Limitations of chlorine dioxide as an alternative disinfectant in comparison with chlorine from the viewpoint of mutagenicity

S. Itoh, H. Murakami, M. Fukuhara and A. Nakano

ABSTRACT

The change in the mutagenicity of water treated with chlorine dioxide was compared with that of chlorinated water to estimate the mutagenicity of drinking water in distribution systems. We carried out chromosomal aberration tests using Chinese hamster lung (CHL) cells to evaluate the mutagenicity. First, the levels of chloroform and TOX produced by chlorine dioxidation were approximately 1% and 5–7%, respectively, of those produced by chlorination. However, in water treated with chlorine dioxide, the activity that induced chromosomal aberrations was stronger than would be expected based on the quantity of by-products. The observed decreasing rate constant of the activity inducing chromosomal aberrations in chlorinated water was 1.4 to 1.9 times greater than that of water treated with chlorine dioxide, indicating that the mutagenicity of water treated with chlorine dioxide is more stable than that of chlorinated water.

The mutagenicity of drinking water treated with chlorine dioxide was estimated to be 70–80% of that of chlorinated drinking water. However, these differences in mutagenicity are reduced when drinking water remains in distribution systems for long periods. The use of chlorine dioxide instead of chlorine can drastically reduce the production of trihalomethanes (THMs). However, the results of this study demonstrate that chlorine dioxide does not have much advantage in terms of the mutagenicity of drinking water. There were no disinfection by-products that demonstrated similar tendencies of change compared to the changes in the activity that induced chromosomal aberrations.

Key words | alternative disinfectant, chlorination, chlorine dioxide, chromosomal aberration test, disinfection by-products

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INTRODUCTION

Since the discovery that chlorination of water can result in the formation of suspected carcinogens such as trihalomethanes (THMs) as well as other hazardous by-products, increased efforts have been made to investigate alternative disinfectants that will not produce such materials (Fielding & Farrimond 1999; Singer 1999; Barrett *et al.* 2000). A primary goal is to find an alternative disinfectant that will produce significantly lower levels of halogenated by-products. Two major alternative disinfectants, chlorine dioxide and chloramines, are generally thought to be suitable for practical disinfection processes. This study discusses the characteristics of water treated with chlorine dioxide in comparison with chlorinated water.

Characteristics of disinfection by-products (DBPs) formation by chlorination and factors affecting the DBPs yield in chlorinated water have been investigated by many researchers (Rockhow *et al.* 1990; Zhuo *et al.* 2001; Liang & Singer 2003). In these studies, numerous models for DBPs formation in chlorination have been proposed to predict concentrations mainly of THMs and haloacetic acids (HAAs) in distribution

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systems (Sung *et al.* 2000; Rossman *et al.* 2001; Gallard & Gunten 2002; Nikolaou *et al.* 2004; Rodriguez *et al.* 2004; Sohn *et al.* 2004). On the other hand, DBPs formed by chlorine dioxide including inorganic by-products such as chlorite and chlorate ions have also been examined (Chang *et al.* 2000*a*,*b*; Dabrowska *et al.* 2003; Veschetti *et al.* 2005). In addition, a few studies show the change or persistence of DBPs formed by chlorine dioxide in distribution systems (Korn *et al.* 2002; Hoehn *et al.* 2003). It is widely believed that increasing the levels of typical DBPs mentioned above imply the increase in the toxicity of drinking water, although this is not clearly described in most studies.

However, we have to pay attention to numerous other DBPs in addition to typical ones formed during disinfection. From this point of view, *in vitro* short-term genotoxicity tests are useful, because they can evaluate the combined action of DBPs present in drinking water as complex mixtures. Actually, many investigations have already been carried out on the mutagenicity in chlorinated drinking water (Rapson *et al.* 1980; Meier *et al.* 1983; Wilcox & Williamson 1986; Donald *et al.* 1989; Kopfler *et al.* 1990; Tanaka *et al.* 1991). As a result, some characteristics on the mutagenicity of chlorinated water have been clarified. One of the representative characteristics is that the mutagenicity easily changes over time after disinfection depending upon pH and temperature of water (Rapson *et al.* 1980; Meier *et al.* 1983; Kinae *et al.* 1992; Itoh *et al.* 2001). These findings suggest that the direction of change in the mutagenicity is inconsistent with that of typical DBPs such as THMs and HAAs in drinking water, which indicates genotoxicity tests are of value for the toxicity detection of water.

There have been some studies on the mutaganicity formation by chlorine dioxide and the comparison between waters treated with chlorine dioxide and chlorine (Donald *et al.* 1989; Anderson *et al.* 1990; Itoh *et al.* 2001; Guzzella *et al.* 2004; Onarca *et al.* 2004), however, no studies have been conducted on the change in the mutagenicity formed by chlorine dioxide over time after the water treatment. We have to consider that there are some differences in the mutagenicity level and the change rate of the mutagenicity over time after disinfection between chlorination and chlorine dioxidation.

This study compares the toxicity of water treated with chlorine and chlorine dioxide. In addition, we examined changes in the mutagenicity of disinfected water in order to estimate the total toxicity of drinking water in distribution systems. Based on the obtained results, we evaluate the advantages of chlorine dioxide. Finally, we also discuss the limitations of chlorine dioxide treatment, which would differ from the generally accepted evaluation.

