Regulations and perspectives on disinfection by-products: importance of estimating overall toxicity

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ABSTRACT

Chemical disinfection of drinking water results in the formation of disinfection by-products (DBPs). This paper reviews evidence on the overall toxicity of disinfected water instead of focusing on the effects of individual DBPs. The possible health effects of ingesting DBPs include development of cancer and adverse reproductive/developmental outcomes. Only a few of the 600–700 chlorinated by-products are regulated, accounting for only a small portion of the overall toxicity of DBPs. This review showed that current water quality management, based on complying with standard values set for individual DBPs, is insufficient in responding to overall toxicity from DBP species. Because water suppliers typically focus their water quality management efforts on meeting the defined maximum concentration standards for individual regulated parameters, current water management practices may not adequately focus on effectively reducing overall DBP toxicity. Therefore, we recommend a progressive shift towards preventive and holistic DBP management based on a comprehensive health-based risk assessment that takes into account the overall toxicity and is supported by a validation of the control processes. We also present a prioritized research agenda that will help determine risk assessment and management and facilitate the development of regulations. This includes the development of an index for overall DBP toxicity.

Key words | carcinogenicity, disinfection by-products, drinking-water quality standards, reproductive/developmental toxicity

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WEAKNESSES IN THE CURRENT REGULATORY APPROACHES ON DBPS

Trihalomethanes (THMs) were originally recognized as a potential health concern in drinking water in the 1970s. Since then, there has been extensive effort by researchers internationally to detect and identify other disinfection by-products (DBPs) (Krasner *et al*. 1989; Stevens *et al*. 1990; Richardson 1998). Although THMs are the most commonly regulated DBP group, they only account for 20–30% of total organic halides (TOX) formed by chlorination. With advances in analytical technologies, 600–700 chlorinated by-products have now been identified. Despite these efforts, it is estimated that detectable by-products account for approximately 50% of TOX. Richardson *et al*. (2007) recently reviewed this issue focusing on carcinogenicity and genotoxicity of DBPs. In addition, the evidence to date has been considered adequate to set health-based values for less than 20 DBPs. As a matter of fact, a total of 18 DBPs currently have health-based values including provisional guideline values that have been derived by the World Health Organization (2006), the US (US Environmental Protection Agency 2006), European Union (1998), Canada (Health Canada 2007) and Japan (Council on Public Welfare Science 2003). No by-product has a health-based value that was determined to account for reproductive and developmental endpoints.

Internationally, current DBP-related norms and regulations (World Health Organization 2006; Karanfil *et al*. 2008) address only a relatively small fraction of the overall DBP toxicity. This proportion cannot be easily increased

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by monitoring increased numbers of DBP species, because regulation and monitoring of more DBPs has both scientific and financial constraints. These constraints are acknowledged in the *Guidelines for Drinking-water Quality* (World Health Organization 2006), which recommend a general shift in emphasis away from monitoring an increasing number of chemical parameters in favour of preventive risk management. Therefore, efforts have focused on the overall toxicity of drinking water.

Here we review the results of studies on the overall toxicity of disinfected water instead of focusing on individual DBPs. The toxicity described in this report includes not only carcinogenicity but also reproductive and developmental toxicity. First, this paper presents evidence that demonstrates the presence of toxicity in disinfected water that cannot be attributed to the currently regulated by-products. This confirms the importance of estimating the overall toxicity of drinking water. Next, we review attempts to evaluate the overall toxicity of disinfected water using *in vivo* bioassays. We discuss problems with these assays and describe ongoing related research by the US Environmental Protection Agency (US EPA). Finally, we highlight requirements of future drinking-water quality regulation and make recommendations.

IMPORTANCE OF ESTIMATING OVERALL TOXICITY OF DISINFECTED WATER

Contribution of individual by-products to the toxicity of chlorinated water

Some researchers have measured the concentrations of by-products and examined the toxicity of individual by-products by *in vitro* bioassays. Table 1 shows an example obtained by Itoh & Echigo (2008). The chromosomal aberration test using Chinese hamster lung cells and the transformation test using mouse fibroblast cells were performed as indices to estimate the initiation and promotion, respectively, in the carcinogenesis process. Three by-products: chloroform, dichloroacetic acid (DCA) and trichloroacetic acid (TCA), contributed 2.9% of the chromosomal aberration-inducing activity and 1.4% to the transformation efficiency. The contributions of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone) and bromate ion were almost negligible (less than 0.1%).

