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

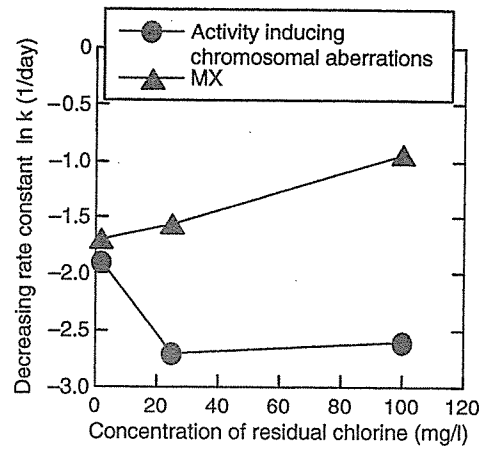

Figure 8 | Decreasing rate constants of activity inducing chromosomal aberrations and MX.

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

＊　Yuko HIROTO, Masahiro OTAKI, お茶の水女子大学大学院人間文化研究科人間環境科学専攻　〒112-8610 東京都文京区大塚2－1－1, Department of Human Environmental Science, Graduate School of Human and Science, Ochanomizu University, 2－1－1 Otsuka, Bunkyo-ku, Tokyo 112-8610 Japan(E-mail：g0570515@edu.cc.ocha.ac.jp)

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/m*l*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。１～4,000m*l*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/m*l*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200m*l*を内径5.5cm，高さ9.0cmのガラスシ

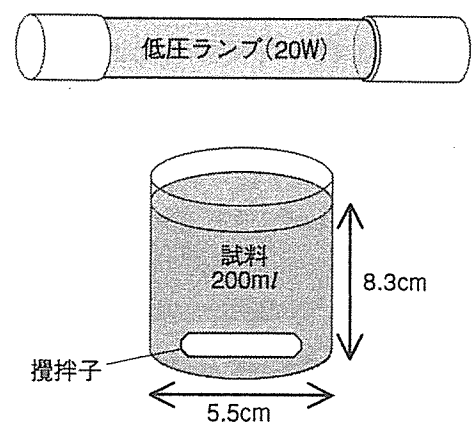


図１　回分式紫外線照射装置

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/cm^2)

k：不活化反応速度定数(mJ/cm^2)

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=5.9mJ/cm^{2}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。

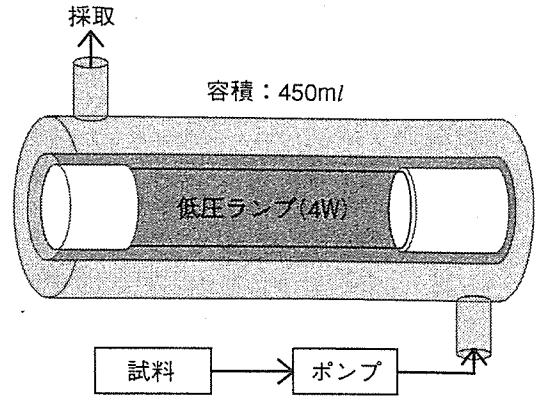


図2　流水式紫外線照射装置

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/cm^{2}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。

