Analytical Characterization of Halobenzoquinones as Emerging Disinfection Byproducts in Disinfected Water

by

Wei Wang

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Medical Sciences - Laboratory Medicine and Pathology
University of Alberta

ABSTRACT

Water disinfection is necessary for killing pathogens, but it causes an unintended chemical risk from the formation of disinfection byproducts (DBPs). Epidemiological studies show a potential association of water disinfection with increased risk of bladder cancer. It is unknown what DBPs are responsible for the observed adverse health effects. The quantitative structure and toxicity relationship analysis predicts halobenzoquinones (HBQs) are potential bladder cancer carcinogens. The objectives of this study are to characterize the occurrence, formation, transformation, removal, and toxicity of HBQs as DBPs.

An analytical methodology that can identify and quantify trace levels of new HBQs in water was essential to achieve the objectives of my research. Therefore, I first developed an analytical method using solid phase extraction–ultra-high performance liquid chromatography–tandem mass spectrometry (SPE-UHPLC-MS/MS) and applied it to study what HBQs are present in swimming pools. 2,6-dichloro-(1,4)benzoquinone (2,6-DCBQ) was widely present in swimming pool water at concentrations up to 100 times higher than its concentrations in the input tap water. Other HBQs 2,3,6-trichloro-(1,4)benzoquinone (TriCBQ), 2,3-dibromo-5,6-dimethyl-(1,4)benzoquinone (DMDBBQ) and 2,6-dibromo-(1,4)benzoquinone (2,6-DBBQ) were also identified in the water of some swimming pools but not in the input tap water. These additional HBQs formed in the pools were due to personal care products, higher levels of dissolved organic contents, higher chlorine doses, and higher water temperatures, as compared to tap water.

To understand what precursors contribute to the formation of HBQs in water, I conducted laboratory-controlled chlorination experiments to characterize precursors of

HBQs in source waters. The biopolymer fraction of natural organic matter (NOM) in source water was identified as the most important source of 2,6-DCBQ precursors based on statistical analysis. Lotions and sunscreens also contain precursors of 2,6-DCBQ and other HBQs in swimming pool water.

To control HBQ formation in drinking water, I evaluated water treatment processes on the removal of HBQ precursors. Common treatments used in drinking water treatment plants (DWTPs), including coagulation, ozonation, and granular activated carbon, can partially remove or destroy HBQ precursors, but not substantially eliminate them. Anthracite/sand filtration and UV irradiation show negligible impact on HBQ formation.

To understand the fate and behavior of HBQs in drinking water distribution systems, I examined the transformation of HBQs in laboratory experiments and in field samples. Using high-resolution quadrupole time-of-flight (QToF) MS, I found that HBQs were transformed to halo-hydroxyl-benzoquinones (OH-HBQs) at neutral pH in the laboratory experiments. An SPE-UHPLC-MS/MS method was developed to quantify HBQs and OH-HBQs in authentic drinking water samples. Using this method, I confirmed that OH-HBQs are DBPs in drinking water and that they increased with decrease of HBQs in several DWTPs. An *in vitro* toxicity study with CHO-K1 cells showed that HBQs are two-fold more toxic than OH-HBQs, and that both HBQs and OH-HBQs are significantly more toxic than the regulated DBPs.

Finally, I studied the interaction of glutathione (GSH) with HBQs to elucidate potential mechanisms of HBQ toxicity. Mono-, di-, tri-, and tetra-GSH conjugates (GS-HBQs) were identified using LC-MS/MS in reaction mixtures. The glutathionylated

conjugates were elevated with the increase in GSH levels. Halosemiquinone (HSQ) free radicals were gradually depleted with the increase in GSH levels using electron paramagnetic resonance spectroscopy. These results suggest that (1) reversible redox reactions between HBQs and halo-hydrobenzoquinones (HHQs) to form HSQ radicals and oxidize GSH to glutathione disulfide; (2) Michael addition of GSH on HBQs; and (3) nucleophilic substitution of the halo groups of HBQs by GSH. Unique desulfurized and disulfide GSH-DBBQ conjugates, and the substitution of the methyl group by GSH on 2,6-dichloro-3-methyl-(1,4)benzoquinone (DCMBQ) were also observed. Finally, the conjugates in HBQ-treated HepG2 cells were identified to be the same as those produced in the reaction of (5:1) GSH/HBQs.

This dissertation confirms HBQs as a group of emerging DBPs and provides the occurrence data and the mechanisms of the formation and transformation of HBQs during water treatment. Evaluation of current treatments for removal of HBQ precursors provides information for DWTPs to control these DBPs. The analytical characterization combined with toxicity evaluation of HBQs stresses the importance of monitoring these DBPs compared to the regulated DBPs. The highly sensitive and specific analytical tools developed here enable future research on assessment of human exposure and health risk of HBQs. The research methodologies are useful for discovery of other potentially important DBPs.