MATERIALS AND METHODS

Chlorination of humic acid

Commercial humic acid (Wako Pure Chemical Industries, Ltd.) dissolved in water was used as a model substrate in this study. The total organic carbon (TOC) of the humic acid solution was 1,030 mg/L. A sodium hypochlorite stock solution (Wako Pure Chemical Industries, Ltd.) was used for chlorination. Available chlorine in the stock solution was analyzed by the iodometric method (Clesceri *et al.* 1998) just prior to use. In order to measure the mutagenicity of chlorinated water and its change without concentrating a disinfected water, the TOC (1,030 mg/L) of the humic acid solution and the concentration of added chlorine were high. A problem of these reaction conditions and the applicability of the obtained results will be discussed in Estimation of the change in mutagenicity. Chlorination was performed by addition of the desired amount of sodium hypochlorite solution diluted with chlorine demand-free water. Chlorine dosage typically used in practice would be approximately 1.0 of Cl_2/TOC. Therefore, 1,000 mg/L of chlorine was added as approximately 1.0 of Cl_2/TOC. In addition, 2,000, 3,000, 3,500, and 4,000 mg/L of chlorine were also added as higher Cl_2/TOC cases. The pH was adjusted to 7.0 by a phosphate buffer with a final concentration of 200 mM, followed by HCl or NaOH. The reaction proceeded in the dark at 20°C. Dechlorination was not carried out so as not to change the activity that induces chromosomal aberrations in the chlorinated water (Donald *et al.* 1989). It was confirmed that chlorine had no influence on the chromosomal aberration test up to a concentration of 50 mg-Cl_2/L in the culture media, which means residual chlorine up to a concentration of 500 mg-Cl_2/L in a sample solution had no influence on the test since substances in a solution were

diluted to one tenth in the media as described in Chromosomal aberration test.

Chlorine dioxidation of humic acid

An aqueous solution of chlorine dioxide was produced by mixing sodium chlorite (Wako Pure Chemical Industries, Ltd.) solution with HCl (1 + 3) (White 1999). Chlorine dioxidation of the humic acid was carried out by adding either a 2% or 4% chlorine dioxide solution prepared just prior to use. In order to measure the mutagenicity of water treated with chlorine dioxide and its change without concentrating a disinfected water, the TOC (1,030 mg/L) of the humic acid solution and the concentration of added chlorine dioxide were high. 800 mg/L of chlorine was added as approximately 0.8 of ClO_2/TOC. In addition, 1,600, 2,000, and 4,000 mg/L of chlorine were also added as higher ClO_2/TOC cases. The pH was adjusted to 7.0 by a phosphate buffer with a final concentration of 200 mM, followed by HCl or NaOH. The reaction proceeded in the dark at 20°C. Residual chlorine dioxide was not removed so as not to change the activity that induces chromosomal aberrations in treated waters. Chromosomal aberration test for some samples cannot be carried out, because cytotoxicity resulted from chlorite and chlorate ions formed during the treatment is strong. The pH of the treated water was adjusted to 2.5 using HCl, and the solution was allowed to stand in the dark at 20°C for 5 days to allow a decrease in the concentration of chlorite ion under acidic conditions. We were able to carry out chromosomal aberration tests using samples containing less than 110 mg/L of chlorite ion.

Chromosomal aberration test

Chromosomal aberration tests using Chinese hamster lung cells (CHL/IU, Dainihon Pharmaceutical Co., Ltd.) were carried out to evaluate mutagenicity (Sofuni 1999). Cells were cultured in Eagle's MEM (Nissui Pharmaceutical Co., Ltd) supplemented with 10% fetal bovine serum (Gibco Oriental Co., Ltd). CHL cultures were grown in 18 ml media in glass silicon-capped bottles. Two ml of chlorinated water was added to 1-day-old cultures. As a result, substances in the treated water were diluted 1:10 in the media. Bacteria in the treated water were eliminated by 0.22 μm filteration. Only activity that induced chromosomal aberrations without activation was measured in this study. Chromosome preparations were made 24 hours after addition of the treated water (Sofuni 1999).

To evaluate the results of the chromosomal aberration test objectively, the shapes of chromosomes were examined with an image analyzer (Nikon LUZEX 2D), as described previously (Itoh *et al.* 1992). Chromosomal aberrations are divided into two categories: broken and exchanged. Exchanged aberrations were detected by the developed method. 50 metaphases in a specimen were analyzed. As a CHL cell has 25 chromosomes, 1,250 of chromosomes were analyzed by each specimen. Image analysis of negative control gave a mean of 4.5 chromosomes/50 metaphases and a standard deviation of 2.6. The activity that induces chromosomal aberrations is expressed as a mean value of test results of triplicate specimens. When the activity that induces chromosomal aberrations of certain chemicals has to be judged, and when a test result has to be compared with data obtained by other laboratories, the standard method (Sofuni 1999) should be used and the method developed by ourselves could not be used. The developed method is effective in order to compare the relative intensity of the activity that induces chromosomal aberrations only in this study.

