlorine on the Behavioural Responses of *Daphnia magna* in the Early Warning of Drinking Water Accidental Events

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Abstract

Biological Early Warning System (BEWS) based on behavioural responses of aquatic organisms was applied to monitor the drinking water quality for the early warning of accidental events. But residual chlorine in drinking water after disinfection might affect the behaviour modes of tested animals. In this research, the results showed that the residual chlorine in drinking water that was more than 0.32 mg/L would affect the behavioural responses of *Daphnia magna* significantly. It was evident dose-effect relationships between concentrations of residual chlorine and the behavioural responses of *Daphnia magna*. After 1.75 mg $Na_2S_2O_3$ was added in 1 L water bodies, the behaviour modes of *Daphnia magna* would maintain the same as in Standard Reference Water (SRW). Therefore, in the early warning of drinking water accidental events, the effects of residual chlorine on the behavioural responses of *Daphnia magna* could be eliminated by on-line dosing of $Na_2S_2O_3$.

Keywords: Biological Early Warning System; accidental events; residual chlorine; *Daphnia magna*; behavioural responses.

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1. Introduction

The severe contamination of water source, such as Songhua River (nitrobenzene), Heilongjiang province, Beijiang River (cadmium), Guangdong province and so on, affect the living of local people greatly[1-3]. These aquatic accidental events may damage public health greatly due to the characteristic of sudden, easy to spread, unknown contaminants.

Biological Early Warning System (BEWS, made in Chinese Academy of Sciences) has been applied in on-line biomonitoring of water source for drinking water in Beijing, China [4, 5]. In BEWS, when the behaviour modes of tested organisms changed significantly, it suggested that something in water body had done harm to the organisms.

Daphnia magna, which is a smaller crustacean (0.5-5.0cm) with a short life history, is a standard organism for toxicity tests and much literature exists on responses to different types of toxins [6-9]. The movement modes of *Daphnia magna* are very multiform and it is very sensitive to changes of the chemical composition in aquatic environment. Due to their easy and economy culture [10], *Daphnia magna* has often been used as bioindicator in toxicological studies and environmental monitoring of aquatic systems. Therefore, as the bioindicator, *Daphnia magna* is fit for the early warning of drinking water accidental events.

At present, in making drinking water, the disinfector applied most broadly all over the world is chlorine [11]. In order to eliminate the germina and the other aquatic microorganisms, keeping certain concentration of chlorine in drinking water is necessary. The main form of chlorine staying in water is residual chlorine, and only a little part may produce some by-products which may bring harm to public health [12, 13]. As in trace concentration, the acute toxic effects of by-products on the results of the on-line biomonitoring of emergency accidents can be ignored [14]. On the contrary, residual chlorine in drinking water may cause significant behavioural changes, but no report is available. Therefore, it is necessary to illuminate the effects of residual chlorine in drinking water on the behavioural responses of *Daphnia magna* in the early warning of aquatic accidental events.

In present study, behavioural responses of *Daphnia magna* were used to discuss the possible effects. According to the effects on the behavioural responses of *Daphnia magna*, some measures were brought forward to eliminate the effects of residual chlorine in the on-line biomonitoring of drinking water accidental events.

2. Materials and methods

2.1. Daphnia magna cultures

All the experimental *Daphnia magna* was at the same age about 24 h young after culturing more than 3 generations in our laboratory. The culture temperature was maintained at 20±2 ℃, and a 16 h light: 8 h dark photoperiod (illumination ranged between 3000 and 4500 lx) was employed in *Daphnia magna* cultures. Culture medium was made by mixing different chemicals at any moment according to components of the Standard Reference Water (SRW) [15]. *Daphnia magna* was fed on *Senedesmus obliqnus*.

Before the exposure-experiments, the female *Daphnia magna* with eggs were often taken out and being cultured individually in SRW in 50 ml glass beakers until they oviposited. Healthy and uninjured neonates (about 24 h) were always taken from the second and following clutches [16], since in the first brood the size of *Daphnia magna* neonates is usually the smallest and is too sensitive to different growth mediums [17]. During the individually culture of female *Daphnia magna* with eggs, the growth medium was renewed regularly and *Daphnia magna* were fed on *Senedesmus obliqnus* three times a week (Monday,

Wednesday, and Friday). Before feeding *Daphnia magna* with the algae, the culture medium of *Senedesmus obliqnus* was filtrated and then diluted by SRW until the concentration reached 1×10^5 cells/ml. The quantity of the algae was about 1% volume of glass beakers.