Previous research has also reported that individual DBPs make small contributions to overall mutagenicity, as reviewed by Donald *et al*. (1989). Meier *et al*. (1985) estimated that the summed mutagenicity of ten chlorinated by-products was only 7–8% on TA100 and less than 2% on TA98 by the Ames test. Research using the Ames test has shown that MX contributes from 0.2 to 60% of the mutagenicity of chlorinated water (Kronberg *et al*. 1988; Kinae *et al*. 2000). On the other hand, the contribution of MX to mutagenicity assessed by the Ames test and by a test using cultured mammalian cells differs. Plewa *et al*. (2002) measured the DNA-damaging activity of several DBPs and MX by alkaline single-cell gel electrophoresis (SCGE, comet assay) using Chinese hamster ovary (CHO) cells. This assay indicated that the genotoxicity of MX was very weak compared with that of bromoacetic acid. This is because MX has high affinity for protein and other nucleophiles to reduce its genotoxicity in mammalian cells. Thus, an estimate of MX based on the result of the Ames test may overestimate its cancer risk (McDonald & Komulainen 2005).

It is widely known that the individual by-products analysed in these studies account for a small proportion of the

Table 1 | Contribution of individual DBPs to the chromosomal aberration-inducing activity and the transformation-inducing activity in chlorinated water (Itoh & Echigo 2008)

DBPs	Chromosomal aberration-inducing activity	Transformation-inducing activity (by the two-stage assay)	Experimental conditions
Chloroform	0.5%	0.9%	
DCA	0.8%	0.25%	Humic acid solution chlorinated with Cl_2/TOC = 1.0
TCA	1.6%	0.25%	
MX	<0.1%	<0.1%	Chlorinated Lake Biwa water
Bromate	<0.1%	–	Humic acid solution treated with ozone/chlorine sequential treatment. Br^- in the humic acid solution; 37.5 mg/L

overall genotoxicity of chlorinated water and the toxicity of chlorinated water can be attributed predominantly to by-products other than those currently regulated. The overall toxicity measured by *in vitro* and *in vivo* bioassays is discussed below in the sections '*in vitro* mutagenicity testing' and '*in vivo* testing', respectively. There is no guarantee that the concentrations of the regulated DBPs track the concentrations of all DBPs of adverse health consequences. An implication is that current water quality management, based on standard values for individual by-products, is insufficient in responding to overall toxicity arising from all DBP species in drinking water.

Contributions of organobromine compounds and bromate ion

In general, the concentrations of brominated by-products formed by chlorination are lower than those of chlorinated by-products. However, brominated low molecular weight by-products such as brominated THMs and haloacetic acids (HAAs) are more toxic than chlorinated by-products (Plewa *et al.* 2002; Richardson *et al.* 2007). A complex mixture of by-products from humic acids formed by hypobromous acid has threefold greater mutagenicity than that formed by hypochlorous acid (Echigo *et al.* 2004).

A previous study assessed the contribution of organobromine by-products to the induction of chromosomal aberrations in chlorinated water (Echigo *et al.* 2004). Total organic chlorine (TOCl) and total organic bromine (TOBr) in TOX were measured separately. This study found that the contribution of TOBr ranged from 28 to 52% in the actual tap water conditions of $[Br^-]/TOC = 0.05–0.1$ mg Br/mg C and $[HOCl]/TOC = 1.0–1.5$ mg Cl_2/mg C. In most chlorinated waters, the concentration of TOBr is far lower than that of TOCl; however, TOBr is more toxic. Thus, the contribution of TOBr can be unexpectedly large. In some parts of the world the concentrations of naturally occurring bromide ions in source waters are often over 100 μg/L. In these cases, the contribution of TOBr to overall toxicity may exceed that of TOCl.

The contribution of bromate ions to the toxicity of ozonated and chlorinated water is very small or negligible, as shown in Table 1.

Figure 1 summarizes these findings on ozonated/chlorinated water. The areas of ellipses approximately show the strength of mutagenicity based on the results of the chromosomal aberration test (Echigo *et al.* 2004). TOCl and TOBr are formed in chlorinated waters. Although the TOBr concentration is low, the contribution of TOBr to overall toxicity is significant. In waters that are both ozonated and chlorinated, oxidized by-products without halogen are formed, including bromate ions that contribute a very small proportion of the overall toxicity.

Figure 2 shows the induction of chromosomal aberrations by humic acid solutions containing bromide ion that had been chlorinated and ozonated/chlorinated (Echigo *et al.* 2004). When ozonation was followed by chlorination, the chromosomal aberration-inducing activity was less than that of water treated only with chlorine. Bromate ion up to 1.1 mg/L was formed by ozonation. Water containing

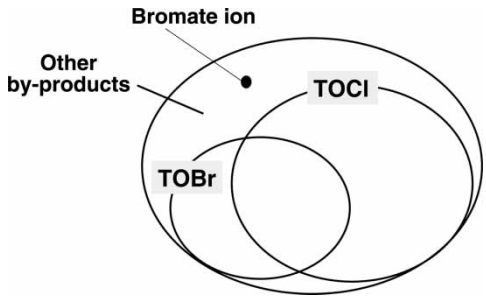

Figure 1 | Contributions of DBPs to the mutagenicity of ozonated/chlorinated water.