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

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

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

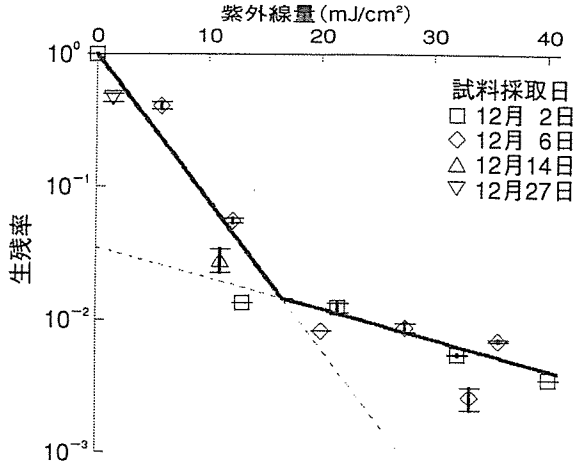


図3　回分式による紫外線照射後の一般細菌の不活化

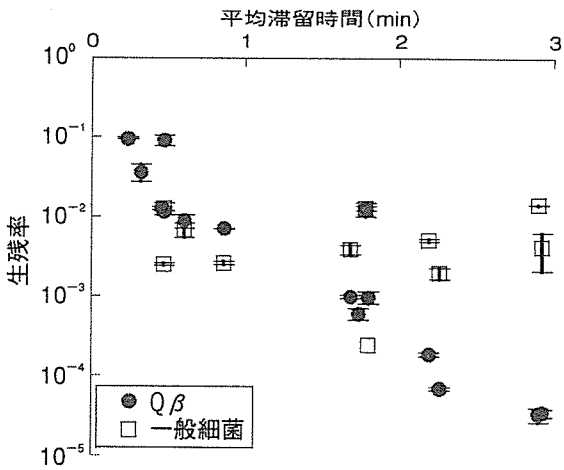
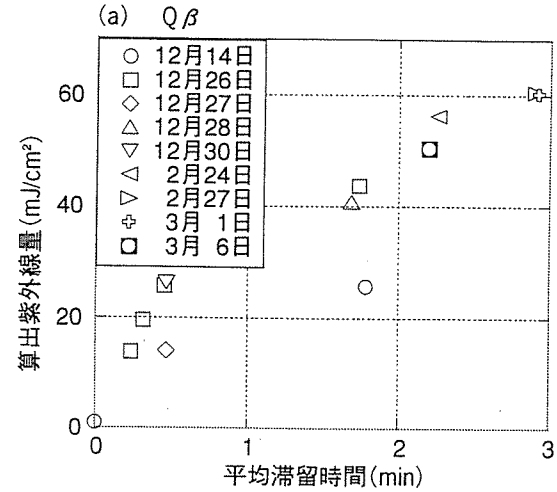


図4　流水式による紫外線照射後の一般細菌とQβの不活化



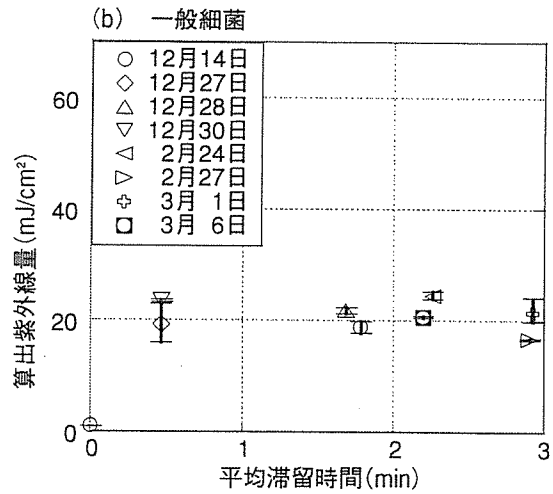


図5　微生物の生残率より算出した紫外線量(流水式)

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20mJ/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。

謝　辞

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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

S. Itoh (corresponding author)
Department of Urban Management,
Kyoto University,
Kyoto 606-8501,
Japan
Tel: +81 75 753 5152
Fax: +81 75 753 5153
E-mail: *itoh@urban.env.kyoto-u.ac.jp*

H. Murakami
Department of Ecosystem Engineering,
The University of Tokushima,
2-1, Minami-jyosanjima-cho, Tokushima 770-8506,
Japan