Analytical procedures

Chloroform in the water treated with chlorine or chlorine dioxide was extracted with hexane, and the concentration was determined by gas chromatography with an electron capture detector (Shimadzu GC-14B) using a 2 m × 2.6 mm i.d. column packed with silicone GE SE-30 on Chromosorb W AW-DMCS 80/100 mesh. The standard operating conditions were as follows. Injector temperature, 150°C; detector temperature, 200°C. The column oven temperature was initially held at 70°C for 3 min, ramped to 145°C at 15°C /min, and held at 145°C for 2 min. Chlorite and chlorate ions were measured by ion chromatography. The standard analytical and operating conditions were as follows. Detector, TOSOH CM25 μSFS; column, TSKgel IC-PW; eluent, 2 mM benzoic acid (pH5.5); eluent flow, 1.2 ml/min; injection volume, 100 μl; column oven temperature, 35°C. Total organic halogen (TOX) was measured by a TOX-10Σ

analyzer (Mitsubishi Chemical Corporation). TOC was measured using a TOC-5000A analyzer (Shimadzu).

RESULTS AND DISCUSSION

Chlorinated water

Figure 1 shows the changes in the activity that induces chromosomal aberrations in chlorinated water. Figure 2 shows the residual chlorine concentration in the chlorinated water. Chromosomal aberration tests could not be carried out for some samples that contained greater than 3,500 mg/L of added chlorine because residual chlorine concentrations were greater than 500 mg/L even two or three days following chlorination, as shown in Figure 2. An activity that induced chromosomal aberrations was produced by chlorination; however, this activity was unstable and gradually decreased over time after the treatment. It must be noted that the activity decreased even under conditions where residual chlorine could be detected in the solution.

Figures 3 and 4 show the levels of TOX and chloroform, respectively, in the chlorinated water. It is known that the levels of typical by-products, such as THMs and HAAs increase after chlorination in distribution systems. This direction of change is not consistent with the direction of change of the activity that induces chromosomal aberrations

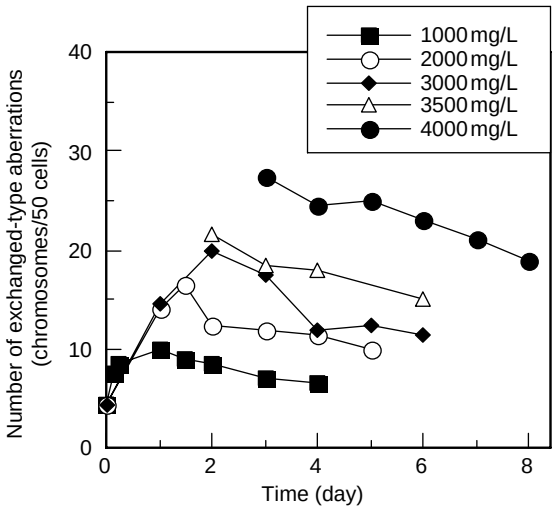

Figure 1 | Changes in the activity that induced chromosomal aberrations in chlorinated humic acid.

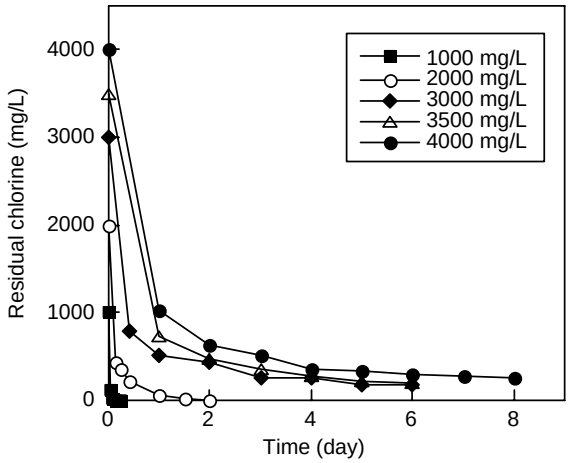

Figure 2 | Residual chlorine in chlorinated water. Initial concentrations of chlorine were 1,000 mg/L, 2,000 mg/L, 3,000 mg/L, 3,500 mg/L, and 4,000 mg/L.

shown in Figure 1. In addition to TOX and chloroform, we measured the concentrations of carbonyl group and low-molecular weight aldehydes (formaldehyde, acetaldehyde, propionaldehyde, and butylaldehyde) (data are not shown). The direction of change of these by-products was also inconsistent with that of the activity that induced chromosomal aberrations. Thus, we were not able to identify a by-product with the same direction of change as the direction of change of the activity that induced chromosomal aberrations. On the other hand, Itoh *et al.* (2006) have discussed the possibility of MX (3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) as an index for comparing the carcinogenicity of tap water near and far from a water purification plant.

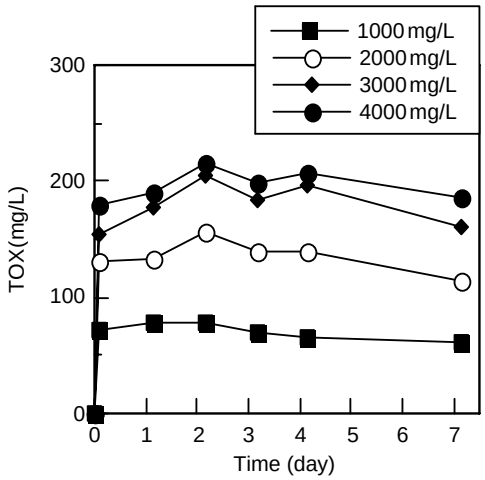

Figure 3 | TOX produced by chlorination.

