Genotoxicity of drinking water treated with different disinfectants and effects of disinfection conditions detected by umu-test

Xuebiao Nie, Wenjun Liu⁎, Liping Zhang, Qing Liu

Division of Drinking Water Safety, School of Environment, Tsinghua University, Beijing 100084, China. E-mail: 363120244@qq.com

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ABSTRACT

The genotoxicity of drinking water treated with 6 disinfection methods and the effects of disinfection conditions were investigated using the umu-test. The pretreatment procedure of samples for the umu-test was optimized for drinking water analysis. The results of the umu-test were in good correlation with those of the Ames-test. The genotoxicity and production of haloacetic acids (HAAs) were the highest for chlorinated samples. UV + chloramination is the safest disinfection method from the aspects of genotoxicity, HAA production and inactivation effects. For chloramination, the effects of the mass ratio of Cl₂ to N of chloramine on genotoxicity were also studied. The changes of genotoxicity were different from those of HAA production, which implied that HAA production cannot represent the genotoxic potential of water. The genotoxicity per chlorine decay of chlorination and chloramination had similar trends, indicating that the reaction of organic matters and chlorine made a great contribution to the genotoxicity. The results of this study are of engineering significance for optimizing the operation of waterworks.

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Introduction

Disinfection is an important final step in the treatment process of drinking water in China and elsewhere. For chlorination, the most common disinfection process for drinking water, many researchers have focused on the reactions of chlorine with natural organic matters (NOMs) that generate typical disinfection byproducts (DBPs), such as trihalomethanes (THMs) and HAAs. The constituents of NOMs in surface water are complex, including humic substances, soluble microbial products and detritus of plants and animals. The dissolved and colloidal forms, referred to as dissolved organic matters (DOMs), are the most challenging and detrimental fractions of NOMs for water treatment and supply. Many researchers have reported that chemical disinfectants such as chlorine react with DOMs to produce numerous DBPs with genotoxic, mutagenic and/or carcinogenic activity (Glaze et al., 1993; Koivusalo and Vartiainen, 1997; King and Marrett, 1996; Koivusalo et al., 1997; Betts, 1998). At the same time, some of the DOMs are toxic. Therefore, only measuring the formation of typical DBPs is not sufficient to investigate the effects of disinfection on the chemical safety of drinking water, and it is imperative to detect comprehensive toxicity indexes such as genotoxicity.

Genotoxicity of drinking water has been investigated by a large number of researchers in many countries. Shen et al. (2003) detected the mutagenic potential in chlorinated tap water in Shanghai, and found that boiled water had stronger mutagenic activity. Li et al. (2006) investigated samples from four locations along the Yangtze River in Nanjing and found that the organic concentrates were genotoxic. Bolognesi et al.
Recent research found that medium-pressure (MP) UV irradiation had persistent impact in the pipelines, so application of UV in water treatment was recommended (Malley et al., 1996; Kashinkunti et al., 2004). However, UV has no chlorine demand and resulting DBPs do not appreciably change after disinfection. Sequential disinfection of low-pressure ultraviolet (LP-UV) has advantages over MP-UV. For reclaimed water disinfection, Wang et al. (2011) found that the genotoxicity of drinking water samples treated by medium-pressure ultraviolet (MP-UV) and chlorine is lower than that treated by MP-UV and chloramine (Plewa et al., 2012). Chloramination, which is considered to be safer than chlorination in drinking water treatment with less production of DBPs (Richardson et al., 2007; Lu et al., 2009), has attracted more attention. However, Wang et al. (2005, 2007a, 2007b) found that ammonia nitrogen could increase the genotoxicity of wastewater during chlorination, which means chloramine disinfection may have great potential to increase the genotoxicity of wastewater. If the same conclusion can be drawn for drinking water treatment, the advantages of chloramination will be challenged.