M. Fukuhara
Nihon Suido Consultants Co., Ltd.,
22-1, Nishi-shinjuku 6, Shinjuku-ku,
Tokyo 163-1122,
Japan

A. Nakano
TOHO GAS Co., Ltd.,
19-18, Sakurada-cho, Atsuta-ku, Nagoya 456-8511,
Japan

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

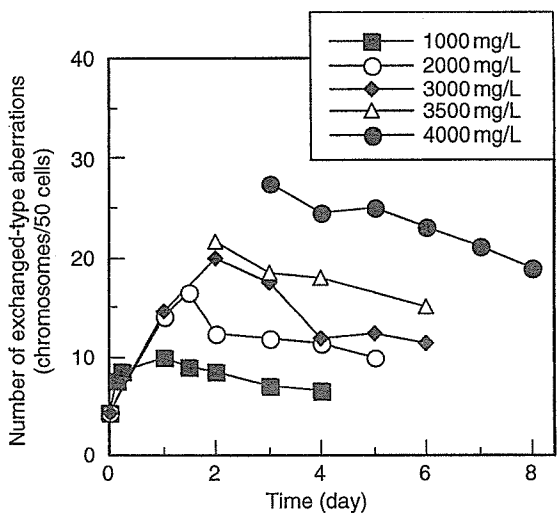

Figure 1 | Changes in the activity that induced chromosomal aberrations in chlorinated humic acid.

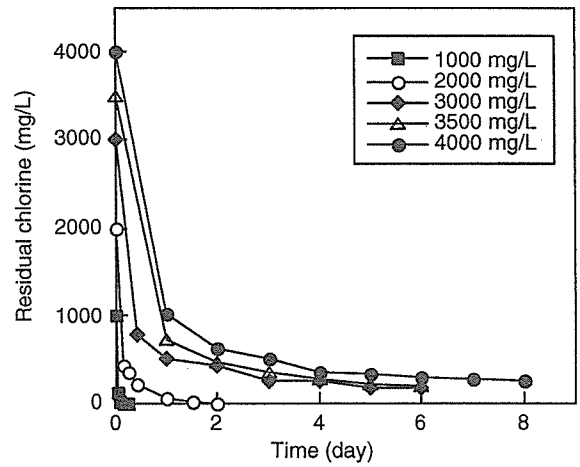

Figure 2 | Residual chlorine in chlorinated water. Initial concentrations of chlorine were 1,000 mg/L, 2,000 mg/L, 3,000 mg/L, 3,500 mg/L, and 4,000 mg/L.

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.

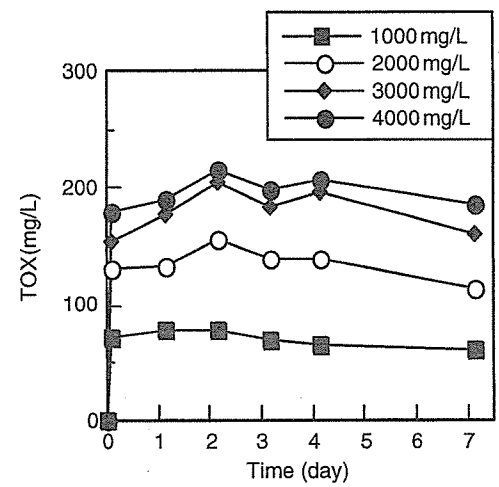

Figure 3 | TOX produced by chlorination.

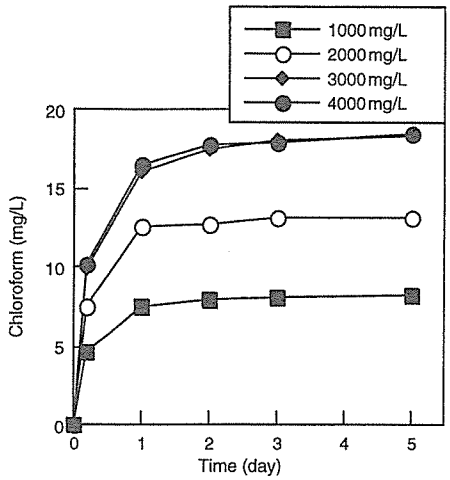

Figure 4 | Chloroform produced by chlorination.

