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G．知的所有権の取得状況

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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研究成果の刊行物・別刷

Reevaluation of the toxicity of chlorinated water and the usefulness of MX as an index

Sadahiko Itoh, Atsushi Nakano and Toshiaki Araki

ABSTRACT

Changes in the toxicity in chlorinated water after chlorine addition were examined. For toxicity evaluation, the chromosomal aberration test and the transformation test were conducted as indexes of initiation activity and promotion activity, respectively, in the carcinogenesis process. Activity inducing chromosomal aberrations in chlorinated Lake Biwa water gradually decreased over time after chlorination. In contrast, activity inducing transformations determined by the two-stage assay gradually increased. Thus, toxicity that decreases or increases is present in chlorinated water. Furthermore, activity inducing transformations determined by the non-two-stage assay gradually decreased over time. This direction of change is opposite to that of activity inducing transformations determined by the two-stage assay and is consistent with that of activity inducing chromosomal aberrations. The drastic decrease in initiation activity detected as activity inducing chromosomal aberrations could be the main cause for the decrease in activity inducing transformations determined by the non-two-stage assay (an index of the sum of initiation and promotion activity). MX change was quantitatively consistent with those of activity inducing chromosomal aberrations and transformations determined by the non-two-stage assay. On the other hand, directions of changes in concentrations of typical by-products such as chloroform were consistent only with that of activity inducing transformations determined by the two-stage assay. Findings of this study suggest that MX is appropriate as an index for comparing the carcinogenicity of tap water near and far from a water purification plant.

Key words | chlorination, chromosomal aberration test, disinfection by-products, MX, transformation test

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INTRODUCTION

In general, concentrations of trihalomethanes and haloacetic acids in chlorinated drinking water increase in water distribution systems. This strongly suggests that the toxicity of disinfected water is not stable and changeable. From this viewpoint, the change in the toxicity of chlorinated humic acid after chlorine injection has been examined by authors (Itoh et al. 2001). For the measurement of toxicity, *in vitro* bioassays as indexes in the carcinogenesis process were carried out. A chromosomal aberration test using Chinese hamster lung cells was carried out as an index to initiation activity, and a transformation test using mouse fibroblast cells was carried out as an index to promotion activity.

As a result, it was found that activity inducing chromosomal aberrations in chlorinated humic acid gradually decreased over time after chlorination. In contrast, activity inducing transformations gradually increased. Thus, toxicity that decreases or increases is present in chlorinated water. Since typical by-products such as trihalomethanes and haloacetic acids increase after chlorination, it is widely believed that the toxicity of drinking water also increases.

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We have pointed out, however, that it might be early to conclude that the toxicity of drinking water increases in water distribution systems.

In this study, MX (3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) known as a strong mutagen is focused on in addition to typical chlorination by-products. MX has received much attention because of the strong genotoxic activity. After Komulainen *et al.* (1997) reported the carcinogenic potency of MX in rat, the necessity of the water quality management of MX has been discussed (Melnick *et al.* 1997; Hirose *et al.* 1999).

In addition, it has been found that MX is unstable in water and reacts with residual chlorine (Meier *et al.* 1987; Kinae *et al.* 1992). This means that a concentration of MX might decrease after it is formed by chlorine. This direction of change is in reverse to those of concentrations of trihalomethanes and haloacetic acids. A problem is which of these carcinogenic by-products is appropriate as an index of the change of the toxicity of chlorinated water. In the previous study (Itoh *et al.* 2001), experiments were conducted using commercial humic acid as the first step. Natural water was used in this study in order to propose an appropriate index for comparing the toxicity of chlorinated drinking water in distribution systems.

MATERIALS AND METHODS

Chlorination and concentration of Lake Biwa water

Lake Biwa is the largest lake in Japan and also the major water source for 14 million people in Kansai area in Japan. The surface water of Lake Biwa was used in this study. Lake Biwa water filtered with a 1.0 μm membrane filter of which DOC (dissolved organic carbon) was 1.9 $mg l^{-1}$ was chlorinated at an initial concentration of 2.0 $mg l^{-1}$. Sodium hypochlorite stock solution (Wako Pure Chemical Industries, Ltd.) was used for chlorination. Available chlorine in the stock solution was analyzed by the DPD ferrous titrimetric method (APHA/AWWA/WEF 1998) just prior to use. The chlorination proceeded at 20°C in a dark room. The pH was not adjusted, however, it was maintained at 7.4 to 7.8 during the chlorination. Residual chlorine was detected during the chlorination, however, dechlorination was not carried out so as not to change the activity inducing chromosomal aberrations and transformations in chlorinated waters. It was confirmed that the trace residual chlorine had no influence on bioassays.

Organic matters in a sample water were concentrated by adsorption and desorption method using Sep-Pak Plus(Long) CSP800 (Nihon Waters K.K.) resin according to the procedure described by Urano *et al.* (1994). 20 l of a sample water adjusted at pH 2 was fed to CSP800 cartridges at a flow rate of 50 $ml\,min^{-1}$. The adsorbed substances were desorbed by DMSO (dimethylsulfoxide). The final volume of DMSO was 2 ml, and the concentration factor was 10000 times.

Chromosomal aberration test

Chinese hamster lung cell (CHL/IU) was obtained from Dainihon Pharmaceutical Co., Ltd. Cells were cultured with Eagle's MEM (Nissui Pharmaceutical Co., Ltd) supplemented with 10% fetal bovine serum (Gibco Oriental Co., Ltd). CHL culture was grown in 12 ml media, in a glass silicon-capped bottle. 0.06 ml of a concentrated solution in DMSO was added to a 1-day-old culture. As a result, a concentration of DMSO in the media was 0.5%. Bacteria in a sample were eliminated by a 0.22 μm filter. Only activity inducing chromosomal aberrations without activation was measured in this study. Chromosome preparation was made after 24 hours culturing of the addition (Sofuni 1999).