2.2. Behavioural responses studies in different water bodies

Daphnia magna are commonly used in the evaluation of the toxicity of chemicals by the ways of 24 h or 48 h acute toxicity tests [18, 19]. As living organism tests offer direct toxicity information on tested chemicals or samples, which analytical methods cannot provide [16], in this study, a 24 h dynamic exposure-experiment to assess the effects of residual chlorine on the behaviour responses of *Daphnia magna* detected by BEWS was carried out.

Water bodies with different concentration residual chlorine were made by adding sodium hypochlorite into SRW. The concentration of residual chlorine was designed as 0.16 mg/L, 0.32 mg/L, 0.64 mg/L and 0.80 mg/L with the control of SRW. The experiment in laboratory was set up in an airtight aquarium in order to decrease the outside effects on the movement behaviour of *Daphnia magna*. In the 24 h exposure-experiment, test chambers (3 cm long, 2 cm in diameter) were all put in an artificial glass stream system. The artificial streams received water bodies with different concentration residual chlorine separately with a flow rate of 2 L/h which was proved to have no influence on the behaviour of swimming *Daphnia magna* [20].

With the help of BEWS, all the movements of organisms in test chambers will affect the high frequency signal of altering current send by one pair of electrodes in test chambers, and the affected signals will be received by the other pair of electrodes as one part of the signal acquisition system of BEWS. Then Behavior Strength (BS) values of the behavioral responses of *Daphnia magna* in different test chambers are got. In these studies, BS, as a scaling factor that changes from 0 (Lose the ability of movement) to 1 (Full behavior express), was introduced to illustrate the behavioral responses of *Daphnia magna*. The behavior data were sampled automatically by the monitoring system every 10 minutes and 6 data records in one hour were used to calculate BS average value. A 30% decrease of the BS was regarded as a significant decrease [21].

No food was added during the experiments. Test organisms were placed in test chambers, which were closed on both sides with nylon nets (250μm), and for each experiment, eight chambers with 10 *Daphnia magna* in each one were used.

The data of the behavioral responses of *Daphnia magna* were analyzed directly in BEWS. The significant BS decrease and the acute toxicity of different water bodies on were calculated with EXCEL.

2.3. Studies in treated water bodies with $Na_2S_2O_3$

In water body Ph>5, hypochlorite and Cl_2 can transform into hypochlorous, and it is the hypochlorous which may engender the main toxicity react of aquatic animals [22]. After treated water bodies with Sodium thiosulfate ($Na_2S_2O_3$), which was purchased from GUOYAO Company (analytical pure, 99%), there will be a reaction as:

$$4ClO^- + S_2O_3^{2-} + H_2O = 2SO_4^{2-} + 4Cl^- + 2H^+ \quad (1)$$

24 h and 48 h static exposure-experiments were applied to evaluate the acute toxicity of $Na_2S_2O_3$ to *Daphnia magna* and 24 h dynamic exposure-experiments were carried out to evaluate the effects of $Na_2S_2O_3$ on the elimination of the acute toxic effects of residual chlorine on the behavioral responses of *Daphnia magna*.

In the 24 h dynamic exposure-experiment, based on the equation 1, 1.75mg $Na_2S_2O_3$ (corresponding with 1mg/L residual chlorine) was added into per liter water bodies with different concentration (0.16

mg/L, 0.32 mg/L, 0.64 mg/L, 0.80 mg/L) of residual chlorine. With the control of SRW, behavioural responses of *Daphnia magna* in different water bodies were monitored by BEWS.

3. Results

3.1. Behavioural responses studies in different water bodies

The amplitude of generated sinus signal caused by the movements of *Daphnia magna* depended on the BS and the size of test organisms relative to the size of test chamber [23]. The behavioural responses were analyzed by a discrete Fast Fourier Transformer (FFT). If the curves standing for different frequencies have high percentage, it shows that BS of *Daphnia magna* is high.