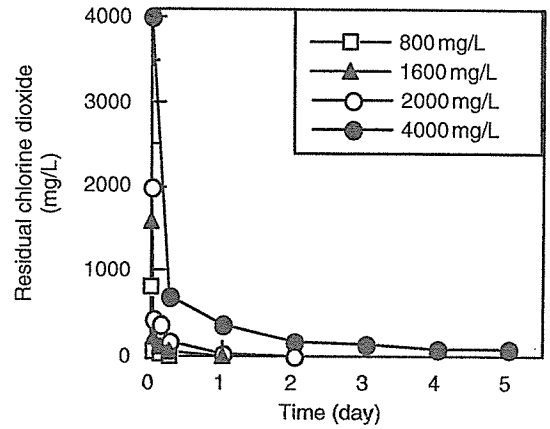

Figure 6 | Residual chlorine dioxide in water treated with chlorine dioxide. Initial concentrations of chlorine dioxide were 800 mg/L, 1,600 mg/L, 2,000 mg/L, and 4,000 mg/L.

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

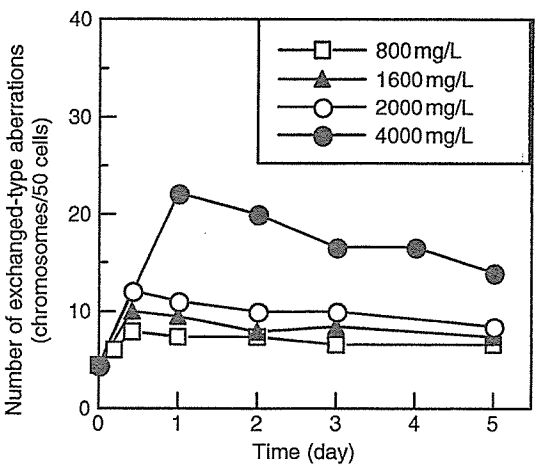

Figure 5 | Changes in the activity that induced chromosomal aberrations in humic acid treated with chlorine dioxide.

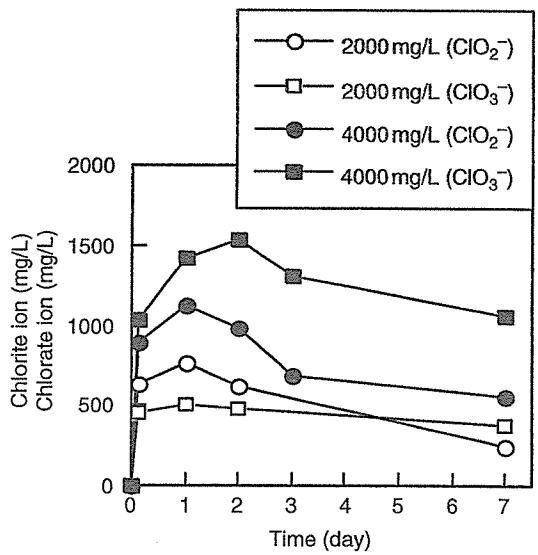

Figure 7 | Chlorite and chlorate produced by chlorine dioxidation. Initial concentrations of chlorine dioxide were 2,000 mg/L and 4,000 mg/L.

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.

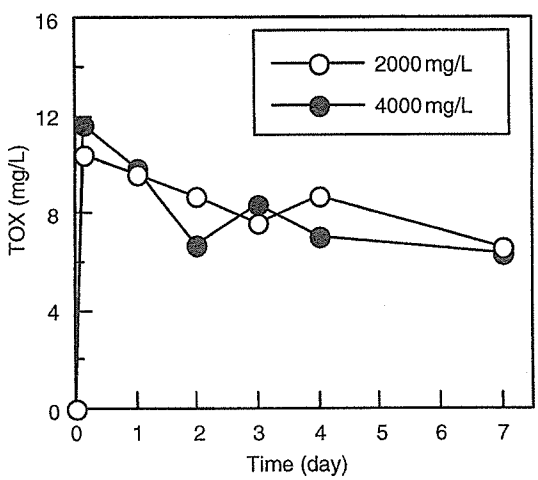

Figure 8 | TOX produced by chlorine dioxidation.