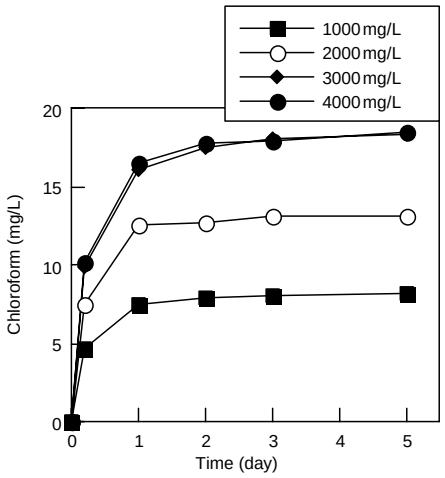

Figure 4 | Chloroform produced by chlorination.

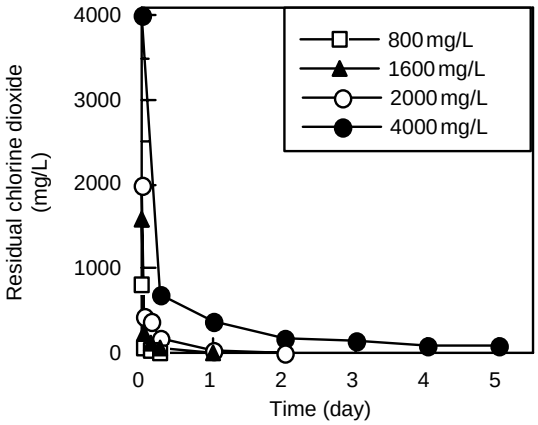

Figure 6 | Residual chlorine dioxide in water treated with chlorine dioxide. Initial concentrations of chlorine dioxide were 800 mg/L, 1,600 mg/L, 2,000 mg/L, and 4,000 mg/L.

Water treated with chlorine dioxide

Figure 5 shows the changes in the activity that induces chromosomal aberrations in water treated with chlorine dioxide. Figure 6 shows residual chlorine dioxide concentrations in the treated water. Comparison of Figures 1 and 5 shows that the activity that induces chromosomal aberrations is approximately 1.3 times greater in chlorinated water than in water treated with chlorine dioxide. An activity that induced chromosomal aberrations was produced by chlorine dioxidation; however, this activity was unstable and gradually decreased over time after treatment. In addition, this activity decreased even under conditions where residual chlorine dioxide could be detected in the solution following a chloride dioxide dose of 4,000 mg/L. These results are qualitatively the same as those obtained in tests of chlorinated water.

Figure 7 shows the concentrations of chlorite and chlorate ions in the water treated with 2,000 mg/L and 4,000 mg/L of chlorine dioxide. The drinking water quality standards (DWQSs) in Japan have been revised in 2003 (Wakayama 2004). The new DWQSs system includes DWQSs (50 items), complementary items to set the targets

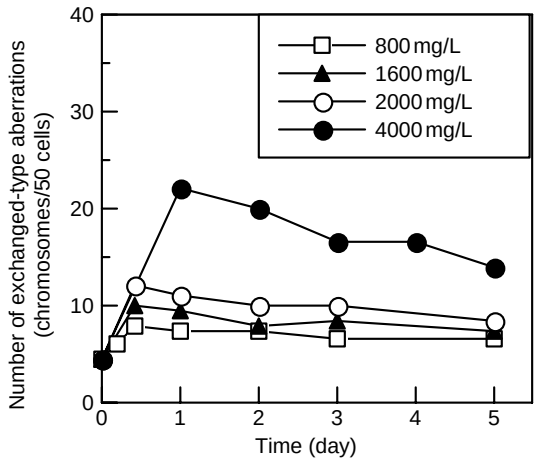

Figure 5 | Changes in the activity that induced chromosomal aberrations in humic acid treated with chlorine dioxide.

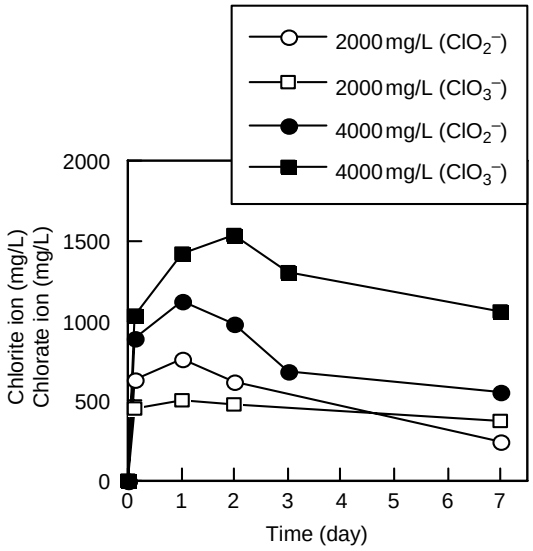

Figure 7 | Chlorite and chlorate produced by chlorine dioxidation. Initial concentrations of chlorine dioxide were 2,000 mg/L and 4,000 mg/L.

for water quality management (27 items), and items for further study (40 items). The target values of chlorine dioxide, chlorite ion, and chlorate ion have been set at 0.6 mg/L in complementary items. Therefore, these inorganic by-products must be monitored after chlorine dioxidation. It must be noted that the concentrations change over time after the treatment, as shown in Figure 7.

Figures 8 and 9 show the levels of TOX and chloroform, respectively, in the treated water. Chloroform and TOX produced by chlorine dioxidation were approximately 1% and 5–7%, respectively, of those produced by chlorination.