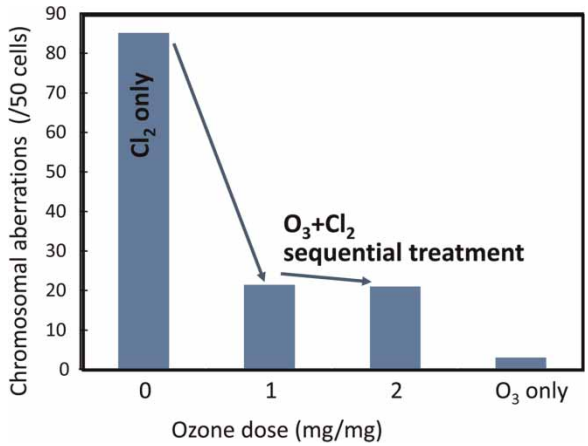

Figure 2 | Effect of ozonation on the chromosomal aberration-inducing activity in chlorinated water with Br^- (Echigo *et al.* 2004); conditions: humic acid concentration, 750 mg C/L; Br^-, 37.5 mg/L; reaction time, 1 day; temperature, 20 °C; chlorine dose, 1,500 mg Cl_2/L; pH, 7.0.

bromated ions that has been treated only with ozone has a weak chromosomal aberration-inducing activity. Because ozonation changes the chemical structures of natural organic matter (NOM), different by-products will be formed and induction of chromosomal aberration is less in chlorinated water if it has been ozonated. Thus, ozonation can produce safer (chlorinated) drinking water even with the formation of bromate ion.

Some water supply utilities reduce bromide ion in raw water by chlorination to decrease the concentration of bromate ions that is formed by subsequent ozonation (Buffle *et al.* 2004). Chlorination before ozonation can result in formation of organobromine by-products (TOBr). However, the contribution of bromate ion to the toxicity of chlorinated water as a final product is negligible, and organobromine by-products have a far greater contribution as shown in Figure 1. Therefore, this procedure may increase overall toxicity of drinking water and a careful safety evaluation should be performed before this is implemented.

Meeting water quality standards for individual DBPs (bromate ion in this case) may result in other potentially significant problems being overlooked, leading to potentially inappropriate and counterproductive treatment measures. Water quality standards for DBPs should be considered as a reference for water quality management. The relative evaluation of the toxicity of brominated organic by-products and bromate ion (Figure 1) and the result indicating the significance of ozonation (Figure 2) present examples of the necessity of measuring the overall toxicity of drinking water.

Change of the toxicity of chlorinated water and its index

As concentrations of THMs and HAAs in chlorinated drinking water increase in water distribution systems (Tanaka *et al.* 1991; Sasaki & Ueda 1992; Summers *et al.* 1996; Arora *et al.* 1997), it is widely believed by water supply utilities that the toxicity of drinking water also increases.

On the other hand, it has been found that mutagenicity of chlorinated water and some chlorinated by-products is not stable. Meier *et al.* (1983) have examined the effect of pH on the stability of mutagenicity of chlorinated water. Mutagenicity of chlorinated humic acids decreases with increasing pH. Nazar & Rapson (1982) have shown that mutagenicity of the known organochlorine mutagens decreases by cleavage of organically bound chlorine. As cleavage of chlorine proceeds by hydroxide ion, mutagenicity decreases faster at higher pH. These findings have shown that the structure of some organochlorine compounds produced by chlorination can be changed by hydrolysis.

Itoh *et al.* (2006) investigated changes in the toxicity in chlorinated water after chlorine addition. Figure 3 illustrates the results. The chromosomal aberration test and transformation test were carried out as indices to initiation activity and promotion activity, respectively. Firstly, it shows that initiation activity just after chlorination is stronger than promotion activity. This was found by a comparison between chlorinated water and various chemicals. Secondly, initiation activity is produced by chlorine; however, it is unstable and decreases sharply over time after chlorination even in the presence of residual chlorine. In contrast, promotion activity produced by chlorine increases slightly over time after chlorination.

Thus, toxicity that decreases or increases is present in chlorinated water. The increasing toxicity (promotion activity) is present in chlorinated water; however, initiation activity dramatically decreases. As the toxicity of water is measured by *in vitro* assays in this study, it is not possible to reach a conclusion on the change of toxicity on the human body. However, it should be noted that the overall

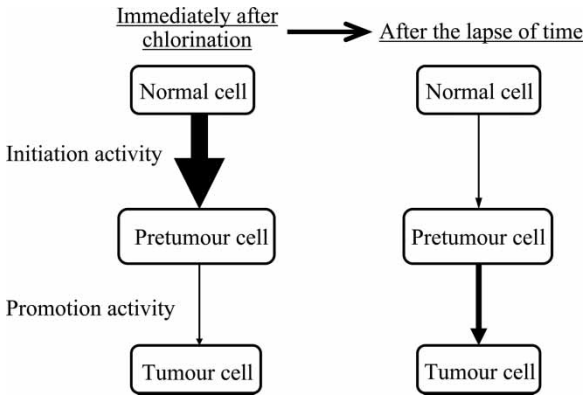

Figure 3 | Proposed change of the toxicity of chlorinated water (Itoh *et al.* 2006). The thickness of arrows shows the strength of initiation activity and promotion activity; changes of the thickness of arrows after the lapse of time indicate changes of initiation activity and promotion activity in the presence of residual chlorine.

toxicity associated with carcinogenic activity can be mainly attributed to initiation activity and presumably decreases over time after chlorination. This was also suggested by the non-two-stage transformation test that is an index of the sum of initiation and promotion activity.