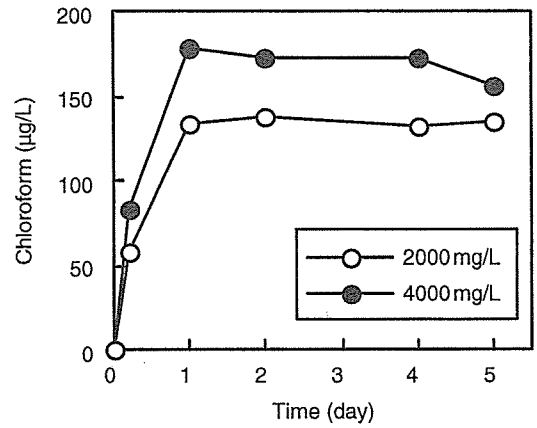

Figure 9 | Chloroform produced by chlorine dioxidation.

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

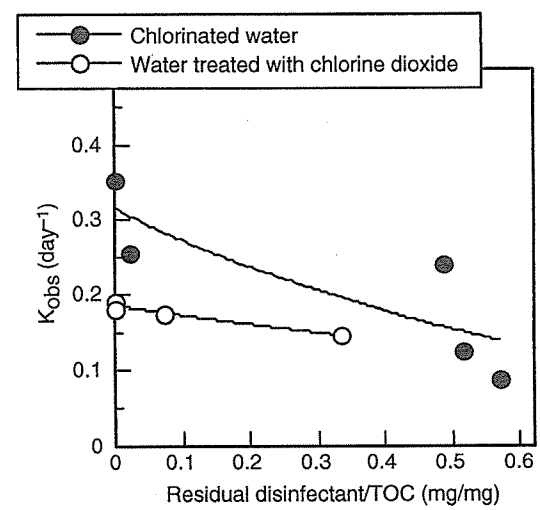

Figure 10 | Effects of residual disinfectant on the decreasing rate constant of the activity that induced chromosomal aberrations.

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

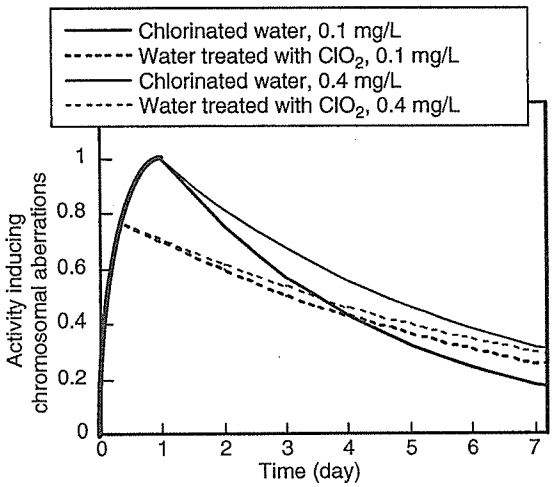

Figure 11 | Estimated changes in the activity that induced chromosomal aberrations in drinking water. DOC of raw water, 2.0 mg/L; Added disinfectant, 1.1 mg/L (disinfectant/DOC = 1).

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.

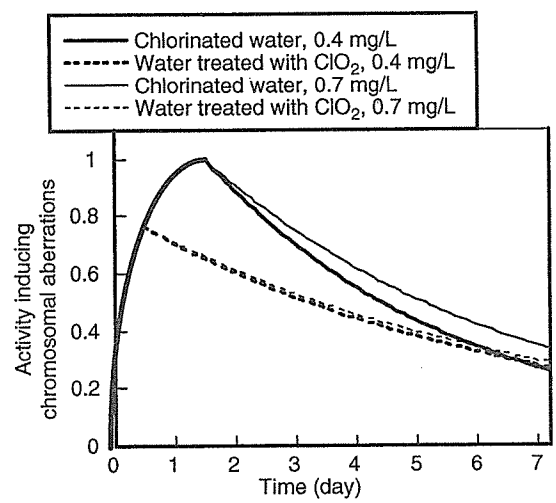

Figure 12 | Estimated changes in the activity that induced chromosomal aberrations in drinking water. DOC of raw water, 3.5 mg/L; Added disinfectant, 3.3 mg/L (disinfectant/DOC = 2).