In recent years, ultraviolet (UV) disinfection, as an alternative disinfection method, is becoming more widespread. The benefits of UV disinfection include reduced risk of microbial pathogens such as Cryptosporidium and minimal production of regulated DBPs (EPA, 2006). Studies have shown that, at practical UV disinfection doses applied to drinking water (40 mJ/cm²), the chlorine demand and resulting DBPs do not appreciably change (Malley et al., 1996; Kashinkunti et al., 2004). However, UV has no persistent impact in the pipelines, so application of UV in water supply works is usually combined with chlorine or chloramine. Recent research found that medium-pressure (MP) UV irradiation of nitrate-containing drinking waters followed by chlorination enhanced the levels of chloropicrin and halonitromethane, especially when followed by chloramination (Reckhow et al., 2010; Shah et al., 2011). The genotoxicity of drinking water treated by medium-pressure ultraviolet (MP-UV) and chlorine is lower than that treated by MP-UV and chloramine (Plewa et al., 2012). For reclaimed water disinfection, Wang et al. (2011) found that sequential disinfection of low-pressure ultraviolet (LP-UV) (8 mJ/cm²) and chlorine (1.5 mg/L) led to less genotoxicity than chlorination alone. From the aspect of chemical safety, low-pressure (LP) UV has advantages over MP-UV.

Most newly built large waterworks in China have applied the approach of UV combined with the traditional chlorination/chloramination, while many existing waterworks have ameliorated the disinfection process by adding UV treatment. The addition of UV brings significant advantages into the disinfection process. If the chlorination needs to be changed to chemically safer chloramination, UV treatment can ensure the inactivation efficiency for pathogenic microorganisms; while for chlorination, with the addition of UV treatment, it is possible to reduce DBP production by lowering the chlorine dose.

As mentioned above, HAAs and THMs are the two most common DBPs in chlorinated drinking water. In addition, there are many other regulated DBPs that have been found to have higher genotoxic risk, such as haloacetonitriles (Plewa et al., 2012), halonitromethane (Liviac et al., 2009) and N-nitrosamines (Liviac et al., 2011; Wagner et al., 2012). In this study, as the source water contained little ammonia, there were almost no N-DBPs formed during the treatment. Between HAAs and THMs, HAAs were reported by some researchers to be the most abundant DBPs and the major source of carcinogenic risk in chlorinated drinking water (Nieminski et al., 1993; Label et al., 1997; Zhang and Li, 2000). Therefore, this study focused on the investigation of HAAs, and a sample concentration method that focuses on nonvolatile substances was used (HAAs are nonvolatile substance while THMs are volatile).

In this study, the genotoxicity of drinking water samples from a waterworks in North China was assessed with the umu-test. Testing results of water samples treated by chlorination alone, chloramination alone, LP-UV alone, and their combinations were compared and evaluated. HAA production was also analyzed. For chloramination cases, the influence of the mass ratio of Cl₂ to N was investigated. The results of this study are of significance for optimizing the operation of waterworks in China, especially waterworks applying UV combined with chlorination/chloramination as the disinfection process.

1. Materials and methods

1.1. Sample collection and preparation

The water samples investigated in this study were collected from a surface water source in Beijing and the effluents of rapid sand filtration (RSF) and ozone-biological activated carbon (O₃-BAC) processes from a waterworks that used this source water. The water samples were immediately delivered to the laboratory after filtering through glass-fiber membranes (0.45 μm, Millipore, USA) to eliminate suspended solids before other water quality parameters were analyzed. The characteristics of the water samples are shown in Table 1.

Chlorine solution was prepared from sodium hypochlorite solution (about 30% (w/w) as Cl₂) and de-ionized water. Chloramine solution was prepared by mixing sodium hypochlorite and ammonium sulfate solutions (the mass ratio of Cl₂:N was 4:1) with continuous stirring for 10 min in an ice bath. The initial pH of the solution was 8.0. The concentration of available chlorine was measured by a Pocket Chlorine Colorimeter (PCII, Hach, USA) to eliminate suspended solids before other water quality parameters were analyzed. All of the chemical reagents used were of analytical grade. The concentrations of available

<table>
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<th>Table 1 – Characteristics of the water samples.</th>
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<td>Water type</td>
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<tr>
<td>Surface source water</td>
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<td>Effluent of RSF</td>
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<td>Effluent of O₃-BAC</td>
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DOC: dissolved organic carbon; UV: ultraviolet; HAAs: haloacetic acids; RSF: rapid sand filtration.
chlorine in the chlorine and chloramine solutions were about 500 mg/L and 250 mg/L, respectively. All the doses of chlorine or chloramine in this paper were calculated as available chlorine in the form of Cl₂.