A major advantage of chlorine dioxide over chlorine is that it produces significantly lower levels of halogenated organic compounds. However, Figure 5 shows that the level of activity that induces chromosomal aberrations in water treated with chlorine dioxide is greater than would be expected based on the quantity of by-products. Therefore, it is important to note that the use of chlorine dioxide instead of chlorine as an alternative disinfectant does not dramatically reduce the mutagenicity of the treated water.

In addition to TOX and chloroform, the concentrations of carbonyl group and low-molecular weight aldehydes (formaldehyde, acetaldehyde, propionaldehyde, and butylaldehyde) were measured (data are not shown). The directions of changes of by-products measured were not consistent with the direction of the change in the activity that induces chromosomal aberrations.

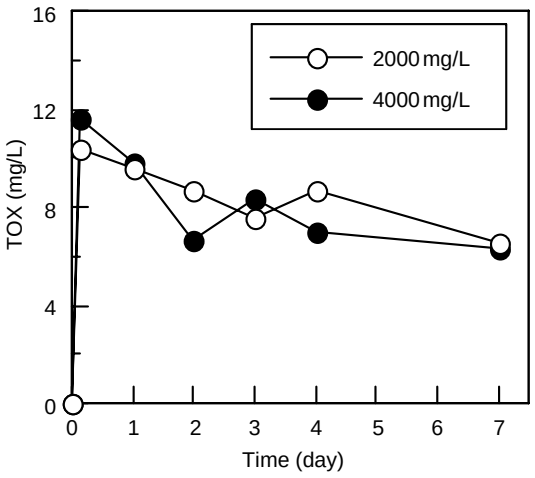

Figure 8 | TOX produced by chlorine dioxidation.

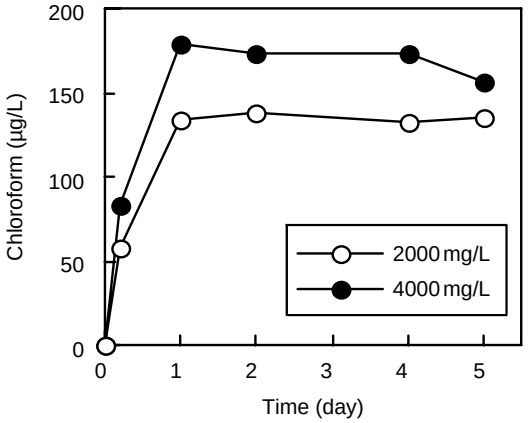

Figure 9 | Chloroform produced by chlorine dioxidation.

Estimation of the change in mutagenicity

Changes in the activity that induced chromosomal aberrations were estimated to compare the safety of drinking water treated with chlorine and chlorine dioxide in distribution systems. Pseudo-first-order rate constants K_{obs} (day^{-1}) were obtained using the integrated first order rate equation:

$$\ln(P_t/P_0) = -K_{obs} \cdot t \quad (1)$$

where P_0 and P_t are the activity that induced chromosomal aberrations in treated water at time 0 and t, respectively. K_{obs} was taken as the slope of the initial decrease.

Figure 10 shows decreasing rate constants for the activity that induced chromosomal aberrations obtained

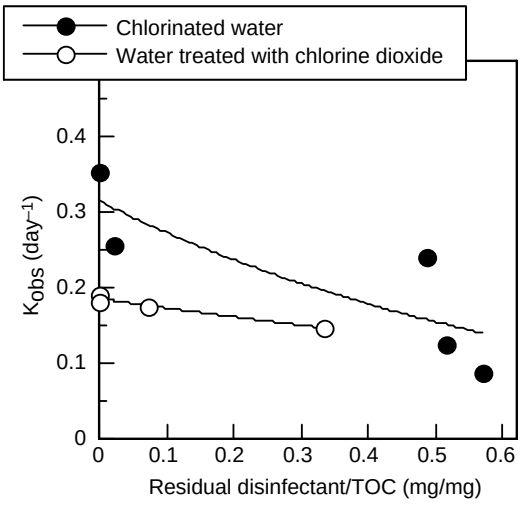

Figure 10 | Effects of residual disinfectant on the decreasing rate constant of the activity that induced chromosomal aberrations.

from the data in Figures 1 and 5. The decreasing rate constant K_{obs} was plotted against residual disinfectant concentration (residual disinfectant/TOC) in the treated water. K_{obs} was then obtained as a function of the concentration of residual disinfectant as follows:

Chlorinated water :

$$K_{obs} = 0.32\exp\{-1.4\times(Cl_2/TOC)\} \quad (2)$$

Water treated with chlorine dioxide :

$$K_{obs} = 0.17\exp\{-0.40\times(ClO_2/TOC)\} \quad (3)$$

The K_{obs} of chlorinated water was estimated to be 1.4 to 1.9 times greater than that of water treated with chlorine dioxide. It is also evident that the decreasing rate constant is smaller, as the residual disinfectant concentration is higher. For example, the K_{obs} of waters treated with chlorine or chlorine dioxide without residual disinfectants were estimated to be 0.32 day^{-1} and 0.18 day^{-1}, respectively, and the half-lives were calculated to be 2.2 days and 4.1 days, respectively. The activity that induced chromosomal aberrations in chlorinated water is greater than that of water treated with chlorine dioxide, as shown in Figures 1 and 5; however, it is noteworthy that this difference decreases over time after the treatment.