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.

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環境水中のエンドトキシン検出と塩素処理による微生物細胞からの生成評価

京都大学　大河内由美子，高橋恭介，小寺恵介，伊藤　禎彦

Detection of endotoxin in environmental water and evaluation of formation from bacterial cells by chlorination

Kyoto Univ.　Yumiko OHKOUCHI, Kyosuke TAKAHASHI, Keisuke KODERA, Sadahiko ITOH

微生物に由来する免疫毒性物質としてエンドトキシンに着目し，大腸菌細胞からのエンドトキシン生成を定量的に調べた。また，琵琶湖・淀川水系におけるエンドトキシン濃度の分布状況と微生物量との関係を調べた結果，下水処理施設放流水の影響により環境水中濃度が上昇することを指摘し，また環境水中の微生物一細胞当たりのエンドトキシン活性は 1.13×10^{-3} (EU/cell) であることを示した。微生物さらに，塩素処理により微生物細胞が不活化された場合にも，エンドトキシン活性の増大が起こりうること，また遊離エンドトキシン化することで溶存態有機物画分へと移行することを示した。

キーワード：エンドトキシン，塩素処理，環境水，大腸菌
Keywords : endotoxin, chlorination, environmental water, *Escherichia coli*

1. はじめに

現行の水質基準では，感染症リスク低減のための微生物指標として一般細菌 / 大腸菌数が設定されており，塩素剤を注入・残留させることで病原性細菌の増殖能を抑制，感染症リスクを低下させている。このように、塩素消毒は微生物の増殖能抑制に優れた効果を発揮する一方で，消毒過程において生成する副生成物による健康影響問題が指摘されてきた。しかしながら，原水中に混入する多種多様な微生物は、浄水処理操作によりある程度除去されるものの，一部は不活化された状態や増殖能が低下した状態 (Viable but nonculturable; VBNC 状態) で水道水中に残存しており，配水過程における再増殖やバイオフィルム形成などの問題が指摘されるとともに，より適切な微生物指標・有機物指標が求められている。

そこで本研究では，微生物が有する高分子化合物，特にグラム陰性細菌・シアノバクテリアの細胞外膜構成物質（リポ多糖；LPS）に起因する生物活性 (エンドトキシン) に着目する。特に，モデル微生物からのエンドトキシン生成量を把握するとともに，環境水あるいは水道水中のエンドトキシン活性量および存在形態を明らかにする。さらに，塩素処理によるこれらの変化を詳細に調べることで，水道水質におけるエンドトキシン管理の必要性について考察する。エンドトキシンは強い免疫反応を惹起することが知られており，本来，生体は微量のエンドトキシン曝露により自然免疫を獲得すると考えられる。しかし，年々アレルギー疾患が増加している現代においては，攪乱された免疫システムが新たなアレルギー症状を誘発する危険をはらんでいるため，日常生活におけるエンドトキシン曝露とアレルギー反応との関連性[1]に強い関心が持たれている。