The previously developed image analysis was used to objectively evaluate results of chromosomal aberration test (Itoh *et al.* 1992): The shapes of chromosomes were analyzed by an image analyzer (Image-Pro Plus Ver4.0). Chromosomal aberrations can be divided into broken-type and exchanged-type. Exchanged-type aberrations are detected by the developed method. 50 metaphases in a specimen were analyzed. As a CHL cell has 25 chromosomes, 1250 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. Activity inducing chromosomal aberrations is expressed as a mean value of test results of triplicate specimens.

When activity inducing chromosomal aberrations of certain chemical 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 cannot be used. The developed method is effective in order to compare the relative intensity of activity inducing chromosomal aberrations only in this study.

Transformation test

The *in vitro* cell transformation assay utilizing BALB/3T3 A31-1-1 cells (Japan Health Sciences Foundation) was carried out as an indicator of tumor-promotion activity. The standard method of transformation test using BALB/3T3 cells had been established by IARC/NCI/EPA Working Group (1985), however, it has been improved by Tsuchiya and Umeda (1995) to enhance transformation frequency and shorten the culture period. Culture conditions of the assay in this study followed the procedure described by Tsuchiya and Umeda (1995). 3-methylcholanthrene (3-MC) was used to induce DNA lesions in the cells as the initiation step. The cells were treated with 0.5 mg l^{-1} of 3-MC for two days. After the initiation, 0.015 ml of a concentrated solution was added to the cells cultured in 6 ml media. As a result, a concentration of DMSO in the media was 0.25%. This testing method is called the two-stage transformation assay, since a chemical or a sample is given to the cells in two steps. Only activity inducing transformations without activation was measured.

In contrast, in the case of the non-two-stage transformation test, a sample is added from the beginning without adding 3-MC. The assay is completed by the continuing addition of a sample. This testing method is useful for measuring the toxicity including genotoxicity of a sample.

The image analysis was also used for detecting transformed foci (Sumitomo *et al.* 1998). Since this method is not the standard method of transformation test, it is effective to compare the relative intensity of activity inducing transformations only in this study. More than 100 colonies were analyzed by each specimen. Image analysis of negative control cells initiated with 3-MC gave a mean of 3.2% transformation foci and a standard deviation of 0.5%. Image analysis of negative control cells without 3-MC in the non-two-stage transformation assay gave a mean of 1.1% transformation foci and a standard deviation of 0.2%. Transformation efficiency is expressed as a mean value of test results of triplicate specimens.

Assays of chemicals

Chromosomal aberration tests and transformation tests of some chemicals including chlorination by-products were carried out. 14 chemicals that are suspected to have initiation and/or tumor-promotion activity were selected from review papers (Ishidate *et al.* 1988; Sakai *et al.* 1993; Budunova & Williams 1994; Sofuni 1999). When ethyl alcohol and DMSO were used to dissolve a chemical, the final concentrations of ethyl alcohol and DMSO are needed less than 1% and 0.5%, respectively, in the media. It has been confirmed that ethyl alcohol and DMSO do not influence the chromosomal aberration test up to concentrations of 1% and 0.5%, respectively (Sofuni 1999). No influence was confirmed in the transformation test either.

Analytical procedures

Chloroform was extracted with hexane, and the concentration was determined by a gas chromatograph with an electron capture detector (GC-ECD, 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 usual operating conditions were as follows. The injector temperature was 150°C. The column oven temperature was initially held at 70°C for 3 min, ramped to 145°C at 15°C min^{-1}, and held at 145°C for 2 min. The detector temperature was 200°C. Haloacetic acids were methylated with diazomethane and analyzed by a GC-ECD. TOX (total organic halides) was measured by a TOX-10Σ analyzer (Mitsubishi Chemical Corporation). TOC (total organic carbon) was measured by a TOC-5000A analyzer (Shimadzu).

Measurement of MX

20 l of a sample water adjusted at pH 2 was fed to CSP800 cartridges at a flow rate of 30 ml min^{-1}. The adsorbed substances were desorbed by 15 ml of ethyl acetate at a flow rate of 0.3 ml min^{-1}. The eluate was concentrated to 200 μl after the addition of mucobromic acid (MBA) as the

internal standard. 100 μl of BSTFA + 1% TMCS (N,O-bis(Trimethylsylyl)trifluoroacetamide with 1% Trimethylchlorosilane, PIERCE) was added to derivatize MX in the residue. This solution was concentrated to 200 μl for the analysis by a gas chromatograph (Agilent, 6890plus) with a mass spectrometer (JOEL, JMS-AX505). 40 μl of the sample was injected by multiple injection (2 μl × 20) with the programmed temperature vaporizing (PTV) mode. The gas chromatography column was a DB-5 capillary column (15 m × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific). The injector temperature was 78°C (10.5 min) → 720° C min^{-1} 200°C (21 min), and the column oven temperature was 80°C (10.5 min) → 6°C min^{-1} → 200°C (0 min) → 25°C min^{-1} → 250°C (2 min). The m/z = 135, 273, 275 ions were selected for TMS-MX and the m/z = 313, 315, 317 ions for TMS-MBA analyses.

RESULTS AND DISCUSSION

Result of bioassays of chlorinated Lake Biwa water

Figure 1 shows the result of bioassays of Lake Biwa water after the addition of chlorine. The residual chlorine after four days was 0.8 mg l^{-1}, and pH was maintained at 7.4 to 7.8 during the chlorination. Activity inducing chromosomal aberrations of Lake Biwa water was produced by chlorine, however, it was unstable and gradually decreased over time after chlorination. In contrast, activity inducing transformations measured by the two-stage assay gradually increased. Thus, the toxicity that decreases or increases is present in chlorinated water.