Next, we tried to estimate changes in the activity that induced chromosomal aberrations in distribution systems. The difficulty here is that the experiments in this study were carried out using commercial humic acid at a high concentration (910 mg/L of TOC as a final concentration). However, it has been confirmed that there was not a large difference in the time to reach the maximum activity and the decreasing rate between humic acid and natural water (Itoh *et al.* 2006). Thus, we could suppose that there is not a large error when the change in tap water is estimated using the results obtained with humic acid solution in this study.

When the disinfection efficiency is estimated, there are two ways to compare the efficiency by the weight of a disinfectant and by the equivalent weight of a disinfectant. Since the purpose of this study is to estimate the change in the mutagenicity of actual drinking water in distribution systems, a comparison by the weight would be more desirable from the practical point of view. In addition, it seems that the injection dose (disinfectant/TOC) of chlorine dioxide in actual water disinfection to achieve the sufficient disinfection efficiency is almost the same as the injection dose of chlorine (Ozawa *et al.* 1991; Inoue *et al.* 2005), although there may be a case in that the injection dose of chlorine dioxide has to be smaller because of the levels of formed chlorite and chlorate ions. Thus, the mutagenicity formed by chlorine and chlorine dioxide was compared by the weight of a disinfectant and with similar concentrations. It is also possible, however, to compare by the equivalent weight of a disinfectant.

Table 1 shows the assumed conditions of supplied tap water in Japan. In the case of polluted raw water, however, it might be difficult for chlorine dioxide to be used because the target values of chlorine dioxide, chlorite ion, and chlorate ion have been set at 0.6 mg/L in the treated water.

The results estimated for a typical case are shown in Figure 11. 1.0 on the vertical axis indicates the maximum activity that induces chromosomal aberrations observed in chlorinated water, and the relative activity is plotted. The time to reach the maximum activity that induces chromosomal aberrations observed in chlorinated water or water treated with chlorine dioxide was set at 24 hours or 10 hours, respectively, based on the data in Figures 1 and 5. The results clearly show that the activity that induces

Table 1 | Conditions of supplied water. The values in the first row are for typical tap water in Japan, and those in the second row are for cases in which the raw water is somewhat polluted

DOC (mg/L)				
Raw water	**Rapid sand filtered water**	**Disinfectant added (mg/L)**	**Disinfectant/DOC**	**Residual disinfectant (mg/L)**
2.0	1.1	1.1	1	0.1, 0.4
3.5	1.65	3.3	2	0.4, 0.7

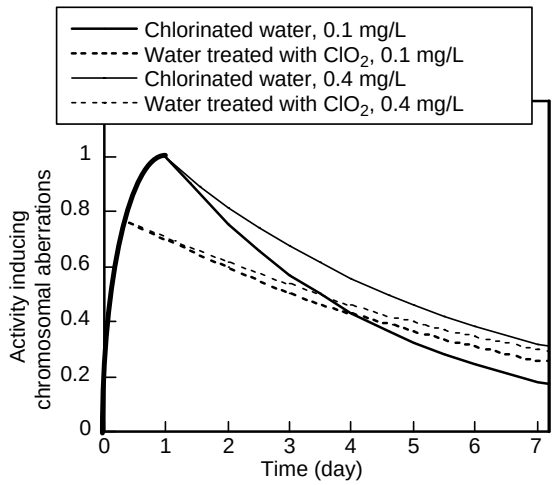

Figure 11 | Estimated changes in the activity that induced chromosomal aberrations in drinking water. DOC of raw water, 2.0 mg/L; Added disinfectant, 1.1 mg/L (disinfectant/DOC = 1).

chromosomal aberrations in water treated with chlorine dioxide is weaker than that in chlorinated water; however, this difference decreases over time after the treatment. In the case of 0.1 mg/L of residual disinfectant, the activity that induced chromosomal aberrations in water treated with chlorine dioxide becomes equal to that in chlorinated water at approximately four days.

The results estimated for polluted water are shown in Figure 12. The time to reach the maximum activity that induces chromosomal aberrations of chlorinated water or water treated with chlorine dioxide was set at 36 hours or 10 hours, respectively, based on the data in Figures 1 and 5. These results show tendencies similar to those observed in typical water, as shown in Figure 11, although the relative activity that induces chromosomal aberrations in water treated with chlorine dioxide was slightly weaker than that shown in Figure 11.

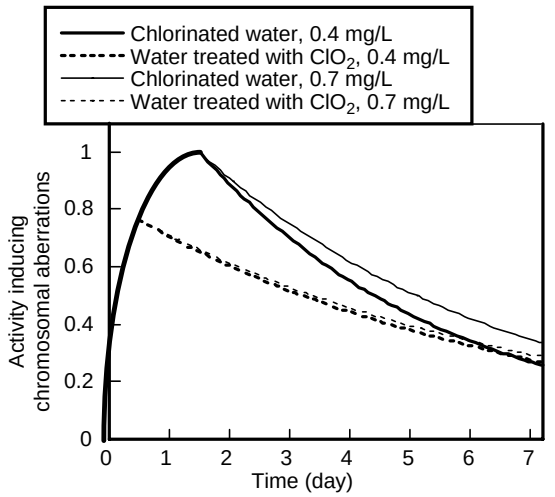

Figure 12 | Estimated changes in the activity that induced chromosomal aberrations in drinking water. DOC of raw water, 3.5 mg/L; Added disinfectant, 3.3 mg/L (disinfectant/DOC = 2).