2. 実験方法

大腸菌細胞 (*E. coli* NBRC 3301) に由来するエンドトキシン

E. coli NBRC3301 を 36°C・120(rpm) で振盪培養し、対数増殖期における培養液中の総エンドトキシン・遊離エンドトキシン・微生物量の定量を行った。エンドトキシン活性は、カブトガニ血球抽出成分 LAL (エンドスペシー ES-50M, 生化学工業) を使用したエンドポイント比色法 (トキシカラー DIA-MP, 生化学工業) により測定し，*E. coli* O113:H10 由来のエンドトキシン標準品を用いて検量線を作成した。培養液全画分を使用して総エンドトキシン活性を測定すると共に，培養液を 14,000 (rpm) で 10 分間遠心分離した上清画分を遊離エンドトキシン測定に供した。微生物量の定量は，標準寒天培地および R2A 寒天培地を用いた平板培養法、ならびに DAPI (4',6-diamino-2-phenylindole) 蛍光染色法により行った。

環境水・水道水中のエンドトキシン調査

図 1 に示す琵琶湖淀川水系 (琵琶湖疏水を含む) の各地点で採水を行い，試料中のエンドトキシン濃度と微生物量 (平板培養計数ならびに DAPI 計数) を測定した。採水は冬季 (2005 年 12 月～ 2006 年 2 月) に実施

した。採水瓶は予め 250°C, 2 時間の乾熱滅菌処理を行って使用し，採水後の試料は低温で輸送後直ちに微生物量・TOC 測定に供した。

塩素処理によるエンドトキシン活性の変化

大腸菌懸濁液および宇治川河川水を塩素処理することにより，エンドトキシン活性の変化を調べた。まず，対数増殖期にある *E. coli* NBRC3301 を遠心分離により集菌した後に，67mM リン酸緩衝液 (pH7.0) を用いて洗菌，同緩衝液に再懸濁した (1.57×10^6 (cfu/mL))。各バイアルに大腸菌懸濁液を分注し，次亜塩素酸ナトリウム溶液を初期濃度が 0 ～ 10.0 ($mgCl_2/L$) となるよう添加し，室温・暗所で塩素処理を行った。反応開始 0.5 および 24 時間後にサンプリングし，チオ硫酸ナトリウム添加により残留塩素を消去した後に，従属栄養細菌数の計数，残留塩素濃度の測定ならびに総エンドトキシン・遊離エンドトキシンの測定をそれぞれ行った。同様に，宇治川試料水に次亜塩素酸ナトリウムを 0 ～ 5.0 ($mg\ Cl_2/L$) となるよう添加し，24 時間の塩素処理を行い，各エンドトキシン測定を行った。

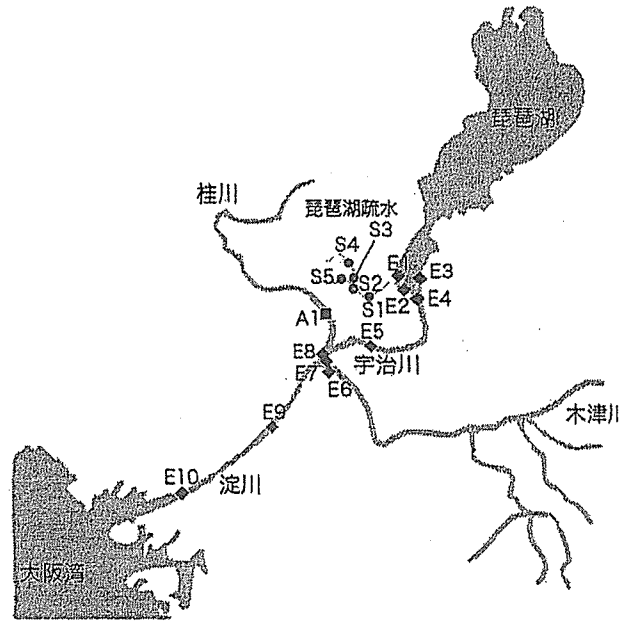

図 1　琵琶湖・淀川水系における採水地点

3. 実験結果と考察

大腸菌細胞 (*E. coli* NBRC 3301) に由来するエンドトキシン

各手法で計数した微生物量と総エンドトキシン活性の相関を図 2 に示す。いずれの計数方法においても，微生物量が増大するにつれてエンドトキシン活性も増大する傾向が確認できる。R2A 寒天培地あるいは DAPI 蛍光染色による微生物計数結果 (x) と総エンドトキシン (y) の間に，比較的高い相関 (それぞれ R^2=0.481, R^2=0.631) が得られた。本実験では，対数増殖期にある微生物細胞を使用しているため，増殖可能菌数 = 全菌数と仮定して R2A 平板培地を用いた微生物量に基づいて大腸菌一細胞当たりのエンドトキシン活性を試算すると，5.64×10^{-3} (EU/cell) (大腸菌 1.0×10^8 (cells/mL) のとき) となった。

