Water Disinfection

DOI: 10.1002/ange.200904934

A Toxic Disinfection By-product, 2,6-Dichloro-1,4-benzoquinone, Identified in Drinking Water**

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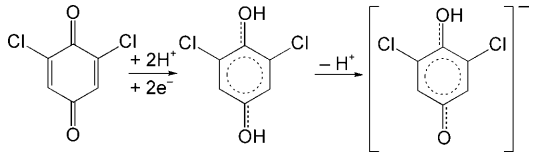
Disinfection of drinking water is a critical public health measure to inactivate pathogens and eliminate waterborne disease outbreaks.[1] However, disinfection of water unintentionally results in the formation of disinfection by-products (DBPs) from the reactions between disinfectants (e.g., chlorine, chloramines, and ultraviolet irradiation) and natural organic matter (NOM) in water. Epidemiological studies have found associations between the consumption of chlorinated water and an increased risk of bladder cancer, and have suggested associations with adverse reproductive effects.[2–6] Safe drinking water requires deactivation of pathogens, while the risks possibly associated with DBP formation have required scientific research and precautionary regulatory attention. Currently, trihalomethanes (THMs) and haloacetic acids (HAAs), the major DBPs that are readily detectable, are regulated.[7,8] However, accumulating evidence suggests that they are not likely causes of the increased bladder cancer risk or adverse reproductive effects.[9] Other as yet unidentified, but more toxic, DBPs produced at much lower levels are more likely contributors.[9,10] Drinking water is a pervasive exposure route for the public, which makes an understanding of potential health risks from DBPs vitally important.

Quantitative structure–toxicity relationship (QSTR) analysis has predicted that haloquinones are highly toxic and may form during water disinfection.[11] The potential toxicity of haloquinones is demonstrated by benzoquinones, which are redox-active, toxicologically important metabolites of other organic molecules that interact with a variety of biologically active molecules (proteins and DNA), thus resulting in various hazardous effects.[12] The chronic lowest observed adverse effect levels (LOAELs) of haloquinones are predicted to be in the low $\mu g\,kg^{-1}$ body weight per day range, which is 1000 times lower than most of the regulated DBPs except for bromate. To date, the formation or occurrence of haloquinones as DBPs in drinking water has not been reported. Consequently, we aimed at determining whether haloquinones are present in treated drinking water. This knowledge is critical because current water utility practices aimed at complying with the regulated, but largely surrogate, DBPs have resulted in increased production of more toxic, but previously unidentified, DBPs.[9] Effective management of DBP health risks requires better knowledge of disinfection chemistry combined with DBP toxicology.[6,9–11]

We initially tested four relevant haloquinones (see the Supporting Information, Figure S1, for their structures): 2,6-dichloro-1,4-benzoquinone (DCBQ), 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ), 2,3,6-trichloro-1,4-benzoquinone (TCBQ), and 2,6-dibromo-1,4-benzoquinone (DBBQ). However, it is difficult to generate stable ionization of $[M+1]^+$ and $[M-1]^-$ ions from these compounds by using electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI). To address this issue, we examined the negative-ionization pathway of haloquinones. We consistently observed $[M+H]^-$ ions, which can be explained based on the electrochemistry of chloroquinones on the ESI tip (Scheme 1). During negative ESI, chloroquinones undergo an electrochemical reduction at the spraying tip, which results in conversion of both C=O groups to C–OH, thus generating an $[M+2H]$ intermediate. This is followed by rapid deprotonation to produce $[M+H]^-$. This process is consistent with the electrochemistry of benzoquinone, namely, quinone $+ 2H^+ + 2e \rightarrow$ dihydroquinone (0.70 V).[13] The precursor and main product ions of the four target haloquinones produced in MS are listed in Table 1. To confirm the formation of $[M+H]^-$, we measured the accurate masses of the precursor and product ions of these compounds by using hybrid quadrupole time-of-flight mass spectrometry. The accurate masses of the precursor and product ions of the four compounds (data not shown) confirm that $[M+H]^-$ ions are indeed formed from the target compounds.



Scheme 1. Proposed ESI pathway for DCBQ.