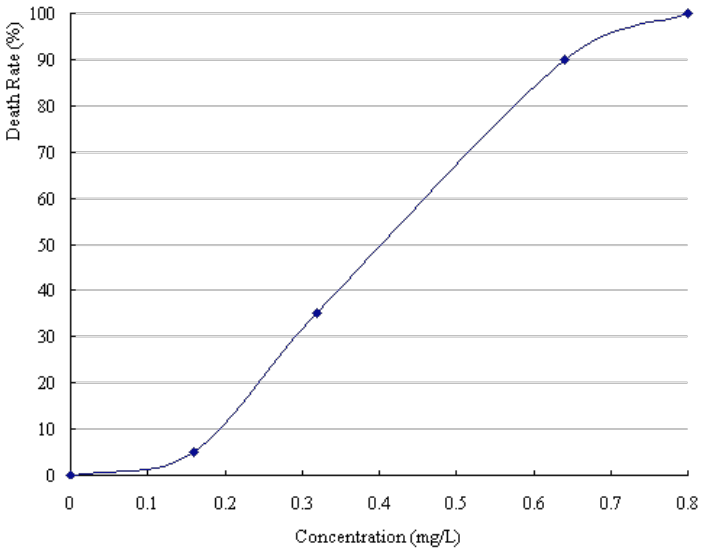

Fig. 1. the dose-effect relationship between the concentration of residual chlorine and death rate of *Daphnia magna*

Acute toxicities of residual chlorine in drinking water on *Daphnia magna* showed that the dose-effect relationship between the concentration of residual chlorine and death rate of *Daphnia magna* is a typical "S-type" (Fig 1.) [24].

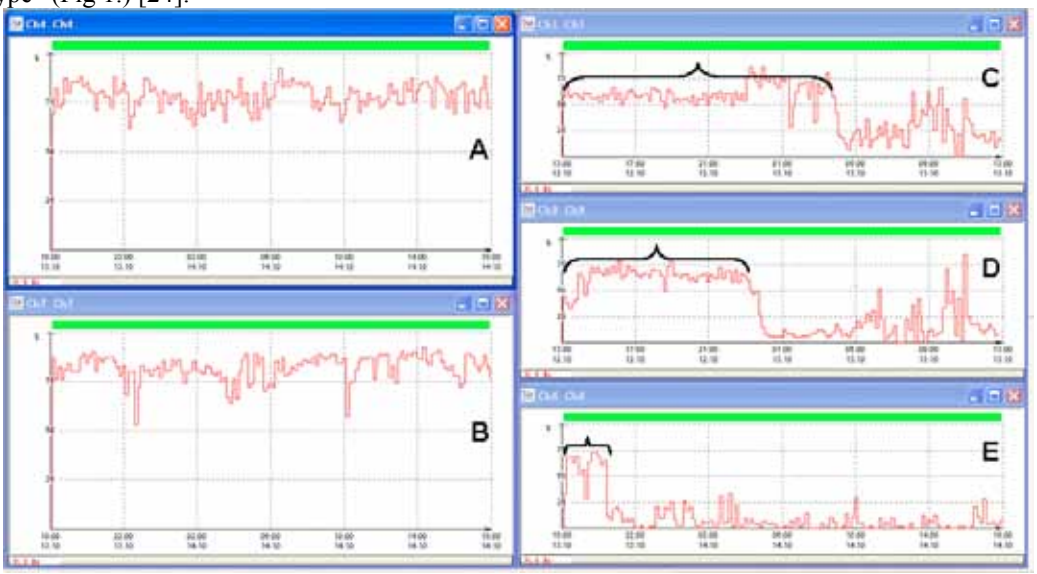

Fig. 2. the results of on-line biomonitoring of the behavioural responses of *Daphnia magna* in different water bodies. The concentrations of residual chlorine were 0.16mg/L (B), 0.32mg/L (C), 0.64mg/L (D), and 0.80mg/L (E) with control SRW (A). The length of the three brackets showed the time that a significant BS decrease of *Daphnia magna* in the three water bodies.