1.2. Disinfection treatments

Samples were disinfected in brown glass bottles on the same day as collection. After chlorine or chloramine was added, the bottles were sealed and kept in a dark chamber at room temperature for 24 hr. The volumes of chlorine/chloramine solutions added were calculated according to the target dose, and the pH of samples was not affected by the addition of the disinfectants. The residual chlorine in each reaction bottle was quenched by sodium thiosulfate (2500 mg/L). In a preliminary experiment, the genotoxicity of the effluent of O₃-BAC dosed by 3 mg/L chlorine or chloramine reached a relatively constant value (data not shown). Also, for most end users of the water supply networks in Beijing, the retention time is no more than 24 hr, which is why the time for disinfection treatment was 24 hr. For the chlorination + chloramination treatment, chlorine was added and kept for 2 hr, then the residual chlorine was detected and NH₄SO₄ solution was added to transform the chloramines into chloramines, with a total disinfection time of 24 hr.

For UV irradiation, water was passed through a bench-scale UV reactor. The reactor contained a LPHO (low-pressure high output) mercury arc lamp (arc length = 30 cm). The UV lamp was allowed to warm up for at least 20 min in the presence of tap water before the treatment of samples. The UV dose was controlled by the inflow flux, which was calculated from a bio-assay (EPA, 2006).

1.3. umu-test

The umu-test was first developed and published by Oda (Oda et al., 1985). As an in vitro short-term bacterial test, the umu-test has many practical advantages compared to the classic Ames-test and other bacterial assays, such as being time-saving, easy to perform, high-sensitivity and low-cost. Moreover, the umu-test has been shown to be suitable for measuring genotoxicity in complex mixtures (Hamer et al., 2000) and to detect the genotoxicity of cytotoxic chemicals (Oda et al., 1985). The results of the umu-test for a large number of mutagens were found to be similar to that of the Ames-test (Nakamura et al., 1987; McDaniels et al., 1990). Therefore, the umu-test appears to be the most promising test system for routine screening of genotoxic activity in environmental mixtures (Whong et al., 1986; Giuliani et al., 1996; Helma et al., 1996; Reiferscheid and Heil, 1996; Shen et al., 2003; Escher et al., 2006; Luo et al., 2006; Xiao et al., 2006; Luo et al., 2007).

In this study, the pretreatment process of water samples was established according to drinking water quality in the Beijing area. Samples were acidified to pH 2 ± 0.2 by 10 mol/L HCl solution and passed through HLB cartridges (500 mg, OASIS, USA), which were previously activated with 5 mL methanol, 5 mL acetone, and 5 mL distilled water. The water flow rate was controlled at 10 mL/min. Two liters of source water or 5 L of each effluent sample of RSF and O₃-BAC was loaded on a cartridge. Then the cartridge was dried under a flow of nitrogen and eluted with 5 mL hexane + dichloromethane (V:V = 1:1), 10 mL methanol + dichloromethane (V:V = 1:9) and 5 mL acetone. The eluate was dried under a nitrogen flow in a water bath of 40 ± 1°C until the volume of the eluate was no more than 100 μL. The residue was stored in the dark at –20°C. Before the umu-test, the residue was dissolved in certain amounts of dimethylsulfoxide (DMSO) and distilled water (the final volume of DMSO in each dilution sample was 12%). All the organic solvents used were of chromatographic purity and purchased from J. T. Baker company.

The umu-test for genotoxicity was performed with Salmonella typhimurium TA1535/pSK1002 without S9 activation according to ISO 13829 (2000). 4-nitroquinoline-1-oxide (4-NQO) was taken as a positive control. During each test, a series of 4-NQO reference samples with different concentrations were run concurrently to obtain a dose-effect curve for 4-NQO (Fig. S1). The dose-effect relationship of two samples is shown in Fig. S2. The genotoxicity of the water samples was standardized to an equivalent 4-NQO concentration by dividing the slope of the dose-effect curve of the samples by the slope of the dose-effect curve of 4-NQO.