From the unique ionization chemistry of haloquinones, we developed and validated a liquid chromatography (LC)–tandem mass spectrometry (MS/MS) method that enabled the

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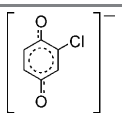
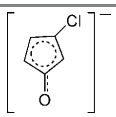
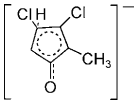
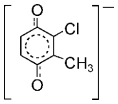
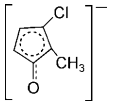
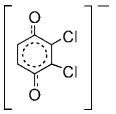
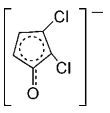
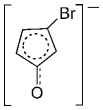
[**] We acknowledge the financial support of the Water Research Foundation, the UK Drinking Water Inspectorate, the Natural Sciences and Engineering Research Council of Canada, the Canadian Water Network, the Alberta Ingenuity Water Research Institute, and Alberta Health and Wellness. We thank Dr. Richard Bull and Dr. Dave Reckhow for providing insights on the potential importance of haloquinones as possible DBPs, Dr. Steve E. Hrudey for his perspectives on the public health risks of DBPs, and Dr. X. Chris Le for his constructive comments and suggestions.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200904934.

Table 1: Precursor and main product ions of the four target haloquinones produced in ESI-MS.

Halo-quinones	Precursor ions [*m*/*z*]	Product ions [*m*/*z*] and their chemical structures		
DCBQ	177	 141	 113	Cl^- 35
DCMBQ	191	 163	 155	 127 Cl^- 35
TCBQ	211	 175	 147	Cl^- 35
DBBQ	267	 157(159)	Br^- 79(81)	

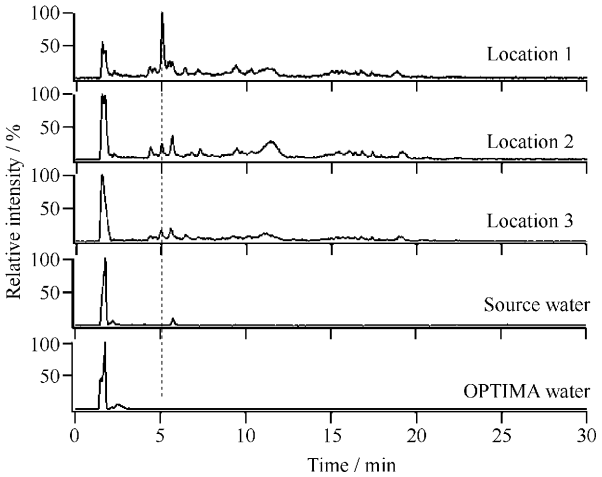


Figure 1. Detection of DCBQ (retention time 5.0 min, see dashed line) in treated drinking water from three locations within a water distribution system (WDS1). Location 1 is the water treatment plant; locations 2 and 3 are sampling points within the water distribution system, with increasing distance from the water treatment plant.

detection of trace concentrations of haloquinones (see the Supporting Information for details). The use of LC separation combined with multiple-reaction monitoring (MRM) of specific ion transitions by MS/MS allowed for highly specific detection of the target haloquinones. Preconcentration of haloquinones from water samples by solid-phase extraction further improved the detection limits. Thus, we were able to detect the four targeted haloquinones at concentrations as low as 3–8.7 ng L^{-1} (Supporting Information, Table S2).

Having established a highly sensitive and specific method, we applied it to the determination of haloquinones in both source water and disinfected drinking water. We consistently found the presence of DCBQ in chlorinated and chloraminated drinking water. Therefore, we subsequently focused on the investigation of DCBQ in drinking-water systems.

Figure 1 shows typical LC–ESI-MS/MS chromatograms of a control sample, a source (raw) water sample, and three disinfected water samples (one from the water treatment plant and two from the distribution system). The disinfection process involved chlorination and UV irradiation, followed by chloramination for secondary disinfection. DCBQ is not detectable in either the control (OPTIMA water) or the untreated source water (Figure 1). In the three water samples collected after the disinfection treatment, the concentrations of DCBQ were 5.3–14.4 ng L^{-1} with a recovery of 97%. The identification of DCBQ in these samples was confirmed by standard addition, matching retention times, and by comparing the signal ratios of the two ion transitions (MRM at *m*/*z* 177/141 and 177/113). The retention time (5.0 min) of DCBQ in the samples was identical to that of the authentic DCBQ standard. The ratio of the peak intensity of *m*/*z* 177/141 to that of *m*/*z* 177/113 in the samples was identical to that of the standard.

To further confirm the presence of DCBQ in treated water, we analyzed another set of source and treated water samples from a different water treatment plant that uses chloramines and UV irradiation for disinfection. Our results consistently show the absence of DCBQ in the untreated source water and the presence of DCBQ (14.3–54.6 ng L^{-1}) in the drinking water after disinfection treatment (Supporting Information, Figure S2). These results indicate that DCBQ is a by-product of drinking-water disinfection.

Figure 2 shows the concentrations of DCBQ in treated water samples collected along two water distribution systems.