In addition to these two kinds of assays, the non-two-stage transformation test, which is useful for measuring the whole toxicity including genotoxicity of a sample, was carried out. Figure 1 shows activity inducing transformations measured by the non-two-stage assay gradually decreased. This direction of change was reverse to that of activity inducing transformations by the two-stage assay and consistent with that of activity inducing chromosomal aberrations. The non-two-stage transformation test can detect the toxicity including initiation and promotion step. It is important to note that the index of the sum of initiation and promotion activity gradually decreases. These results are qualitatively consistent with those in the case of commercial humic acid conducted by authors (Itoh *et al.* 2001).

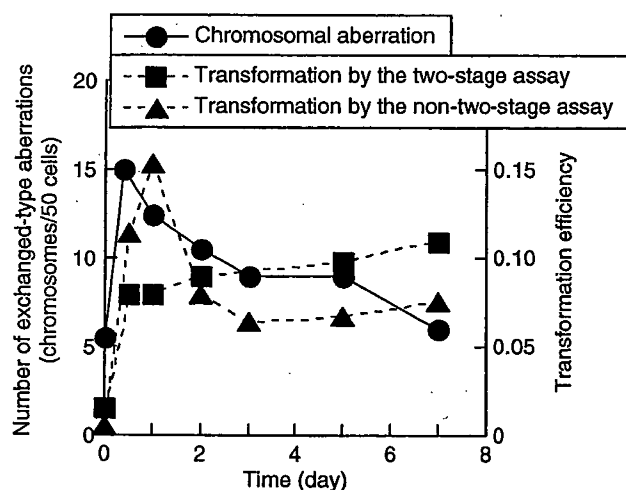

Figure 1 | Activity inducing chromosomal aberrations and transformations of chlorinated Lake Biwa water.

Comparison with activity of some chemicals

Figure 2 shows activity inducing chromosomal aberrations and transformations measured by the two-stage assay of 15

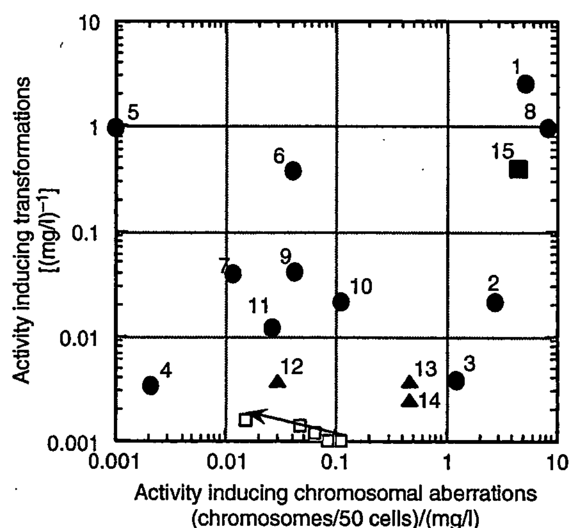

Figure 2 | Relationship between activity inducing chromosomal aberrations and transformations. Activity inducing transformations were measured by the two-stage assay. (□→□) change of activity of chlorinated Lake Biwa water. 1, 4-NQO; 2, quercetin; 3, 5-fluorourasil; 4, sodium nitrite; 5, TPA; 6, mezerein; 7, BHA; 8, 3-MC; 9, teoferin; 10, urea; 11, caffein; 12, chloroform; 13, dichloroacetic acid; 14, trichloroacetic acid; 15, MX.

tested chemicals including MX. The horizontal axis shows activity inducing chromosomal aberrations expressed as (chromosomes/50 cells)/$(\mathrm{mg\,l^{-1}})$, and the vertical axis shows activity inducing transformations expressed as $(\mathrm{mg\,l^{-1}})^{-1}$. $\mathrm{mg\,l^{-1}}$ in these units means the weight per liter of the 15 tested chemicals in the culture media and TOC of Lake Biwa water before chlorination.

The change in activity inducing chromosomal aberrations and transformations by the two-stage assay of chlorinated Lake Biwa water shown in Figure 1 is plotted in Figure 2. The toxicity changed in the direction of the arrow. Firstly, it was found that activity inducing chromosomal aberrations of chlorinated water is relatively stronger than activity inducing transformations, when the plotting position of initial chlorinated water (the initial point of the arrow) is compared with those of other chemicals. Secondly, activity inducing chromosomal aberrations decreases sharply, while activity inducing transformations increases slightly.

Since the chromosomal aberration test and transformation test are carried out as indexes to initiation activity and promotion activity, respectively, it appears that initiation activity in chlorinated water is stronger than promotion activity. It also seems that initiation activity of chlorinated water decreases sharply and promotion activity increases slightly. It is assumed that the decrease of activity inducing transformations measured by the non-two-stage assay shown in Figure 1 can be attributed to this reason.

This phenomenon is illustrated in Figure 3. The increasing toxicity (promotion activity) is present in chlorinated water, however, initiation activity drastically decreases. Since the toxicity of water is measured by *in vitro* assays in this study, it is not possible to get a conclusion on the change of toxicity on the human body. However, it should be noted that the whole toxicity associated with carcinogenic activity can be mainly attributed to initiation activity and presumably decreases over time after chlorination.

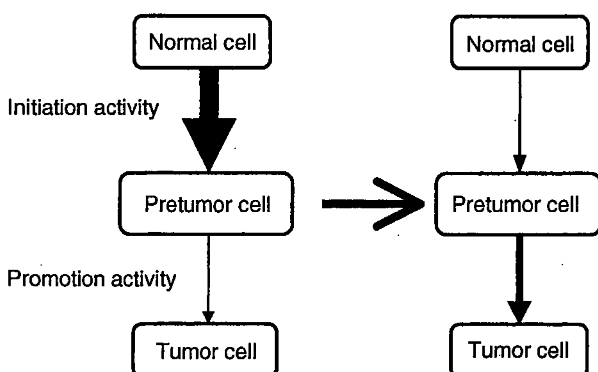

Figure 3 | Proposed change of the toxicity of chlorinated water.