**Fig. 1** – Comparison of mutagenicity of source water detected by umu- and Ames-tests.
The disinfection treatment of samples in the Ames-test was the same with that in the umu-test. For each sample, 100 L was loaded on XAD2/XAD8 resin (V:V = 1:1), which was eluted with 60 mL ethyl acetate 3 times. The eluate was dried by N2 flow until the volume was no more than 200 μL.

The Ames-test in this study used S. typhimurium TA98 and TA100 strains to detect directly mutagenic compounds, without in vitro microsomal activation (S9 mix) (Ames et al., 1975; Maron and Ames, 1983). Water samples were tested using doses of 0.5, 1, 2, 3 and 4 L equivalent/plate. Water samples without disinfection were tested at lower doses (0.25, 0.5, 0.75, 1, 1.25 L equivalent/plate) to avoid toxicity to bacteria. Positive controls were 2-nitrofluorene for TA98-S9 (10 mg/plate) and sodium azide for TA100-S9 (10 mg/plate). DMSO was tested as negative control.

According to the guidelines published in “Standard Methods for the Examination of Water and Wastewater” (1998), the results of the Ames-test are considered positive if two consecutive dose levels or the highest non-toxic dose level produce a response at least twofold that of the solvent control doses, and at least two of these consecutive doses show a dose–response relationship (APHA, 1995, 1998). The mutation ratio (MR) was used to calculate the ratio of sample revertants to spontaneous revertants. Linear regression analysis of the dose–response curves was used to calculate the MR value per liter of tested water, a specific index of mutagenic activity.

1.5. HAA detection

The HAAs in samples were detected by the method of liquid–liquid extraction, derivatization, and gas chromatography (GC) with electron capture detection (ECD). The pretreatment procedure of samples and parameters of GC were based on the EPA 522.3 method (2003) and adapted (Liu et al., 2004). The HAA9 was detected by this method, but for most samples, no bromated HAAs were detected. The detailed procedures and parameters are referred by Liu et al. (2004). The limit of detection and recovery for HAA detection is listed in Table S1.
2. Results and discussion

2.1. Comparative study of Ames- and umu-tests

The Ames-test is a classic method to detect mutagens in water, so it was imperative to compare the results of Ames- and umu-tests according to the sample characteristics in this study. Comparisons of Ames- and umu-tests have been reported by some researchers, most of which focused on the mutagenicity of pure chemicals (Reifferscheid and Heil, 1996) or just on a qualitative comparison of the two methods (Helma et al., 1996). In this study, quantitative comparison of the two tests was conducted using surface source water in North China as samples.

The mutagenicity of (disinfected) source water detected by the Ames-test is shown in Table S2. Samples without disinfection treatment had no mutagenicity for both TA98 and TA100 strains. Samples treated by 1 mg/L chlorine or chloramine showed positive results for TA98, while the results were negative for TA100. Samples treated by 3, 5 mg/L chlorine or chloramine showed positive results for both TA98 and TA100. The above results showed that the chlorination and chloramination of source water produced frameshift mutagenicity at dosages of 1, 3, and 5 mg/L, and produced base replacement mutagenicity at dosages of 3 and 5 mg/L.

The genotoxicity of the same water samples was detected by the umu-test, and the comparison of the umu-test and Ames-test results is shown in Fig. 1. According to the two tests, the mutagenicity (or genotoxicity) of source water treated by chlorine was higher than that treated by chloramine. With the chlorine dosage increasing from 1 to 5 mg/L, the mutagenicity (or genotoxicity) first increased and then decreased, as shown by both the Ames-test (TA 98) and umu-test. With the chloramine dosage increasing from 1 to 5 mg/L, the mutagenicity (or genotoxicity) decreased slightly and then increased, as shown by Ames- (TA 98) and umu-tests. The above analysis implied that the umu-test (based on HLB SPE) was in good accordance with the classic Ames-test (based on XAD SPE).