It is well known that concentrations of typical by-products such as THMs and HAAs increase after chlorine injection based on studies on characteristics of DBP formation by chlorination and factors affecting the DBP yield (Rockhow *et al.* 1990; Zhuo *et al.* 2001; Liang & Singer 2003). Since many investigations have been carried out on the mutagenicity in chlorinated drinking water, some characteristics of the mutagenicity have been clarified. One of the representative characteristics is that the mutagenicity easily changes and decreases over time after disinfection depending upon pH and temperature of water (Rapson *et al.* 1980; Meier *et al.* 1983; Kinae *et al.* 1992; Ueda 1996; Itoh *et al.* 2001). These findings suggest that the direction of change in the mutagenicity is inconsistent with those of THMs and HAAs. In addition to these previous views, Figure 3 obtained by *in vitro* tests as indices of initiation activity and promotion activity shows that the toxicity of chlorinated water is not consistent with concentrations of THMs and HAAs. These by-products are widely measured; however, they would not be appropriate as indices to compare the toxicity of chlorinated drinking water in distribution systems.

The stability of some DBPs after production by chlorine has been examined and discussed (Glezer *et al.* 1999; Nikolaou *et al.* 2001; Lekkas & Nikolaou 2004; Xie 2004). MX, a strong mutagen and carcinogen (McDonald & Komulainen 2005), is also produced by chlorination; however, it has been found that it decreases over time after it is formed by chlorine (Meier *et al.* 1987; Kinae *et al.* 1992). This decrease could be attributed to hydrolysis and the reaction of MX with residual chlorine. This direction of change is the reverse of those of THMs and HAAs. In addition, the change in concentration of MX was quantitatively consistent with the change of the toxicity (Itoh *et al.* 2006). Consequently, MX is appropriate as an index for comparing the carcinogenicity of tap water near and far from a water purification plant.

This example suggests that we have to focus on the overall toxicity of chlorinated water and indicator by-products have to be selected in view of the purpose of water quality management.

Toxicity and characteristics of chlorine dioxide-treated water

The use of so-called 'alternative' (meaning non-chlorine) disinfectants can markedly reduce the levels of halogenated organic compounds, including THMs, in drinking water (Fielding & Farrimond 1999; Singer 1999; Barrett *et al.* 2000). DBPs formed by chlorine dioxide including inorganic by-products such as chlorite and chlorate ions have also been examined (Chang *et al.* 2000a, b; Dabrowska *et al.* 2003; Veschetti *et al.* 2005). Chlorine dioxide is generally thought to be suitable for practical disinfection processes with reducing levels of halogenated DBPs (Gates 1998). However, the use of alternative disinfectants has had unexpected consequences, including the production of a different set of toxic DBPs (Sedlak & Von Gunten 2011). For this reason, we have to consider the overall level of toxicity of water that is formed by these disinfectants, in addition to typical halogenated DBPs.

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, there have been some studies on mutagenicity formation by chlorine dioxide and 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). As described above in the section 'Change of the toxicity of chlorinated water and its index', the mutagenicity in chlorinated water changes over time after chlorination. 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); 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.

In one study the toxicity of chlorine dioxide-treated water and associated changes were examined and compared with that of chlorinated water (Itoh *et al.* 2007). The

chromosomal aberration-inducing activity is produced by chlorination and chlorine dioxidation; however, this activity is unstable and gradually decreases over time after the treatments. Moreover, this activity decreases even under conditions where residual chlorine and chlorine dioxide can be detected. Changes in the chromosomal aberration-inducing activity were estimated to compare the safety of drinking water treated with chlorine and chlorine dioxide in distribution systems. The time to reach the maximum chromosomal aberration-inducing activity observed in chlorinated water or chlorine dioxide-treated water was set at 24 hours or 10 hours, respectively, based on the data obtained. Decreasing rate constants for the chromosomal aberration-inducing activity were calculated as a function of the concentration of residual disinfectants. It has been found that the decreasing rate constant is smaller, as the residual disinfectant concentration is higher. Residual concentrations in distribution systems were set at 0.1 and 0.4 mg/L. Figure 4 shows an estimated result based on typical drinking water in Japan. The 1.0 on the vertical axis indicates the maximum chromosomal aberration-inducing activity in chlorinated water.

The levels of chloroform and TOX formed by chlorine dioxidation were approximately 1% and 5–7%, respectively, of those formed by chlorination (Itoh *et al.* 2007).