Changes of typical by-products and MX

Figure 4 shows changes in concentrations of chlorination by-products and TOX. All by-products and TOX increased after chlorine injection. These directions of changes of typical by-products are consistent only with that of activity inducing transformations by the two-stage assay and reverse to those of activity inducing chromosomal aberrations and transformations by the non-two-stage assay. By-products shown in Figure 4 are widely measured, however, they would not be appropriate as indexes to compare the toxicity of chlorinated drinking water in distribution systems.

In contrast, Figure 5 shows the change in concentration of MX. It was found that MX decreases over time after it is formed by chlorine. This decrease could be attributed to hydrolysis and the reaction of MX with residual chlorine (Meier *et al.* 1987; Kinae *et al.* 1992). Figures 1 and 5 show that the change of MX is qualitatively consistent with those of activity inducing chromosomal aberrations and

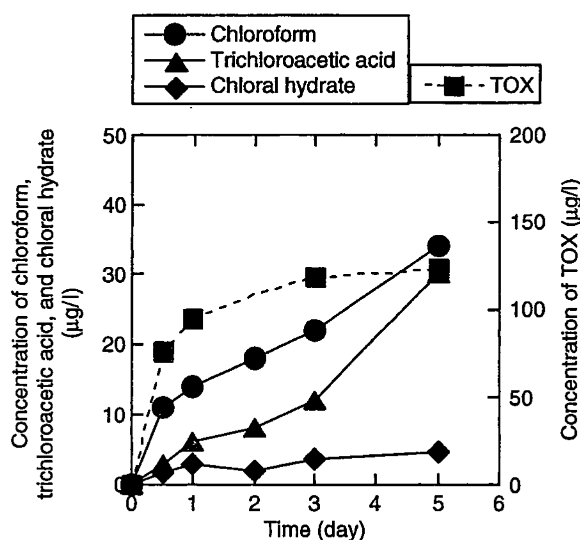

Figure 4 | Concentrations of by-products in chlorinated Lake Biwa water.

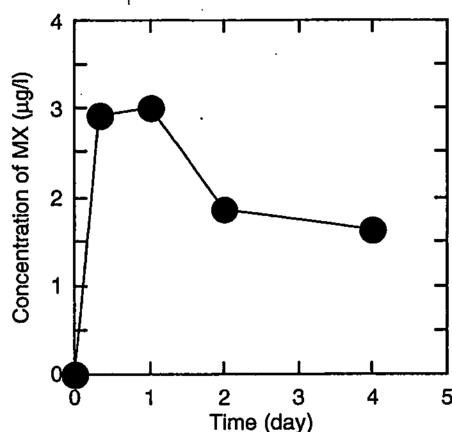

Figure 5 | Concentration of MX in chlorinated Lake Biwa water.

transformations by the non-two-stage assay. This suggests that MX can be one of the indexes for the toxicity detected by these bioassays.

Behavior of MX in water

The stability and the toxicity change of MX in distilled water and chlorine aqueous solution were examined. The MX aqueous solution in a phosphate buffer of 67 mM at pH 7.0 was treated with chlorine. The chlorination proceeded at 20°C in a dark room. Figure 6 shows the change in concentration of MX. It shows MX decreases slightly even in water without chlorine, and the decreasing rate of MX increases with increasing chlorine added to the solution.

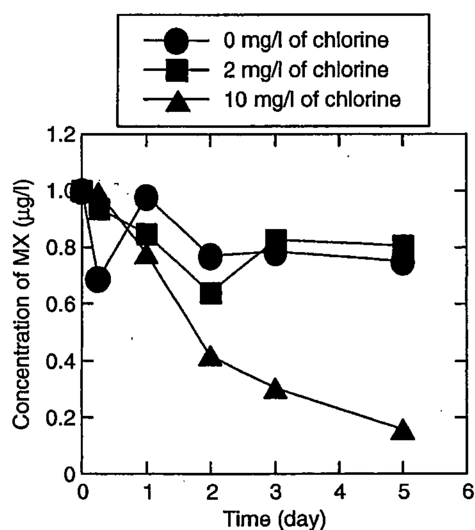

Figure 6 | Change of concentration of MX in water and chlorinated water.

Figure 7 shows the change in activity inducing chromosomal aberrations of MX after chlorine was added to the MX aqueous solution. It shows that activity inducing chromosomal aberrations of MX decreases slightly even in water without chlorine, and it decreases gradually after chlorine is added to the solution. This change is almost correspondent to the change of MX concentration shown in Figure 6. It is reasonable to suppose that activity inducing chromosomal aberrations of MX decreases while MX is decomposed by reacting with residual chlorine.

The usage of MX as an index

To evaluate the usage of MX as an index, reaction rates of changes in the toxicity and the concentration of MX are determined. Decreasing rate constant k for MX is given by assuming first-order reaction at the initial stage after reaching the maximum.

$$\frac{dC}{dt} = -kC \tag{1}$$

where C is concentration of MX (ng l^{-1}), t is reaction time (day), and k is decreasing rate constant (day^{-1}). Decreasing rate of MX was taken as the slope of the decrease in Figure 5, and calculated constant k was 0.19 day^{-1}. Subsequently, decreasing rate constants were calculated with Figure 1, by replacing C in the equation (1) with results of bioassays. Obtained observed rate constants of activity

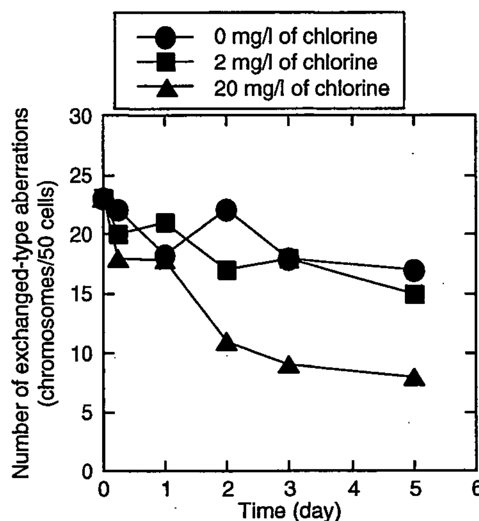

Figure 7 | Change of activity inducing chromosomal aberrations of MX in water and chlorinated water.

inducing chromosomal aberrations and transformations by the non-two-stage assay were 0.14 day^{-1} and 0.18 day^{-1}, respectively. It is apparent that these rate constants are similar to the constant for MX. Consequently, the change of MX was quantitatively consistent with those of activity inducing chromosomal aberrations and transformations by the non-two-stage assay.