The 24 h on-line biomonitoring results of *Daphnia magna* behavioural responses in different water bodies were shown in Fig 2. At the beginning of the exposure-experiments, the amplitude caused by the movement behaviour of *Daphnia magna* in the four kinds of growth mediums was almost the same. But with time past, the effects of residual chlorine on the behavioural responses of *Daphnia magna* were clear. The behavioral responses of *Daphnia magna* exposed in low concentrations of residual chlorine (0.16mg/L and 0.32mg/L) were smooth. In 0.16mg/L residual chlorine, the behavioral responses of *Daphnia magna* kept similar to those in SRW (Fig 2. (A)). The higher the concentrations of residual chlorine in water body were, the higher degree the movement behaviour of *Daphnia magna* changed and the shorter the time that the intensive behavioural changes needed was.

These results suggested that it was the significant dose-effect relationship between the concentration of residual chlorine and the behavioral responses of *Daphnia magna*.

3.2. Studies in treated water bodies with $Na_2S_2O_3$

After 1.75 mg/L $Na_2S_2O_3$ was added into different water bodies, there would be superfluous $Na_2S_2O_3$ in water based on equation 1. In SRW, the concentration of $Na_2S_2O_3$ could reach 1.75 mg/L. So if $Na_2S_2O_3$ in SRW had no evident effects on *Daphnia magna*, it suggested that the dose of $Na_2S_2O_3$ less than 1.75 mg/L would not affect the movement behaviour of *Daphnia magna*.

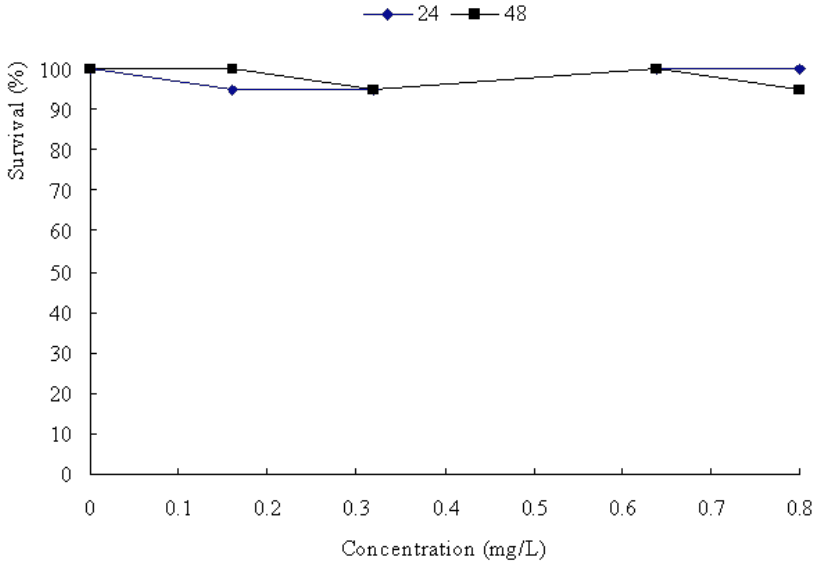

Fig. 3. the acute toxicity of 1.75 mg/L $Na_2S_2O_3$ treated water to *Daphnia magna*

The survival rate of *Daphnia magna* exposed for 24 h and 48 h in $Na_2S_2O_3$ treated water bodies was shown in Fig 3. Comparing with the death rate of *Daphnia magna* in residual chlorine of different concentration (Fig 1.), the survival rate in treated water did not vary with the increase of the concentration of residual chlorine, but maintained between 95% and 100%. The results showed that there was no acute toxicity on *Daphnia magna*.