Assuming that the typical retention time of typical drinking water in the distribution system is within two days, Figure 11 shows that the mutagenicity of drinking water treated with chlorine dioxide would be 70–80% of that of chlorinated water. In the case of polluted water, Figure 12 shows that the mutagenicity of chlorine dioxide treated water would be 65–70%. This decreased mutagenicity is an advantage of chlorine dioxide. However, the difference in mutagenicity is small when drinking water remains in distribution systems for a long period.

The use of chlorine dioxide instead of chlorine can solve the THMs problem. Judging from the findings of this study, however, it should be noted that chlorine dioxide does not have much advantage over chlorine in terms of the mutagenicity of drinking water.

CONCLUSIONS

The change in the mutagenicity of water treated with chlorine dioxide was compared with that of chlorinated water to estimate the mutagenicity of drinking water in distribution systems. Major findings of this study are as follows.

The levels of chloroform and TOX produced by chlorine dioxidation were approximately 1% and 5–7%, respectively, of those produced by chlorination. However, it was revealed that the activity that induces chromosomal aberrations in water treated with chlorine dioxide is stronger than would be expected based on the quantity of by-products produced.

The observed decreasing rate constant of the activity that induced chromosomal aberrations in chlorinated water was 1.4 to 1.9 times greater than that of water treated with chlorine dioxide. This indicates that the mutagenicity of water treated with chlorine dioxide is more stable than that of chlorinated water.

The mutagenicity of drinking water treated with chlorine dioxide was estimated to be 70–80% of that of

chlorinated drinking water. This is an advantage of using chlorine dioxide. However, the difference in mutagenicity would be small when drinking water remains in distribution systems for long periods. The use of chlorine dioxide instead of chlorine can solve the THMs problem. The findings of this study, however, demonstrate that chlorine dioxide does not have much advantage in terms of the mutagenicity of drinking water.

There were no disinfection by-products that demonstrated similar tendencies of change compared to the changes in the activity that induced chromosomal aberrations.

REFERENCES

Anderson, W. B., Huck, P. M., Daignault, S. A. & Irvine, G. A. 1990 Comparison of drinking water disinfectants using mutagenicity testing. In: Jolly, R. L. (ed.) *Water Chlorination: Chemistry, Environmental Impact and Health Effects*, (6). Lewis Publishers, Michigan, USA, pp. 201–225.

Barrett, S. E., Krasner, S. W. & Amy, G. L. (eds) 2000 *Natural Organic Matter and Disinfection By-products, Characterization and Control in Drinking Water*. Am. Chemical Society, Washington, DC, USA.

Chang, C. Y., Hsieh, Y. H., Hsu, S. S., Hu, P. Y. & Wang, K. H. 2000a The formation of disinfection dy-products in water treated with chlorine dioxide. *J. of Hazardous Materials* **B79**, 89–102.

Chang, C. Y., Hsieh, Y. H., Shih, I. C., Hsu, S. S. & Wang, K. H. 2000b The formation and control of disinfection by-products using chlorine dioxide. *Chemosphere* **41**, 1181–1186.

Clesceri, L. S., Greenberg, A. E. & Eaton, A. D. (eds) 1998 *Standard Methods for The Examination of Water and Wastewater*, 20th edition. American Public Health Association, Washington DC, USA.

Dabrowska, A., Swietlik, J. & Nawrocki, J. 2003 Formation of aldehydes ClO_2 disinfection. *Wat. Res.* **37**, 1161–1169.

Donald, K. D., William, B. A., Susan, A. D., David, T. W. & Peter, M. H. 1989 Evaluting treatment processes with the ames mutagenicity assay. *J. Am. Wat. Wks. Assoc.* **89**(9), 87–102.

Fielding, M. & Farrimond, M. (eds) 1999 *Disinfection By-products in Drinking Water. Current Issues*. Royal Society of Chemistry, UK.

Gallard, H. & Gunten, U. 2002 Chlorination of natural organic matter: kinetics of chlorination and of THM formation. *Wat. Res.* **36**(1), 65–74.

Guzzella, L., Monarca, S., Zani, C., Feretti, D., Zerbini, I., Buschini, A., Poli, P., Rossi, C. & Richardson, S. D. 2004 In vitro potential genotoxic effects of surface drinking water treated with chlorine and alternative disinfectants. *Mutation Res.* **564**, 179–193.

Hoehn, R. C., Ellenberger, C. S., Gallagher, D. L., Wiseman, E. V., Benninger, R. W. & Rosenblatt, A. 2003 ClO_2 and by-product persistence in a drinking water system. *J. Am. Wat. Wks. Assoc.* **95**(4), 141–150.

Inoue, H., Ito, T., Tsutsumi, Y. & Nishimoto, S. 2005 Pilot study for improving water treatment system using chlorine dioxide. *J. Japan Wat. Wks. Assoc.* **74**(9), 10–21 (in Japanese).

Itoh, S., Sumitomo, H. & Matsuoka, Y. 1992 Detection of activity-induced chromosomal aberrations using image analysis. *Wat. Sci. Technol.* **25**(11), 227–234.