Next, the effect of residual chlorine concentration on the reaction rate of MX and the toxicity change was examined in order to clarify an application range of MX as an index. As shown in Figure 6, MX decreases rapidly with a higher concentration of residual chlorine as a result of decomposition by the reaction with chlorine. On the other hand, Itoh *et al.* (2003) have confirmed that activity inducing chromosomal aberrations of chlorinated water decreases slowly with increasing concentration of residual chlorine. This means that activity inducing chromosomal aberrations decreases mainly by hydrolysis. Thus, mechanisms of changes would be different between MX and activity inducing chromosomal aberrations. Therefore, it is supposed that the change in MX concentration and results of bioassays could be correspondent within a limited range of residual chlorine.

In the following examination, only activity inducing chromosomal aberrations was measured, because on the basis of results obtained by authors (Itoh *et al.* 2001), it can be assumed that the change in activity inducing chromosomal aberrations would indicate the change in the toxicity of chlorinated water.

Sodium hypochlorite was added to Lake Biwa water filtered with a 1.0μm membrane filter. Added chlorine was 25 $mg\,l^{-1}$ and 100 $mg\,l^{-1}$ as initial concentrations, since this experiment is the addition to the one by which Figure 1 and Figure 5 were obtained. Other conditions in chlorination are described in MATERIALS AND METHODS. After the change in activity inducing chromosomal aberrations and MX concentration in chlorinated waters were measured, decreasing rate constants were calculated. All decreasing rate constants calculated in this study were plotted in Figure 8 as a function of concentration of residual chlorine.

The result of activity inducing chromosomal aberrations shows that the decreasing rate constant is small, that is, the activity is slow to decrease with increasing concentration of residual chlorine. In contrast, the result of MX

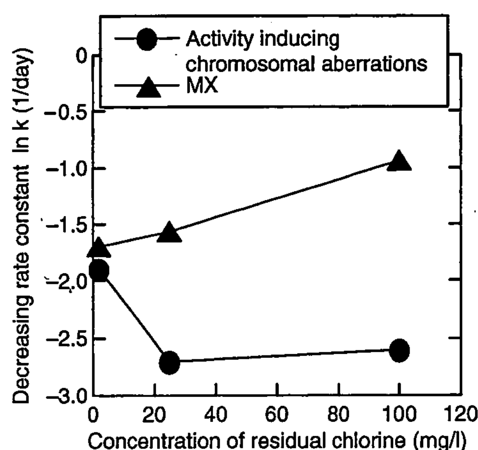

Figure 8 | Decreasing rate constants of activity inducing chromosomal aberrations and MX.

concentration shows that the decreasing rate constant is large, that is, MX is rapid to decrease with increasing concentration of residual chlorine. This phenomenon suggests that MX is not appropriate as an index in drinking water with a higher concentration of residual chlorine, since the difference in rate constants between activity inducing chromosomal aberrations and MX concentration becomes large with increasing concentration of chlorine.

Under the condition of actual drinking water, however, decreasing rate constants of MX and activity inducing chromosomal aberrations were 0.19 day^{-1} and 0.14 day^{-1}, respectively. It seems to be possible to use MX as an index for usual drinking water, since a concentration of residual chlorine is approximately 0.5 $mgCl_2\,l^{-1}$ in actual tap water.

On the other hand, it has been revealed that pH affects the stability of MX in water. Kinae *et al.* (1992) and Meier *et al.* (1987) have shown that MX is unstable under alkaline condition compared to acidic condition. In addition, it was pointed out that there is a discontinuous region where MX is more stable at pH 8 than at pH 6. In contrast, Itoh *et al.* (1993) have shown that there is not a discontinuous region in the effect of pH on activity inducing chromosomal aberrations and it decreases faster under alkaline condition than under acidic condition. That is, the effect of pH on behaviors of MX and activity inducing chromosomal aberrations suggests the difference in mechanisms of their changes in water.

It follows from what has been described that pH and concentration of residual chlorine have to be limited for

utilizing MX as an index. In this study, it was pointed out that MX would be an appropriate index under the condition of neutral pH and chlorine dosage typically used in practice.

This study demonstrates that MX can be utilized for comparing the toxicity of tap water near and far from a water purification plant. When polluted raw water is chlorinated, however, higher concentrations of trihalomethanes and haloacetic acids are formed. In this sense, these typical by-products are still useful as indexes. The important point would be that indicator by-products have to be selected in view of the purpose of water quality management.

CONCLUSIONS

Activity inducing chromosomal aberrations in chlorinated Lake Biwa water gradually decreased over time after chlorination. In contrast, activity inducing transformations determined by the two-stage assay gradually increased. Thus, toxicity that decreases or increases is present in chlorinated water. Furthermore, activity inducing transformations determined by the non-two-stage assay gradually decreased over time. This direction of change is opposite to that of activity inducing transformations determined by the two-stage assay and is consistent with that of activity inducing chromosomal aberrations.

It was found that activity inducing chromosomal aberrations of chlorinated water is much larger than activity inducing transformations. The drastic decrease in initiation activity detected as activity inducing chromosomal aberrations could be the main cause for the decrease in activity inducing transformations determined by the non-two-stage assay (an index of the sum of initiation and promotion activity). An important finding is the toxicity presumably decreases over time after chlorination because of the drastic decrease in initiation activity and the slight increase in promotion activity.