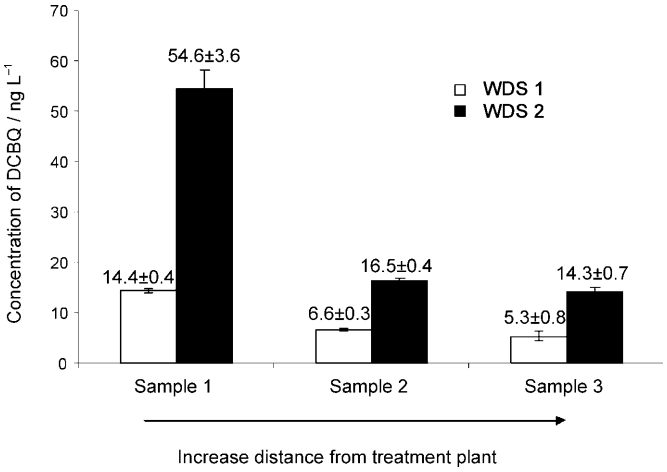


Figure 2. Changes in DCBQ concentration within two water distribution systems (WDS1 and WDS2). Sample 1 was collected from the water treatment plant. Samples 2 and 3 were collected from locations 2 and 3, respectively, within the water distribution system. Sample 2 was collected at a point (location 2) closer to the water treatment plant than sample 3 (location 3).

In both cases, the concentrations of DCBQ decrease with increasing distance from the treatment plants studied above.

To understand the decrease in concentrations of DCBQ in the distribution systems, we further monitored the stability of DCBQ in disinfected water and tested how pH affected its stability. DCBQ is unstable at basic and neutral pH, whereas its stability is improved under acidic conditions (e.g., pH 4.5; Supporting Information, Figure S3). The pH of water from the distribution system sampled in this study ranged between 7 and 8. Therefore, the water samples were acidified with formic acid to maintain the stability of DCBQ prior to analysis. Figure S4 in the Supporting Information confirms that DCBQ is stable for a minimum of 5 days in tap water acidified immediately after collection. The DCBQ determined in the treated water is the result of its formation and degradation.

The finding of DCBQ as a DBP is significant because of its potential toxicity and relevance to bladder cancer risk.[11] Although THMs and HAAs are commonly formed during water disinfection and are relatively easy to measure, their LOAELs are around 1000 times less toxic than the predictions for some haloquinones. The predicted LOAEL for DCBQ is 49 $\mu g\,kg^{-1}$ body weight per day.[11] The regulated DBPs cannot explain the observed epidemiological estimates for human health risk from consuming disinfected drinking water.[9]

The use of the unique electrochemistry of haloquinones at the ESI tip enables the development of the LC–MS/MS method for these compounds. The finding of DCBQ provides the first evidence that some chloroquinones are produced from water disinfection, in agreement with the QSTR prediction. A previous study on the reactions of phenols and chlorophenols with chloroamines showed the formation of dichloroquinone through a radical reaction mechanism, which supports the notion that DCBQ can form as a DBP of chlorination and chloramination.[14] The chemistry and toxicology of quinones support the suggestion that the finding of this novel and toxicologically relevant DBP points to a new direction of research in identifying other potential DBPs, which may offer more plausible agents to control or avoid the potential for increased risk of bladder cancer.[12] The scientific understanding of drinking-water quality has advanced substantially since THMs were first discovered in 1974.[15] Knowledge of the formation chemistry of more toxicologically relevant DBPs is essential to control these chemicals to achieve safe drinking water for the public.[16]

Experimental Section

Source and treated water samples were collected at different points within two water distribution systems (WDS1 and WDS2). Location 1 was the water treatment plant, while location 3 was farther than location 2 from the water treatment plant within the same distribution system. Water samples were collected in precleaned 4 L amber glass bottles, to which formic acid (20 mL, 50% in water) was added immediately after water collection.

Haloquinones were extracted from the water samples by solid-phase extraction (Oasis HLB cartridges, 6 mL, 200 mg per cartridge; Waters, Milford, MA (USA)). The final eluent (500 μL) consisted of 20% methanol and 80% aqueous formic acid (0.5%, v/v), and was subjected to LC–MS/MS analysis.

LC–MS/MS analyses of haloquinones were performed with a Luna $C_{18}(2)$ column (100 × 2.0 mm i.d., 3 μm; Phenomenex, Torrance, CA, USA) for separation and electrospray MS/MS (API5000, Applied Biosystems/MDS Sciex, Concord, ON, Canada) for detection in the negative-ionization mode. MRM in combination with the standard addition method was used for quantification.

Details of the materials, methods, and experimental procedures are given in the Supporting Information.

Received: September 3, 2009
Published online: December 18, 2009

Keywords: disinfection by-products · drinking water treatment · mass spectrometry · quinones · toxicology

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