PREFACE

Parts of Chapter 2 have been published as Wang, W.; Qian Y.; Boyd, J.M.; Wu, M.; Hrudey, S.E.; Li, X-F. Halobenzoquinones in swimming pool waters and their formation from personal care products. *Environ. Sci. Technol.* **2013**, 47(7), 3275-3282. Copyright (2013) with permission from American Chemical Society.

Some of the research conducted for Chapter 3 was part of a research collaboration with Professor R. Andrews's group at the University of Toronto. They collected and coagulated source water samples, and performed LC-OCD analysis. I was responsible for the chlorination reaction, LC-MS analysis and PCA data analysis. Parts of this chapter have been published as Diemert, S.; Wang, W.; Andrews, R.C.; Li, X-F. Removal of halo-benzoquinone (emerging disinfection by-product) precursor material from three surface waters using coagulation. *Water Res.* **2013**, 47 (5), 1773-1782. Copyright (2013) with permission from Elsevier.

Parts of Chapter 4 have been published as Wang, W.; Qian, Y.; Jmaiff, L.K.; Krasner, S.W.; Hrudey, S.E.; Li, X-F. Precursors of halobenzoquinones and their removal during drinking water treatment processes. *Environ. Sci. Technol.* **2015**, 49(16), 9898-9904. Copyright (2013) with permission from American Chemical Society.

Parts of Chapter 5 have been published as Wang, W.; Qian, Y.; Li, J.; Moe, B.; Huang, R.; Zhang, H.; Hrudey, S.E.; Li, X-F. Analytical and toxicity Characterization of halo-hydroxyl-benzoquinones as stable halobenzoquinone disinfection byproducts in treated water. *Anal. Chem.* **2014**, 86 (10), 4982-4988. Copyright (2014) with permission from American Chemical Society.

The experiment of electron spin resonance for Chapter 6 was conducted with help from Professor Arno Siraki's group at the University of Alberta.

ACKNOWLEDGEMENTS

I'd like to express my sincere gratitude to all who have helped, supported and encouraged me over the past five years.

First, I want to thank my supervisor, Dr. Xing-Fang Li. She herself is an excellent example for me, succeeding in balancing both family life and academic research as a female professor. Thank you for your invigorating encouragement and thorough training, helping me pursue my academic career, and kind suggestions for sound management of my personal life.

I'd also like to thank my co-supervisor, Dr. X. Chris Le. Thank you for always sending me in the right direction when I was puzzled by details, and for your great patience and consistent support, helping me to progress more than I otherwise would. I would also like to thank my mentor, Dr. Steve E. Hrudey, who always reminded me to look at the big picture and think about the far-reaching significance of my work.

The members of the AET Division have contributed immensely to my personal and professional life over the past five years. The group has been a constant source of camaraderie, friendship, and collaboration. I am especially grateful for the Li and Le groups who always gave me valuable suggestions on my research, and accompanying me in daily life like a family. I'd like to thank Ms. Katerina Carastathis for her substantial help, editing my manuscripts and ordering chemicals. Thank you to Ms. Dianne Sergy for all the help on the registration and document work. I'd like to thank Dr. Hongquan Zhang for insightful suggestions on my experiments and manuscripts; thank you to Dr. Yuli Zhao, Dr. Rongfu Huang, Dr. Jessica Boyd and Ms. Xiufen Lu on training me in instrument operations; thank you to Jinhua Li, Dr. Birget Moe and Lindsay Jmaiff for all the substantial collaboration you have done with me on my work. Thanks for help from Dr. Minghuo Wu, Dr. Beibei Chen, Dr. Yanming Liu, Dr. Zhixin Wang, Dr. Feng Li, Dr. Chuan Wang, Dr. Xiaowen Yan, Dr. Hanyong Peng, Dr. Lingling Yang, Dr. Yanan Tang, Qi Zhang, Qingqing Liu, Yanwen Lin, Xuan Sun, Katherine Fu, and Ian Vander Meulen.

My thanks and appreciation also goes out to Dr. Monika Keelan and Ms. Cheryl Titus for their constructive guidance on my professional development, leadership ability,

and network skills. Thanks to Dr. Arno Siraki and Dr. Derrick Clive for their help on my experiment. Thanks for continuous encouragement from Professsor Lizhong Zhu.