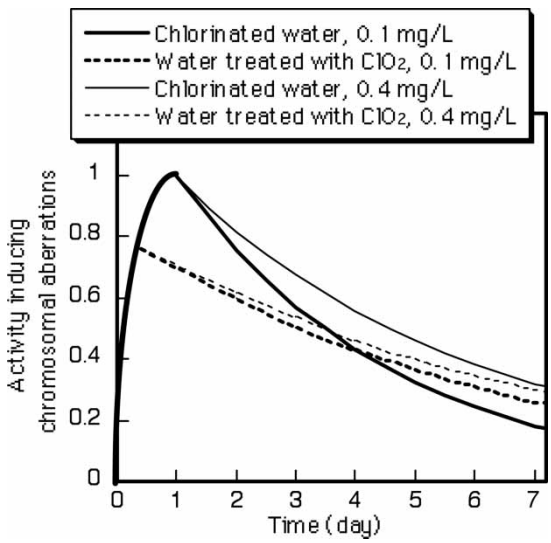

Figure 4 | Estimated changes in the chromosomal aberration-inducing activity in drinking water (Itoh *et al.* 2007). DOC of raw water, 2.0 mg/L; DOC after rapid sand filtration, 1.1 mg/L; added disinfectant, 1.1 mg/L (disinfectant/DOC = 1); assumed residual disinfectant concentrations, 0.1 and 0.4 mg/L.

A major advantage of chlorine dioxide over chlorine is that it produces significantly lower levels of halogenated organic compounds. Figure 4 shows, however, that the chromosomal aberration-inducing activity produced by chlorine dioxidation is stronger than would be expected based on the quantity of the formed 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.

Figure 4 shows that the activity in chlorine dioxide-treated water that induces chromosomal aberrations decreases more slowly, indicating that the mutagenicity of chlorine dioxide-treated water is more stable. The chromosomal aberration-inducing activity in chlorine dioxide-treated water is weaker than that in chlorinated water just after treatments; however, the difference in the two activities decreases over time after treatment. In particular, when the residual disinfectants are 0.1 mg/L, the activity in chlorine dioxide-treated water that induces chromosomal aberrations becomes equal to that in chlorinated water at approximately four days. After that, the relationship is reversed. When the residual disinfectants are 0.4 mg/L, the difference in the two activities does not rapidly decrease.

Assuming that the drinking water is retained in distribution systems typically for less than two days, Figure 4 also suggests that the mutagenicity of chlorine dioxide-treated water would be 70–80% of that of chlorinated water – a potential advantage of chlorine dioxide treatment. In addition, although chlorine dioxide-treated water is less mutagenic than chlorinated water, the difference is small when the drinking water remains in the distribution system for a long period of time.

Thus, while at face value chlorine dioxide treatment can 'solve' the THMs problem, it should be noted that it is similar to chlorine in terms of the mutagenicity of drinking water.

Chlorate ion and chlorite ion are formed as inorganic by-products by chlorine dioxide and standard values have been set for these by-products that prevent its widespread use because they are not easy to achieve. The finding presented here is an additional limitation in using chlorine dioxide.

Contribution of DBPs to the estrogenic effects of drinking water

The potential health risks of endocrine disrupting chemicals (EDCs) were of great public interest in the mid-to-late 1990s. Many epidemiological studies have been conducted to examine the relationship between adverse reproductive and developmental outcomes and exposure to chlorinated drinking water. Some reviews of these studies (Zavaleta *et al*. 1999; International Programme on Chemical Safety 2000; Nieuwenhuijsen *et al*. 2000; US Environmental Protection Agency 2006) have suggested that adverse outcomes, such as spontaneous abortion, stillbirth, low birth weight, neurotoxicity and birth defects, can be associated with THMs and chlorinated by-products. These associations were not reported in other studies and further research would be needed to confirm any association.

Hundreds of compounds have been listed as suspected EDCs (Endocrine Disruptor Screening and Testing Advisory Committee 1998), and most research on EDCs focuses on these individual micropollutants. In contrast, the relationship between the consumption of chlorinated water and reproductive and developmental toxicity has been explored in epidemiological studies as mentioned above. Therefore, chlorinated by-products formed from NOMs should be of interest in addition to typical EDCs.

We consider it is important to measure the estrogenic effects of raw water containing both micropollutants and NOMs, and of chlorinated by-products in addition to suspected EDCs. The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) (1998) established by the US EPA also recommended that a mixture of DBPs be evaluated for their potential to cause endocrine disruption.