2.2. Genotoxicity of drinking water treated with six disinfection methods

The water samples investigated were collected from the effluent of the ozone-biological activated carbon (O3-BAC) process of waterworks in Beijing. Five liter water samples were treated with six different disinfection methods: chlorination, chloramination, UV (a LP mercury lamp), UV + chlorination, UV + chloramination, chlorination + chloramination). Fig. 2 shows the genotoxicity of water samples treated by the six disinfection methods at different doses (the dose of UV was 40 mJ/cm²). At different doses of available chlorine (0.5–5 mg/L), the genotoxicity of samples treated by chlorination or UV + chlorination was much higher than that of samples treated by the other four disinfection methods, which were almost the same. The dose of chloramine did not affect the genotoxicity noticeably within the range of 0.5–5 mg/L. For samples treated by chlorination, the genotoxicity decreased slightly when the chlorine dose was higher than 1 mg/L. This may be due to the fact that, at a relatively high chlorine dose, some genotoxic substances (or intermediate products) will be chlorinated further and change to less-genotoxic substances. Such a phenomenon has been found by Yang et al. (2000) for the chlorination of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone), which is regarded as a strongly toxic substance (Hemming et al., 1986; Xie, 1989; McDonald and Komulainen, 2005). When treated by UV + chlorination, however, the...
genotoxicity had a positive correlation with chlorine dose (0.5–5 mg/L).

Fig. 3 shows the effects of UV dose on the genotoxicity of water samples treated by UV, UV + chlorine (1 mg/L) and UV + chloramine (1 mg/L). With increasing UV dose (0–100 mJ/cm²), the genotoxicity increased slightly. For UV and UV + chloramination, the genotoxicity at UV dose of 40 mJ/cm² was almost the same as that with no UV treatment. 40 mJ/cm² is the most widely used UV dose in waterworks. Thus it is safe to apply LP-UV from the aspect of genotoxicity.

Figs. 4 and 5 show the effects of chlorine dose on HAA production. Similar to the genotoxicity results, samples treated by chlorination and UV + chlorination had the most HAA production, and the HAA production had a positive relation with the chlorine dose. The HAA production of samples treated by chlorination + chloramination was higher than that of chloramination and UV + chloramination. UV had no effect on HAA production, even at the dose of 300 mJ/cm² (Fig. 5).

The above results indicate that, compared to disinfection methods applying chlorination, chloramination and UV + chloramination are much safer from the aspect of genotoxicity and HAA production. Considering that UV inactivates most microorganisms, including chlorine-resistant Giardia lamblia and Cryptosporidium, UV + chloramination is an ideal disinfection method. Moreover, the ammonia nitrogen content in many surface water sources in China is relatively high and MP-UV will increase N-DBP risk (Reckhow et al., 2010; Shah et al., 2011), so there is a promising prospect for the application of LP-UV + chloramination in China.

2.3. Effects of the mass ratio of Cl₂ to N for chloramination

For chloramination cases, the effects of the mass ratio of Cl₂ to N (4:1, 7:1 and 9:1) on the genotoxicity and HAA production of effluents of O₃-BAC are shown in Fig. 6. In the dosage range of 0–10 mg/L, both genotoxicity and HAA production increased with increasing mass ratio of Cl₂ to N. At the mass ratio of 4:1, most chloramine molecules are monochloramine, while most chloramine molecules are dichloramine or trichloramine at the ratio of 7:1 or 9:1. The disinfection efficacy of monochloramine is higher than that of dichloramine and trichloramine. So for waterworks, the water source of which contains ammonia, it is better to remove the ammonia to a moderate degree according to the chlorine dosage.