Finally and most importantly, I'd like to thank my husband, Dr. Yichao Qian, who gave me infinite love, support, help, encouragement, and company. Thank you for being my lover, friend, partner, mentor, and refuge. Thanks to my beloved parents and parents-in-law for their boundless encouragement and understanding. You are always the source of strength and faith that raises me up to go forward.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

1.1 DRINKING WATER SAFETY

Access to clean drinking water is the most basic and essential human right to guarantee health all around the world. The World Health Organization (WHO) defines "drinking-water safety" including "minimum procedures and specific guideline values and how these are intended to be used".[1] The concept and content of "safety" necessarily evolves with the emergence of new knowledge and subsequent requirements for "safety". The WHO published the first edition of the WHO International Standards for drinking water in 1958, and further edited them in 1963, 1971, 1983, 1993, and 2004. To date, 90% of the world's population has gained access to clean drinking water. However, nearly 663 million people worldwide are still unable to access clean water and 842000 global death per year is because of waterborne diseases.[2,3]

1.2 DRINKING WATER TREATMENT

Waterborne pathogens represent a great risk to human health. Because of this, disinfection of drinking water is necessary to remove, deactivate or kill pathogenic microorganisms, limiting waterborne diseases. Chlorine disinfection has been successfully used for over a century since its first use in Middelkerke, Belgium in 1902. Today chlorination is still the most widely used disinfection method. However, it has been found that some microorganisms, such as *Cryptosporidium*, are resistant to chlorine. In addition, disinfection byproducts (DBPs) are unintentionally produced from the reaction between chlorine, natural organic matter (NOM) and other substances. Long-term consumption of chlorinated drinking water has been associated, not necessarily

causally, with an increased incidence of bladder cancer[4–7] and adverse reproductive outcomes.[8–11]

In addition to chlorination, other techniques are applied as alternative or supplementary disinfection. Chloramines are often employed as a secondary disinfection step because they provide longer-lasting residual protection against pathogens. However, some non-halogenated DBPs, such as nitrosamines,[12] are formed after chloramine disinfection. In addition, chloramination has been found to induce the release of lead from pipes.[13,14] Another alternative, chlorine dioxide is an effective agent against protozoa (e.g., *Cryptosporidium* and *Giardia*) and other pathogens, as well as producing fewer halogenated DBPs compared with chlorination.[15] However, chlorite, which can be toxic to blood cells, is produced in the disinfection process.[16] Ozone, a strong oxidizing agent, can be applied as the primary disinfectant, but lacks any residual protection.[17] Ultraviolet (UV) irradiation is an effective supplementary disinfection method to deactivate chlorine-resistant microorganisms (e.g. *Cryptosporidium parvum* oocysts) and decrease chemical input.[18] But UV may result in the photochemical transformation of contaminants, and UV lacks lasting effectiveness.[19]

Because no alternative/combined disinfection method has been found to eliminate the formation of DBPs, efforts are also made to remove DBP precursors through physical-chemical treatment processes, such as filtration, coagulation, adsorption, and disinfection.[14] Coagulation is used to remove particles suspended in water by the addition of alum/iron salts or other chemicals, which is generally more effective to remove large molecular weight, hydrophobic components of NOM.[20] Filtration physically removes solid material from water by passing water through sand or other porous materials.

Granular activated carbon can remove contaminants through physical adsorption or biodegradation.[21,22] These techniques can partially remove, but not eliminate DBP precursors.[23,24]

1.3 DISINFECTION BYPRODUCTS (DBPS)

Four trihalomethanes (THMs), chloroform, bromodichloromethane, dibromochloromethane, and bromoform, were the first DBPs identified by Rook and by Bellar et al. in 1974.[25,26] In the next year, a national survey in the U.S. confirmed the widespread presence of THMs in drinking water. In 1976, Symons reported the carcinogenic properties of chloroform, based on the result of a two-year exposure study on rodent cancer bioassay.[27] In 1980, Quimby discovered haloacetic acids (HAAs) in drinking water.[28] In the decades that followed, THMs and HAAs have been recognized as the two major classes of DBPs because of their widespread occurrence and high concentrations.[29]

The discovery of DBPs led to regulations for limiting exposure to these chemicals on precautionary grounds.[30] Canada first regulated the maximum total THMs at 350 µg/L in 1978.[31] In the next year, the U.S. set the maximum running annual average level of THMs at 100 µg/L. The US EPA set the enforceable maximum contaminant level (MCL) for total THMs at 80 µg/L, and for total level of HAAs (HAA_5) at 60 µg/L in Stage 2 of the Disinfectant and Disinfection By-product Rule.[32] Health Canada set the guideline value of both total THMs and HAA_5 at 80 µg/L.[33] However, animal toxicity studies found that these regulated DBPs do not fully account for the increased risk of disease posed by DBPs in epidemiological studies.[34] Because of this discrepancy, recent efforts have been

made to identify DBPs of toxicological significance to explain the epidemiologically observed bladder cancer risk related to drinking water.[35]

To date, more than six hundred DBPs have been discovered due to the advanced analytical techniques developed.[36] In addition to the most abundant DBPs-THMs and HAAs-the scope has been expanded to include nitrosamines, halonitronmethanes, haloacetronitriles, haloketones, haloaldehydes, haloacetates, and halomides, to name a few. However, reported DBPs only account for approximately 40% of the total halogen compounds in drinking water according to the analysis of total organic carbon (TOC) and total organic halide (TOX),[37] suggesting that a large portion of DBPs remains unknown. Identification of toxicologically relevant DBPs is currently a critical issue that requires further research.