Figure 5(a) illustrates the components of water that induce estrogenic effects and how they are changed by chlorination (Itoh *et al*. 2009). First, NOMs have a weak estrogenic effect that increases after chlorination. Itoh *et al*. (2000a) found that commercial humic acid exhibits the estrogenic effect, which increases upon chlorination. In addition, this study demonstrated that the estrogenic effect of concentrated Lake Biwa water using the XAD7HP resin increases up to 2.3 times upon chlorination. The reasons that chlorination increases the estrogenic effect could be: (1) chlorine produces by-products such as organochlorine substances, which are estrogenic; (2) a low molecular weight fraction, which may bind to the estrogen receptor in a cell, increases as a result of the oxidation and hydrolysis caused by chlorination; and (3) chlorine releases estrogenic substances, which interact with humic substances in the aqueous environment. Itoh *et al*. (2000b) revealed that the main factor affecting the increase in the estrogenic effect is the effect of chlorination by-products. However, it has not been successful in detecting specific by-products contributing to the increase in the estrogenic effect.

In addition, coagulation and activated carbon treatment decreased the estrogenic effect of the source water, but chlorination increased the estrogenic effect of the source water and treated waters (Itoh *et al*. 2009). These results suggest that the estrogenic effect is formed by the reaction of chlorine with organic matter that remains after water

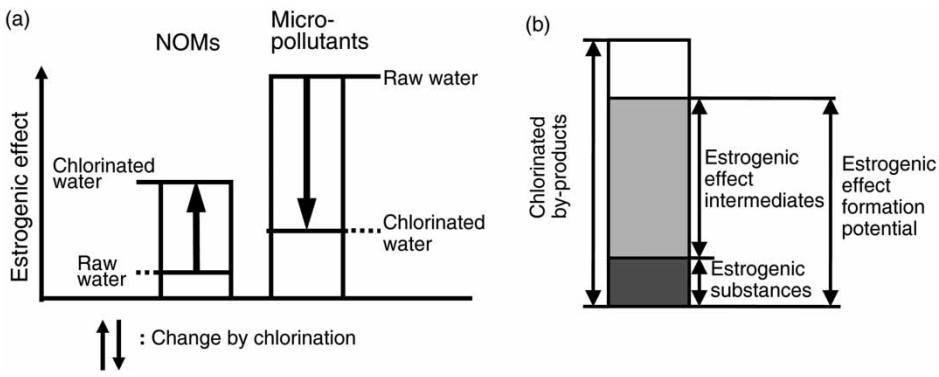

Figure 5 | Components of the estrogenic effects in chlorinated drinking water (Itoh *et al*. 2009).

treatment. It should be emphasized that this phenomenon is very similar to the formation of THMs in the drinking water treatment process; that is, NOMs are major precursors for both the estrogenic effect and THMs.

On the other hand, the estrogenic effects of most micropollutants decrease after chlorination as shown in Figure 5(a). The effects of chlorination of bisphenol A (BPA), 4-nonylphenol (4-NP), estrone (E_1), 17β-estradiol (E_2), estriol (E_3), and 17α-ethynylestradiol (EE_2) on the estrogenic effect have been reported (Hu *et al.* 2002; Kuruto-Niwa *et al.* 2002, 2007; Lenz *et al.* 2003; Tabata *et al.* 2003; Deborde *et al.* 2004; García-Reyero *et al.* 2004; Lee *et al.* 2004; Nakamura *et al.* 2006). In fact, some chlorinated derivatives or intermediates during chlorination of BPA and 4-NP show stronger estrogenic effect than parent compounds; however, the estrogenic effect of these compounds eventually decreases after chlorination with chlorine dosage typically used in practice.

Different results have been reported about the effect of chlorination on the estrogenic effect of river water and treated wastewater. The estrogenic effect decreased by chlorination in some studies (Takigami *et al.* 1998; Akatsuka *et al.* 2000); however, it increased in another study (Yakou *et al.* 2000). Figure 5(a) indicates that organic matter of which the estrogenic effect increases or decreases after chlorination is present in raw water. The findings demonstrate that the overall estrogenic effects in chlorinated drinking water are the sum of the increased and decreased activities of individual constituents after chlorination. The effect of chlorination depends on the quantity of the estrogenic effect that increases and decreases by chlorination.

In addition, the estrogenic effect originated from NOMs shown in Figure 5(a) following chlorination increased gradually over time, even in the absence of residual chlorine (Itoh *et al.* 2009). It is known that the concentration of THMs and HAAs increases while in the distribution system. The obtained result suggests that some part of the estrogenic effect in drinking water also increases over time after chlorination. The increase in estrogenic effect is faster at a higher pH than at a neutral pH, which is reasonable because the hydrolysis rate increases as the pH increases. Based on this finding, Figure 5(b) illustrates the components of the estrogenic effect originated from NOMs. It shows that the components, which form the 'estrogenic effect formation potential' and 'estrogenic effect intermediates', can be defined. The estrogenic substances formed just after chlorination are part of the chlorinated by-products. The 'estrogenic effect intermediates' change into estrogenic substances over time, explaining why the increased estrogenic effect shown in Figure 5(a) continues to increase over time after chlorination.