Itoh, S., Ikeda, D., Toba, Y. & Sumitomo, H. 2001 Changes of activity inducing chromosomal aberrations and transformations of chlorinated humic acid. *Wat. Res.* **35**(11), 2621–2628.

Itoh, S., Nakano, A. & Araki, T. 2006 Reevaluation of the toxicity of chlorinated water and the usefulness of MX as an index. *J. Water and Health* **4**, 523–531.

Kinae, N., Sugiyama, C., Nasuda, M. Y., Goto, K., Tokumoto, K., Furugori, M. & Shimoi, K. 1992 Seasonal variation and stability of chlorinated organic mutagens in drinking water. *Wat. Sci. Technol.* **25**(11), 333–340.

Kopfler, F. C., Ringhand, H. P., Meier, J. R. & Kaylor, W. 1990 Comparison of mutagenic activity of chlorinated aquatic and commercial humic substances. In: Jolly, R. L. (ed.) *Water Chlorination: Chemistry, Environmental Impact and Health Effects*. Lewis Publishers, Michigan, USA, pp. 147–158.

Korn, C., Andrees, R. C. & Escober, M. D. 2002 Development of chlorine dioxide-related by-product models for drinking water treatment. *Wat. Res.* **36**(1), 330–342.

Liang, L. & Singer, C. 2003 Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water. *Environ. Sci.* **37**, 2920–2928.

Meier, J. R., Longg, R. D. & Bull, R. J. 1983 Formation of mutagens following chlorination of humic acid : a model for mutagen formation during drinking water treatment. *Mutation Res.* **118**, 25–41.

Nikolaou, A. D., Lekkas, T. D. & Golfinopoulos, S. K. 2004 Kinetics of the formation and decomposition of chlorination by-products in surface waters. *Chemical Eng. J.* **100**, 139–148.

Onarca, S. M., Zani, C., Richardson, S. D., Thruston, A. D., Moretti, M., Feretti, D. & Villarini, M. 2004 A new approach to evaluating the toxicity and genotoxicity of disinfected drinking water. *Wat. Res.* **38**, 3809–3819.

Ozawa, S., Aizawa, T., Tomisawa, T., Saito, M. & Magara, Y. 1991 Formation of by-products by chlorine dioxide. *J. Japan Wat. Wks. Assoc.* **60**(4), 10–18 (in Japanese).

Rapson, W. H., Nazar, M. A. & Butsky, V. 1980 Mutagenicity produced by aqueous chlorination of organic compounds. *Bull. Environ. Contam. Toxicol.* **24**, 590–596.

Rockhow, D. A., Singer, P. C. & Malcolm, R. L. 1990 Chlorination of humic materials: byproduct formation and chemical interpretations. *Environ. Sci.* **24**, 1655–1664.

Rodriguez, M. J., Serodes, J. B. & Levallois, P. 2004 Behavior of trihalomethanes and haloacetic acids in a drinking water distribution system. *Wat. Res.* **38**(20), 4367–4382.

Rossman, L. A., Brown, R. A., Singer, P. C. & Nuckols, J. R. 2001 DBP formation kinetics in a simulated distribution system. *Wat. Res.* **35**(14), 3483–3489.

Singer, P. C. (ed.) 1999 *Formation and Control of Disinfection By-products in Drinking Water*. Am. Wat. Wks. Assoc., Denver, USA.

Sofuni, T. (ed.) 1999 *Data Book of Chromosomal Aberration Test in vitro*, 1998 (revised edition). Life-science Information Center, Tokyo, Japan.

Sohn, J., Amy, G., Cho, J., Lee, Y. & Yoon, Y. 2004 Disinfectant decay and disinfection by-products formation model development: chlorination and ozonation by-products. *Wat. Res.* **38**, 2461–2478.

Sung, W., Matthews, B. R., O'Day, D. K. & Horrigan, K. 2000 Modeling DBP formation. *J. Am. Wat. Wks. Assoc.* **92**(4), 53–63.

Tanaka, K., Shikada, Y., Matsuda, A. & Takahashi, Y. 1991 Occurrence of organic halogens and mutagenicity in drinking water in Niigata District of Japan. In: Matsui, S. (ed.) *Proceedings of the First IAWPRC International Symposium on Hazard Assessment and Control of Environmental Contaminants in Water, Otsu, Japan, Nov. 25–28*. T.I.C. Ltd., Osaka, Japan, pp. 468–475.

Veschetti, E., Cittadini, B., Maresca, D., Citti, G. & Ottaviani, M. 2005 Inorganic by-products in waters disinfected with chlorine dioxide. *Microchemical J.* **79**, 165–170.

Wakayama, H. 2004 Revision of drinking water quality standards and QA/QC for drinking water quality. *Japan-U.S. Joint Conference on Drinking Water Quality Management and Wastewater Control, 12–15 July, 2004*, Hawaii, USA.

White, G. C. 1999 *Handbook of Chlorination and Alternative Disinfectants*, 4th edition. John Wiley & Sons, Inc., New York, USA.

Wilcox, P. & Williamson, S. 1986 Mutagenic activity of concentrated drinking water samples. *Environ. Health Perspect.* **69**, 141–149.

Zhuo, C., Chengyong, Y., Junhe, L., Huixian, Z. & Jinqi, Z. 2001 Factors on the formation on disinfection by-products MX, DCA and TCA by chlorination of fulvic acid from lake sediments. *Chemosphere* **45**, 379–385.

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