2.4. Effects of water characteristics and disinfectant dosage

Three kinds of water samples were collected to investigate the effects of water characteristics on genotoxicity: the source water, the effluent of RSF and the effluent of O₃-BAC. The water volume for each sample of source water and RSF and O₃-BAC effluents was 2, 5 and 5 L, respectively. The genotoxicity of samples after being treated by different doses of chlorine or chloramine is shown in Fig. 7. The genotoxicity of all samples treated by chlorination was much higher than that of those treated by chloramination at the same dose. For all 3 kinds of water samples, the dose of chloramine had no obvious effect on the genotoxicity. After chlorination, the source water samples showed much higher genotoxicity than the other two kinds of samples; the effluent of O₃-BAC showed the lowest genotoxicity, near that of source water without disinfection treatment. The reason is that the higher concentration of NOMs in source water (DOC and UV254 listed in Table 1) leads to more production of genotoxic substances.

As shown in Fig. 7, the genotoxicity of source water without disinfection ranged from 59 to 107 ng 4-NQO/L, and it increased obviously after chlorination. The curves “Chlorine 1” and “Chloramine 1” show the genotoxicity of samples disinfected immediately after collection. The curves “Chlorine 2” and “Chloramine 2” show the genotoxicity of samples disinfected one week later (stored at 4°C). The genotoxicity of
samples stored for one week before treatment was lower than for those treated immediately at the same dose. As no disinfectant was added before storage, it is reasonable that microbial activity changed the content and constitution of DOMs. All the other samples in this study were disinfected on the same day as collection. As the TOC of source water did not change obviously during storage, it seems that the precursors of genotoxicity are microbially available.

For the source water and the effluent of RSF, samples treated by 5 mg/L chlorine showed the highest genotoxicity. For the effluent of O3-BAC, the “peak” dose was lower (about 3 mg/L). One reasonable explanation is that part of the genotoxic substances can be chlorinated further into non-genotoxic or less-genotoxic substances. The DOMs in the effluent of O3-BAC were at a much lower level than in source water and the effluent of RSF (Table 1). Thus an effective way to reduce genotoxicity risk is to remove the NOMs as much as possible.

The effects of water characteristics and disinfectant dosage on HAA production are shown in Fig. S3. For all 3 kinds of water samples, HAA production increased with chlorine dosage, which was different from the changes in genotoxicity. HAAs are a class of ultimate products hard to chlorinate further, so assessment of genotoxicity by HAA production alone is of doubtful accuracy, especially for drinking water.

2.5. Relationship between chlorine decay and genotoxicity
Chlorine decay represents the ability of organic substances in water to react with chlorine. Genotoxicity per chlorine decay is the ratio of genotoxicity (equivalent of 4-NQO) and chlorine decay (mg/L) relative to the initial dosage, meaning genotoxicity produced by every unit (mg/L) of chlorine decay. As shown in Fig. 8, for the source water and the effluent of O3-BAC, the curves of genotoxicity per chlorine decay of chlorination and chloramination were almost the same, which implied that the reaction of organic matter and chlorine made a major contribution to genotoxicity, so chlorine decay is a good fast tool for waterworks to evaluate the genotoxicity risks according to an effluent’s chlorine demand characteristic.

2.6. Analysis of chlorinated products with different polarity
As mentioned in Section 2.3, the cartridge was eluted by 3 kinds of solvents, eluting substances with weak-polarity, medium-polarity and strong polarity, respectively. The 3 kinds of eluates were not mixed and the genotoxicity was detected separately. Results are shown in Fig. 9. After chlorination or chloramination, the genotoxicity of source water derived mainly from medium-polarity substances, and the genotoxicity from weak-polarity substances was higher.
than that from strong polarity substances. The summed genotoxicity of the 3 eluates was less than the genotoxicity of the mixed eluates, which implied that there are synergistic effects among substances with different polarity.

3. Conclusions

The genotoxicity risk and HAA production of (UV+) chlorination were the highest among all the methods investigated. The UV + chloramination method is the safest disinfection process from the aspects of genotoxicity, total HAAs and inactivation efficiency.

With increasing chlorine dose, the genotoxicity of source water and effluents in RSF and O3-BAC increased and reached a peak value at the dose of 3–5 mg/L, whereas HAA production was positively correlated with chlorine dose in the range of 0–10 mg/L. The curves of genotoxicity per chlorine decay of chlorination and chloramination coincided and implied that the reaction of organic matters (especially medium-polarity substances) and chlorine made a major contribution to the production of genotoxicity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.07.016.

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