This phenomenon is similar to the formation of THMs because NOMs are major precursors of both estrogenic effect and THMs. The 'THM formation potential' and the 'THM intermediates' in the formation process of THMs have definitions that are similar to those illustrated in Figure 5(b) (Xie 2004). To decrease the estrogenic effects of drinking water, NOMs in addition to suspected EDCs should be removed before chlorination. Furthermore, it is important to assess the reproductive and developmental toxicity of mixtures of by-products that originated from NOMs.

ATTEMPTS TO ESTIMATE THE OVERALL TOXICITY OF DISINFECTED WATER

In vitro mutagenicity testing

As discussed above, we have to pay much attention to numerous other DBPs in addition to typical ones formed by disinfection. It has been emphasized for many years that it is important to measure and evaluate the toxicity of complex DBP mixtures in chlorinated water. *In vitro* short-term bioassays such as the Ames test can evaluate the combined action of DBPs. Many studies have investigated the mutagenicity of organic extract in disinfected water, including chlorinated water (Loper *et al.* 1978; Donald *et al.* 1989). As a mutagenicity test, the Ames test was mainly carried out until the 1980s; however, various kinds of *in vitro* bioassay such as assays using cultured mammalian cells have been performed since then.

Our review of studies that compared the mutagenicity of water treated with different disinfectants (Zoeteman *et al.* 1982; Backlund 1985; Meier & Bull 1985; Cognet *et al.* 1986; Kamei *et al.* 1989; Anderson *et al.* 1990; Sayato *et al.* 1991; DeMarini *et al.* 1995; Monarca *et al.* 1998; Guzzella *et al.* 2004; Maffei *et al.* 2005) found that a study by Meier &

Bull (1985) yielded typical results. This study showed that the mutagenicity of chlorinated water was the strongest and that chloramine-treated water was also mutagenic. The mutagenicity of chlorine dioxide-treated water was minimal, and ozonated water had no detected mutagenicity. DeMarini *et al.* (1995) showed that different types of disinfected water had mutagenicity in the following order: chlorination > ozonation plus chlorination > chloramination > ozonation plus chloramination > ozonation > raw water. There are additional findings on ozonation such as: ozone has the effect of reducing the mutagenicity of raw water (Zoeteman *et al.* 1982); and the mutagenicity of ozonated water is detected in some cases (Cognet *et al.* 1986) and not in others (Meier & Bull 1985; Anderson *et al.* 1990). In addition, these results may vary with raw water quality and sample preparation procedure. For example, Sayato *et al.* (1991) showed that chlorination reduces mutagenicity because the mutagenicity of raw water is strong.

Performing *in vitro* mutagenicity testing is not constrained by chemical analysis, which quantifies the concentration of individual chemicals, thus providing one of the indicators of the overall toxicity of water. As a matter of fact, epidemiological studies have reported associations between the mutagenicity of chlorinated drinking water and increased risk of cancers of the bladder, rectum, kidney, pancreas and lymphatic system (Koivusalo *et al.* 1995; Koivusalo *et al.* 1997; Koivusalo *et al.* 1998). The results of *in vitro* mutagenicity testing can be employed for reducing the risk of drinking water, and can contribute to the development of a better water treatment process. On the other hand, these tests have the limitation that toxicity to the human body cannot be assessed and a health-based value cannot be derived by extrapolating the results for humans.

In vivo testing

It is essential to estimate the overall toxicity of disinfected water with *in vivo* assays so that the toxicity of TOX (i.e. complex mixtures of chlorinated water) can be estimated. However, only a few carcinogenicity studies using experimental animals have been conducted.

Bull *et al.* (1982) showed an increased number of tumours when concentrations of US drinking water were applied to mouse skin as tumour initiators in initiation/promotion studies. The same study also showed that water disinfected by chlorine, ozone and chloramine resulted in a greater number of papillomas compared with non-disinfected water. Van Duuren *et al.* (1986) administered a chlorinated humic acid solution (1 g TOC/L) as drinking water to mice for two years. There were no increases in tumours. Similarly no adverse effects relevant to carcinogenicity have been detected in other studies (Kool *et al.* 1985; Miller *et al.* 1986; Condie *et al.* 1994). Condie *et al.* (1985) carried out a sub-chronic toxicity test administering chlorinated humic acid solution in drinking water for 90 days. NOAEL (no-observed adverse effect level) was derived as 0.5 g TOC/L. Daniel *et al.* (1991) conducted a sub-chronic toxicity test in male and female rats. A provisional NOAEL of untreated humic acid solution, ozonated water and ozonated/chlorinated water was set to be 1.0 g TOC/L.

In summary, no studies have shown evidence of the carcinogenic effects of complex DBP mixtures via drinking water consumed by rodents. There have been many epidemiological studies of associations between consumption of chlorinated drinking water and increased risk of various cancers (International Agency for Research on Cancer 2004; US Environmental Protection Agency 2006). The US EPA has concluded that the available data indicates a potential association between consumption of drinking water and bladder cancer, and it also suggests a potential association between consumption of drinking water and rectal and colon cancers. Although an epidemiological study is useful as a means to observe adverse effects on human health, there is no attempt to date to derive health-based values of DBPs based on epidemiological evidence. *In vivo* assays using experimental animals should be given a higher priority to derive a health-based value of a DBP mixture.