MX change was quantitatively consistent with those of activity inducing chromosomal aberrations and transformations determined by the non-two-stage assay. On the other hand, directions of changes in concentrations of typical by-products such as chloroform were consistent only with that of activity inducing transformations determined by the two-stage assay. Findings of this study suggest that MX is appropriate as an index for comparing the carcinogenicity of tap water near and far from a water purification plant. It was also pointed out that indicator by-products have to be selected in view of the purpose of water quality management.

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[報　文]

流水式紫外線照射装置における一般細菌を用いた生物線量計の実用性

Practicability as Biodosimeter of the Standard Plate Count Bacteria in Flow UV Reactor

廣戸　裕子*　　大瀧　雅寛*

流水式紫外線照射装置において，装置内において微生物が受ける紫外線量を測定する方法として，紫外線耐性が既知の微生物を投入する方法が提案されている。しかし，この手法は装置内に外部から微生物を投入しなくてはならないため，浄水場で稼働中の装置には適用するのは現実的に難しく，装置内紫外線量を継続的に測定するよりよい手法が求められている。原水中に存在する微生物が生物線量計としての役割を果たせれば，新たに微生物を装置内に投入することなく，装置内の紫外線量を測定することが可能になると考えられる。本研究では，原水中に存在する微生物として一般細菌を対象とし，実験室的に回分式と流水式によって生物線量計としての実用性を調べた。とくに計測の正確性を確保するために培養後の微生物数を十分に得ること，紫外線耐性およびその再現性に注目した。

一般細菌を含む原水として地下水を用いた。正確な装置内紫外線量を測定するため，生物線量計として実績のある大腸菌ファージQβを用い，同時に紫外線を照射して実験を行なった。紫外光源には低圧ランプを用い，回分式紫外線照射装置にはガラスシャーレを，流水式紫外線照射装置には二重円筒管を用いた。実験条件として，照射時間と流量を変えて一般細菌の生残率を測定した。また，紫外線照射後の一般細菌濃度はとくに低濃度になる場合が多いため，その測定法についても検討した。

結果として，低濃度の一般細菌測定法を確立することができた。回分式の実験結果に再現性がみられ，紫外線量に対して2段階による直線近似ができた。したがって，一般細菌の生物線量計としての利用可能性が示唆された。しかし，流水式においては，紫外線量が変化しているはずの場合でも，それに応じた紫外線量測定をするには至らないという課題が残った。

Key words：紫外線，流水式，生物線量計，一般細菌，大腸菌ファージQβ

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1. はじめに

一般的に広く利用されている塩素消毒は，トリハロメタン等の有害副生成物[1]や，塩素耐性をもつクリプトスポリジウム等の病原微生物の存在[1)2)]など，さまざまな問題が指摘されており，塩素消毒の代替法が検討されている。塩素消毒の補完技術として，とくにクリプトスポリジウムやジアルジアの原虫対策として，「紫外線消毒ガイドライン」が㈶水道技術研究センターによって明確にされた。

紫外線消毒の特徴としては，有害副生成物生成の可能性が少なく，すべての微生物に有効であることが挙げられ，塩素消毒の有効な代替手法として考えられるが[1)3)]，紫外線消毒の導入のさいには，紫外線消毒装置が十分な消毒効果を有しているのか，継続的に測定する方法の確立が必要であると考えられる。

紫外線強度の測定には紫外線照度計が一般的に用いられるが[4)]，これは装置内の一部分を継続的に測定するにすぎない。現在，装置内全体での必要紫外線量が確保されているのかを評価するための方法として，化学線量計[3)5)]や生物線量計が提案されている[3)4)]。化学線量計は，光反応性の化学物質を装置内に投入し，化学変化量によって照射紫外線量を測定するものであり，生物線量計は，大腸菌ファージQβ[6)~8)]や芽胞菌[7)9)]などを装置に投入し，紫外線照射前後の濃度変化から微生物が受けた紫外線量を求める手法である。この方法は微生物に効果のある紫外線量を直接測定することができるという利点をもつ。しかし，この方法では指標となる微生物を装置内に投入する必要があるため，浄水場においては設置前の事前評価には適用できるものの，稼働中の装置を継続的に測定するには適用できないという欠点がある。

原水中に存在する微生物を生物線量計として利用することができれば，新たに微生物を装置内に投入することなく浄水場等でも広く応用できるのではないかと考えられる。

本研究では，水道水質基準項目の1つである一般細菌を生物線量計の指標微生物として利用することが可能であるかを検討した。生物線量計として用いられている大腸菌ファージQβとの整合性をさまざまな条件下において検討した。

2. 実験方法

2.1 試料の調整

埼玉県大里郡寄居町の地下水を対象試料とし，大腸菌ファージQβ（以下，Qβとする）を濃度が10^7PFU/ml程度となるように加え，よく攪拌したものを試料とした。地下水は平成17年12月2日から平成18年3月6日にわたって計11回採水した。

2.2 低濃度一般細菌の測定法

紫外線照射後に得られる一般細菌はとくに低濃度になったため，その測定法の検討も併せて行なった。

測定は，メンブレンフィルター法に準じて行なった。1～4,000mlの試料を真空ポンプで吸引ろ過し，微生物をろ紙上に捕集した（Millipore社製，セルロースアセテート膜，直径5cm，孔径0.45μm）。ろ紙を普通寒天培地に密着させ，37℃で24時間程度培養し，ろ紙上の一般細菌数を計数した。同法によって，0.005CFU/ml程度まで測定可能となる。