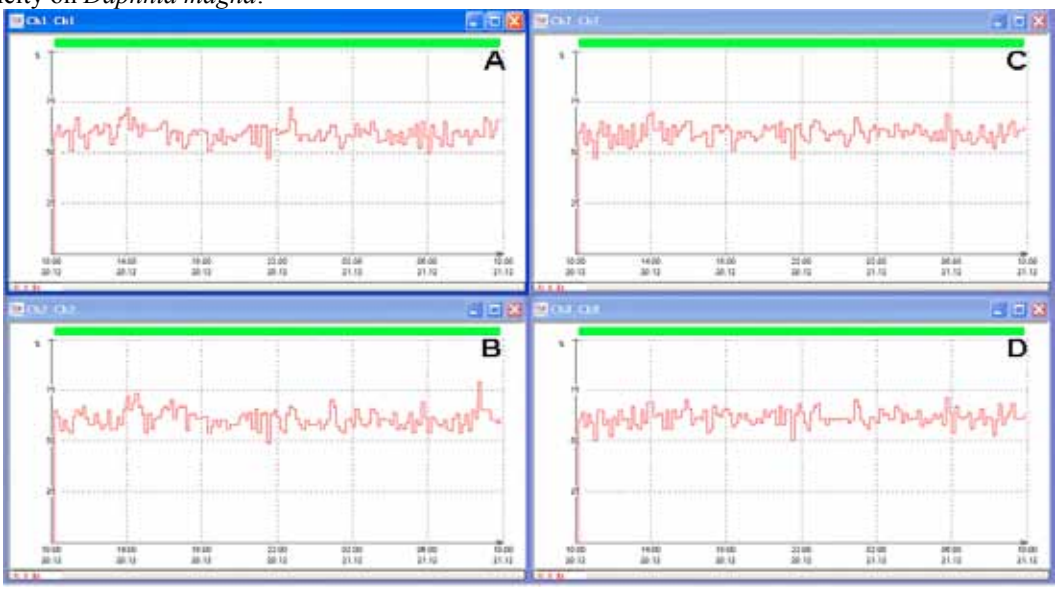

Fig. 4. the results of 24-h on-line biomonitoring of 1.75 mg/L $Na_2S_2O_3$ treated water. The concentrations of residual chlorine before treated were 0.16mg/L (A), 0.32mg/L (B), 0.64mg/L (C), and 0.80mg/L (D)

Based on these results, the behavioral responses of *Daphnia magna* in different water bodies were shown in Fig 4. It suggested that BS of *Daphnia magna* were all about 0.5-0.75, and they were lower than BS in SRW, which changed from 0.6 to 0.9. However, the behavioral responses of *Daphnia magna* shown in Fig 4. kept similar to each other, which advised that the superfluous $Na_2S_2O_3$ ($\leq$1.75mg/L) in water bodies would not affect the movement behaviour of *Daphnia magna*. As the signal of the behavioral responses of *Daphnia magna* was made from the high frequency signal of altering current in test chambers sent by the signal acquisition system in BEWS, the decrease of BS might due to the increase of water conductivity after $Na_2S_2O_3$ was added [25], because the increase of water conductivity would decrease the signal strength as the quadrupole impedance principle of the signal acquisition system in BEWS [26].

These results advised that the measure by adding certain dose $Na_2S_2O_3$ to eliminate the effects of residual chlorine in finished water on the movement behaviour of *Daphnia magna* was possible.

4. Discussions

The studies on the effects of residual chlorine on the behavioural changes of *Daphnia magna* advised that the residual chlorine in finished water affected the movement modes, and it was evident dose-effect relationships between concentrations of residual chlorine and the behavioral responses of *Daphnia magna*.

At present, in making drinking water in P. R. China, the residual chlorine in finished water bodies should be more than 0.3 mg/L, and usually the water supply plants kept the concentration of residual

chlorine in finished water 0.8 mg/L more or less. Therefore, on the one hand, the research results suggested that *Daphnia magna* could be regard as bioindicator in the on-line biomonitoring technology in monitoring the variety of the concentration of residual chlorine in finished water. On the other hand, when *Daphnia magna* was applied in monitoring drinking water accidental events to warn the deterioration of water quality or in setting up the biological early warning systems (BEWS), the effects of residual chlorine must be removed at first.

By the way of eliminating residual chlorine with 1.75mg $Na_2S_2O_3$, the limit of the sudden increase of residual chlorine concentration in finished water detected by *Daphnia magna* using BEWS could reach 1.16mg/L (according to equation 1, 1.00mg/L could be eliminated by1.75mg $Na_2S_2O_3$ and the MLD of residual chlorine to *Daphnia magna* is 0.16mg/L). Once the concentration of residual chlorine reached 1.16mg/L, it showed that something wrong with the disinfection system in the process of making drinking water may happen.

Accidental events in water supply were only a little probability, but once they happened, they would bring great damage to the health and wealth of public. According to the research results, in the early warning of drinking water accidental events, the effects of residual chlorine on the behavioral responses of *Daphnia magna* could be eliminated by on-line dosing of $Na_2S_2O_3$.

Acknowledgments

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