Toxicity estimation project initiated by the US EPA

Available evidence suggests that it will be essential to perform *in vivo* toxicity tests on disinfected water to obtain results that can be used to derive water quality standards for TOX (μg Cl/L). In the future, monitoring and managing drinking water quality using a standard value of TOX should be implemented in the case of chlorinated water. The US EPA has initiated the Integrated Disinfection Byproducts

Mixture Research Project for this purpose (Simmons *et al.* 2002, 2004).

In this project, the following *in vivo* toxicology tests will be performed: reproductive and developmental toxicity, mutagenicity, carcinogenicity, immunogenicity, hepatic/renal toxicity, neurotoxicity, developmental neurotoxicity, and kinetics/metabolism. *In vitro* bioassays on similar types of toxicity have also been designed to be performed. It is very valuable that, in addition to *in vitro* bioassays, *in vivo* toxicity studies that are associated not only with carcinogenicity but also with several other types of toxicity have been planned.

This project confronts challenging technical issues such as the development of a concentration procedure using a reverse osmosis membrane (Speth *et al.* 2008), preparation of water concentrates that are drinkable by laboratory animals (Narotsky *et al.* 2008), and ensuring the chemical stability of water concentrates (McDonald *et al.* 2010). As multi-disciplinarity is needed to tackle these technical issues, specialists from different fields have designed and initiated this huge project.

The reproductive and developmental endpoints are being given first priority in this project. The results obtained to date showed that 130-fold concentrates of both chlorinated and ozonated/post-chlorinated water appeared to exert no adverse developmental effects (Narotsky *et al.* 2008). Cancer endpoints, however, were assigned a lower priority because of the difficulty in obtaining enough water concentrate for a two-year cancer bioassay. In addition, water is disinfected either by chlorination or by ozonation/post-chlorination, and there is no plan to research adverse effects of water that has been treated with chlorine dioxide or chloramines (Simmons *et al.* 2008). Future research progress is highly encouraged.

Since obtaining useful information for actual regulation depends on the progress and success of *in vivo* bioassays, they should be given a higher international priority.

CONCLUSIONS AND RECOMMENDATIONS

The regulation of DBPs has played a great role in producing safe drinking water; however, there are numerous limitations with the current system. Only a few of the 600–700 chlorinated by-products are regulated, accounting for only a small portion of the overall toxicity represented by DBPs.

Water suppliers typically focus their water quality management efforts to comply with defined maximum concentration standards for individual regulated parameters. As a result, toxicity from causes other than regulated by-products is overlooked, leading to potentially inappropriate and counterproductive treatment measures. The contribution of bromate ion to overall water toxicity (Figure 1) and the toxicity and changes in chlorine dioxide-treated water (Figure 4) are good examples. Standard values are never sufficient as golden rules as far as DBPs are concerned. Instead, they should serve as important points of reference for water quality management.

We recommend a paradigm shift towards preventive and holistic DBP management based on a comprehensive health-based risk assessment that takes into account the overall toxicity. This approach is recommended in the WHO Guidelines for Drinking-water Quality as 'Water Safety Plans' (WSPs). WSPs require assessment of risks from catchment to consumer, and implementation of control measures that are validated to effectively mitigate risks. Moreover, the WSP approach puts more emphasis on monitoring of control measures rather than on monitoring at end-of-pipe against an ever-growing list of standards. The implication for DBP management is to focus efforts on the implementation and monitoring of preventive control measures such as removal of DBP precursor compounds, the consideration of the costs and benefits of using alternative or non-chemical disinfection processes, and if appropriate, to establish and validate removal of DBPs prior to distribution. Care must be taken to not compromise disinfection efficacy in efforts to reduce DBP toxicity, and this should also be demonstrated in the WSP risk management plan.

Other than a progressive shift to promotion of WSPs, there may be a limited number of immediately implementable policy or regulatory actions. One step would be to keep standard values that have been derived with sufficiently large safety (uncertainty) factors. For example, first, an alternative approach such as the benchmark dose method has been introduced to derive tolerable daily intakes (TDIs). This method may give a new health-based value that differs from a previous value, even when the same

toxicity data are analysed. Second, it has been emphasized that a standard value should be set using an appropriate allocation of the TDI to drinking water. An actual measurement of the proportion of intake from drinking water may give a new allocation of intake instead of the default value, ultimately resulting in a new health-based value. Even in these cases, however, any changes in the present standard values should be considered carefully and the overall toxicity of water should be considered.

International organizations and authorities charged with reviewing and revising national drinking water standards should collect information on the overall toxicity of disinfected water. Obtaining useful information for actual regulation depends on the progress and success of *in vivo* bioassays that can be used to derive health-based values. Therefore, *in vivo* assays with experimental animals should be given a higher international priority.

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