2.3 回分式紫外線照射実験

一般細菌の生物線量計としての可能性を検討するため，まず一般細菌の紫外線耐性を回分式による紫外線照射実験にて求めた。

紫外光源には20Wの低圧ランプ（東芝ライテック㈱製，GL20）を用いた。

試料200mlを内径5.5cm，高さ9.0cmのガラスシ

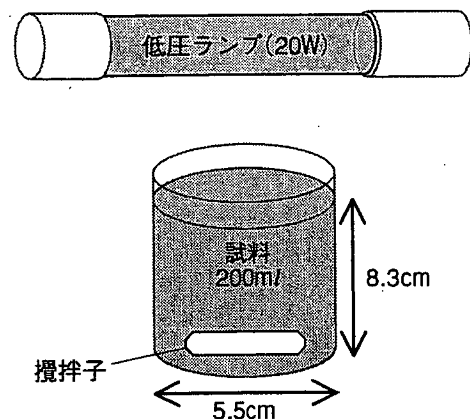


図1 回分式紫外線照射装置

ャーレに入れ，攪拌させながら紫外線を照射した。回分式の実験装置を図1に示した。このときの水深は8.3cmであった。

紫外線照射前後の試料中の一般細菌濃度を測定した。4回の実験中，初期濃度を平均すると3.9CFU/m*l*であった。

試料の波長254nmにおける吸光度は0.024～0.030/cmで幅があり，試料内の平均紫外線量はそのつど変わっていた。そこで，Qβを生物線量計として用い，紫外線量の正確な値を算出した。投入後のQβの初期濃度の平均は4.1×10^7PFU/m*l*であった。

算定式には次式を用いた。

$$I=-k\times\ln(S_{UV}) \quad \cdots\cdots(1)$$

ここで，

I：装置内平均紫外線量(mJ/cm²)

k：不活化反応速度定数(mJ/cm²)

S_{UV}：生残率$=\dfrac{\text{微生物濃度(照射後)}}{\text{微生物濃度(照射前)}}(-)$

である。

Qβの場合$k=5.9$mJ/cm²であることがわかっている[1]。

Qβは宿主菌に*E. coli* K12(NBRC 13965)を用いた重層寒天培地法により測定した。培養時間は37℃で24時間とした。

2.4　流水式紫外線照射実験

Qβと一般細菌の濃度変化を比較することにより，回分式と同様に一般細菌が生物線量計として適用可能かについて検討した。

紫外光源には4Wの低圧ランプ（岩崎電気㈱製QCGL 4W-2)を用い，装置には，内管外径2.3cm，外管内径6.3cm，液層厚さ2cm，長さ16.5cmで容積450m*l*の二重円筒管装置を用いた。試料には，回分式と同様に地下水にQβを混合したものを用いた。

装置内を試料で満たし，定常流を仮定するため紫外線を照射しながら試料を1*l*程度流下させた後に試料を採取した。流水式の実験装置を図2に示す。流量は150～2,000m*l*/minとし，流量を変化させて8回行なった。

装置通過前後の試料中の，一般細菌とQβの濃度を測定した。一般細菌は前述のメンブレンフィルター法に従い，ろ紙上に捕集し普通寒天培地で測定した。Qβは重層寒天培地法にて測定した。

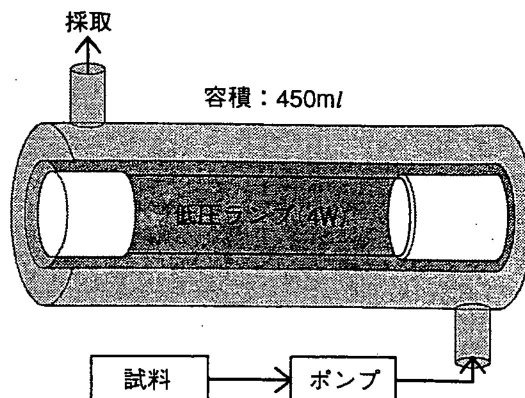


図2　流水式紫外線照射装置

なお，一般細菌の初期濃度の平均は3.7CFU/m*l*であり，Qβの初期濃度の平均は5.0×10^7PFU/m*l*であった。試料の波長254nmにおける吸光度は0.015～0.031/cmの範囲であった。

3.　結果と考察

3.1　回分式紫外線照射実験

回分式紫外線照射実験による一般細菌の生残率と紫外線量の関係を，図3に示す。

異なる日に採取した試料においても，プロット点は同じ傾向を示し，実験結果に再現性がみられた。同一水源においては，一般細菌は一定の紫外線耐性をもつことがわかった。また，2段階ではあるものの，不活化が一次反応で近似できるため，生物線量計への適用が可能であると考えられた。一般細菌の生残率が15mJ/cm²付近を境界として2段階に分かれたのは，一般細菌中に紫外線耐性の強い種がある割合で存在したためと考えられた。

今回の結果から，一般細菌の生残率と紫外線量の関係をつぎの2式で近似した。

$$I=-3.92\times\ln(S_{UV})\ (S_{UV}>1.26\times10^{-2})$$
$$I=-19.1\times\ln(S_{UV})+64.2\ (1.26\times10^{-2}>S_{UV}) \quad \cdots\cdots(2)$$