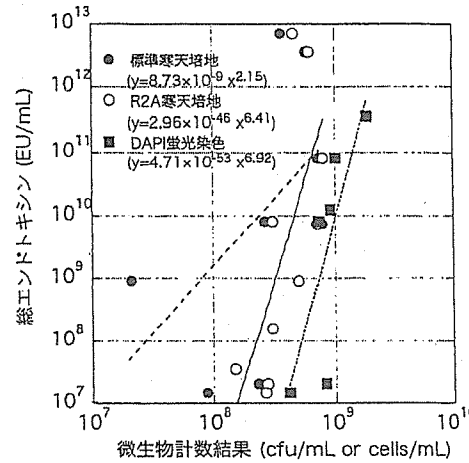

図 2　各手法で計数した大腸菌数と総エンドトキシンの関係

環境水・水道水中のエンドトキシン調査

各試料のエンドトキシン測定結果を表 1 に示す。琵琶湖および河川水中のエンドトキシン濃度は 3.11×10^2 ～ 2.43×10^3 (EU/mL) の範囲に分布した。一方，下水処理場放流水のエンドトキシン濃度は 1.08×10^4 (EU/mL) と環境水と比べて高い値を示し，自然水で 2000 EU/mL 前後の高い値を示した地点，E3,E7 および E8 はいずれも下水処理放流水の影響を強く受けうる地点であった。この結果より，取水口が下水処理放流口近くに存在する場合には，原水中のエンドトキシン濃度が 1 オーダー程度増大する可能性があることから，水資源の循環利用を行っていくうえでは，生菌数のみならずエンドトキシンなどの微生物由来化学物質にも着目することが望ましいと考えられる。

表 1　各採水地点におけるエンドトキシン濃度

	水系	採水地点	総Et (EU/mL)	遊離Et(EU/mL)	遊離Et/総Et比
E-1	琵琶湖南湖	大津港	4.28×10^2	2.20×10^2	0.514
E-2	琵琶湖南湖	なぎさ公園	3.31×10^2	2.30×10^2	0.695
E-3	琵琶湖南湖	矢橋	2.43×10^3	3.30×10^2	0.136
E-4	瀬田川	瀬田唐橋	3.11×10^2	2.25×10^2	0.723
E-5	宇治川	宇治橋	3.11×10^2	1.92×10^2	0.617
E-6	木津川	三川合流地点	6.21×10^2	1.77×10^3	>1
E-7	宇治川	三川合流地点	2.03×10^3	8.26×10^2	0.407
E-8	桂川	三川合流地点	1.80×10^3	4.80×10^2	0.267
E-9	淀川	枚方大橋	1.51×10^3	2.87×10^2	0.190
E-10	淀川	豊里大橋	3.40×10^2	2.50×10^2	0.735
S-1	琵琶湖第一疏水	洛東高校前	6.27×10^2	1.18×10^3	>1
S-2	琵琶湖疏水	蹴上	1.55×10^2	1.82×10^2	>1
S-3	疏水分線	若王子橋	1.46×10^2	1.77×10^2	>1
S-4	疏水分線	一乗寺西橋	1.16×10^2	2.95×10^2	>1
S-5	琵琶湖疏水	冷泉橋	2.57×10^2	1.94×10^2	0.755
A-1		下水処理場放流水	1.08×10^4	1.26×10^3	0.117