3.2　流水式紫外線照射実験

流水式紫外線照射実験における各平均滞留時間と微生物の生残率の関係を，図4に示す。Qβの生残率は，平均滞留時間が増加するほど減少傾向

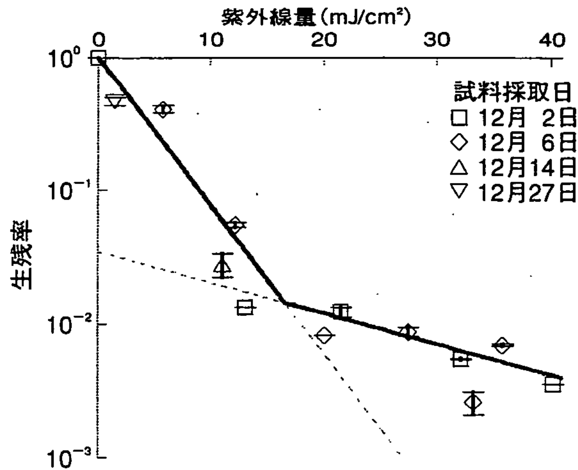


図3　回分式による紫外線照射後の一般細菌の不活化

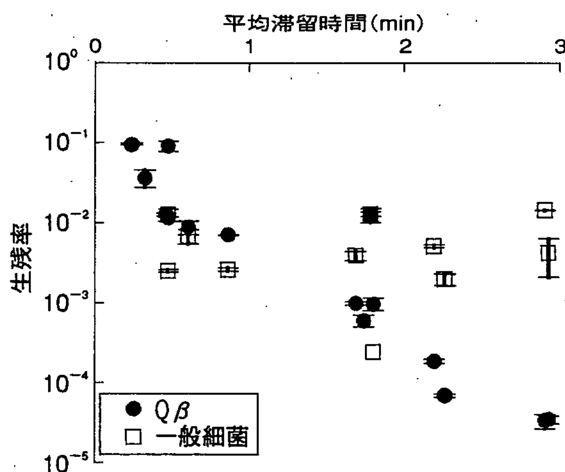
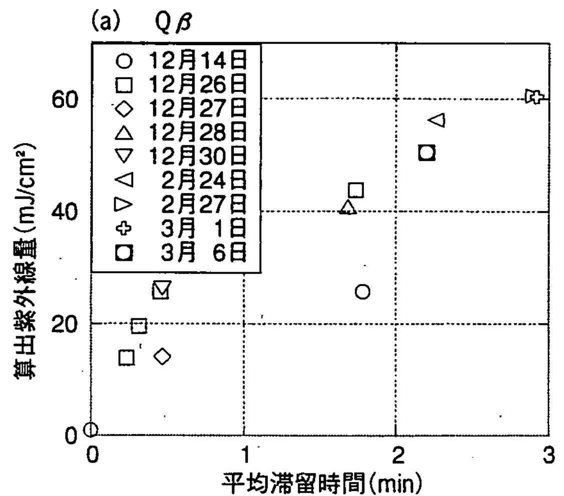


図4　流水式による紫外線照射後の一般細菌とQβの不活化



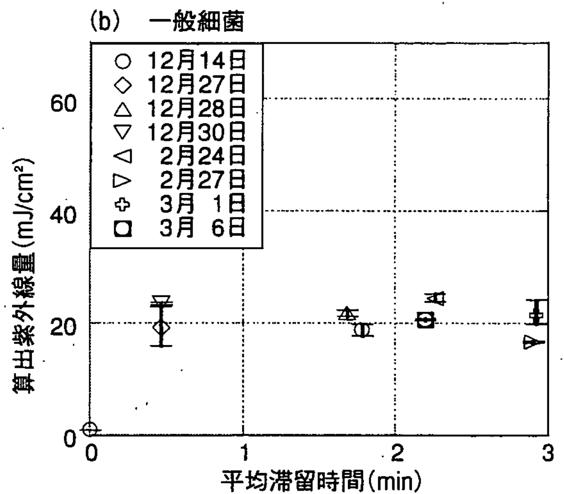


図5　微生物の生残率より算出した紫外線量(流水式)

がみられたが，一般細菌はQβと比較してそれほどこの傾向が大きくみられなかった。

この結果から得られた生残率より各平均滞留時間ごとの装置内紫外線量を算出した。Qβは式(1)を用い，一般細菌は回分式の実験結果から得られた近似式(2)を用いた。

流水式による一般細菌，Qβのそれぞれの生残率から算出された紫外線量と，平均滞留時間の関係を図5に(a)にQβを，(b)に一般細菌を示す。

Qβの場合は，平均滞留時間が増すにつれ算出紫外線量も増加する結果となった。一般細菌は，紫外線量の算出結果が低平均滞留時間においてはQβと一致するものの，平均滞留時間を延ばしても1 min以下と同程度の紫外線量しか示さなかった。

一般細菌がQβと一致する結果となったのは紫外線量20mJ/cm²のときであったが，一般細菌の生残率からは紫外線量20mJ/cm²が確保されていることは測定できても，それ以上の紫外線量の測定には不向きであるといえる。

このような結果になった原因としては，一般細菌はろ紙上に捕集した数に差がみられ，かつ計数した個体数が少ないことが考えられる。滞留時間が大きく，不活化率が大きな条件ではこの傾向が高くなり，紫外線量の信頼性が著しく低下していた。したがって，生物線量計として適用するためには，この点を改善して再度実験を行なう必要がある。

また，20mJ/cm²以下の紫外線量に対する測定の正確性，および今回与えた以上の紫外線量域において一般細菌がどのような反応を示すかについて，今後の検討課題といえる。

4. まとめ

流水式紫外線照射実験では，Qβの結果から20 mJ/cm²以上の紫外線量だと判断できる場合においても，一般細菌の生残率は20mJ/cm²程度であると示していたため，生物線量計として課題が残った。この原因を考えると，20mJ/cm²以上の紫外線量域では，一般細菌の生残率が小さくなり，生残率から紫外線量を求める式においては傾きが小さかったため，感度が悪くなるためと考えられた。また，今回実験したさいに照射した紫外線量より，多くの紫外線量を与えた場合，さらに紫外線耐性の高い種が存在する可能性が考えられた。

低い生残率の条件下において，データの信頼性が低下するという課題が残るが，この点を改善することにより，一般細菌が回分式および流水式紫外線照射装置においても生物線量計として用いられる可能性が示された。

本研究では，同一の地下水を試料として用いたが，応用可能な範囲を調べるためには他の水道水源においても一般細菌が同様に紫外線感受性を示すか確認する必要がある。

また，その他の検討事項としては，今回の一般細菌は紫外線耐性が高く，20mJ/cm²以上の紫外線量域での生物線量計としての感度が低かったため，他の指標微生物で生物線量計として適当なものを検討する必要があると考えられた。

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

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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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