1.4 HALOBENZOQUINONES (HBQS) AS DBPS

1.4.1 Quantitative structure toxicity relationship (QSTR)

A quantitative structure toxicity relationship (QSTR) analysis examined the toxicological data of 489 established and novel DBPs. Four groups of compounds: (1) halobenzoquinones (HBQs), (2) halocylcopentenoic acids, (3) N-haloamines, and (4) N-nitrosamines and nitrosamides were prioritized for DBP research because of the low values of their lowest observed adverse effect level (LOAEL).[35] The LOAEL of HBQs are predicted to be in the low μg/kg day range, and several HBQs were predicted to be carcinogenic.[38] It is widely accepted that compounds with structures similar to HBQs, such as benzoquinones (BQs), interact through multiple pathways, such as redox reactions, alkylation and free radical reactions with a variety of biologically active molecules (e.g., DNA or proteins), causing undesirable effects.[35]

1.4.2 Toxicity of HBQs

HBQs were first confirmed as an emerging class of DBPs in 2010.[39] Four HBQs, 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ), 2,3,6-trichloro-1,4-benzoquinone (TriCBQ), and 2,6-dibromo-1,4-benzoquinone (2,6-DBBQ), were identified in treated tap water at ng/L levels.[39,40]

A few *in vitro* studies have been conducted regarding the cytotoxicity and the mechanism of toxicity of HBQs. HBQs have been found to be cytotoxic to T24 bladder cancer cells, with 24-h IC_{50} values determined to be 95 μM for 2,6-DCBQ, 110 μM for DCMBQ, 151 μM for TriCBQ, and 142 μM for 2,6-DBBQ.[41] The cytotoxicity of HBQs is hypothesized to be due to the formation of reactive oxygen species (ROS), resulting in oxidative damage to DNA and proteins. It was found that exposure to HBQs produced increased levels of 8-hydroxydeoxyguanosine (8-OHdG) and protein carbonylation in T24 cells, indicating oxidative damage to genomic DNA and proteins.[41] Furthermore, HBQ exposure depleted cellular glutathione (GSH) in cells. The depletion of GSH by other chemicals was found to sensitize cells to HBQs, while extracellular GSH supplementation could reduce HBQ-induced cytotoxicity, emphasizing the role of GSH and GSH-related enzymes in the detoxification of ROS after HBQ exposure.[42] These findings correlate strongly to the toxic hazards of quinones observed in organisms, particularly those involved in disrupting protein handling systems.[43] *In vivo* studies to confirm the toxic effects observed in *in vitro* studies have not yet been conducted. In addition, further investigation into molecular mechanisms of the toxicity of HBQs and assessment of human exposure are necessary to better understand the health risks of HBQ exposure through drinking water.

1.4.3 Analytical characterization of HBQs

1.4.3.1 Benzoquinone-related chemistry

The basic structure of HBQ compounds consists of halogen and/or alkyl and hydroxyl groups on a benzoquinone (BQ) ring (Figure 1.1). To understand the chemical properties of HBQs, it is important to first discuss the unique chemical properties of BQ, particularly its related redox and addition chemistry.

The reversible redox property of BQ is well known.[44] BQ can undergo two-electron reduction to hydroquinone (HQ) or be converted via one-electron reductions into semiquinone radicals ($SQ^{\bullet-}$) and HQ in two sequential steps. HQ can be oxidized back to BQ by molecular oxygen. Thus, in aqueous solution BQ has six redox states through the electrochemical processes of its two electrons and two protons.[45] The six redox states of BQ have been shown to be analogous for HBQ (Figure 1.1).

The second key feature of BQ chemistry is its ability to undergo the nucleophilic addition reaction, Michael addition.[46,47] Michael addition is the 1,4-addition of a doubly stabilized carbon nucleophile to an α,β-unsaturated carbonyl compound. It involves the addition of a nucleophile to the —C=C— bond and delocalization of the charge among the electronegative elements to form an anion. The addition of oxygen nucleophiles such as H_2O_2 or O_2 to BQ forms a quinone epoxide. Alternatively, a reduction process occurs when the addition of sulfur nucleophiles forms a thioether derivative.

1.4.3.2 Chemical properties of HBQs

To illustrate the redox reactions of HBQs, we may consider Figure 1.1 to replace any of R_1, R_2, R_3, and R_4 with halogen groups and to show the reversible conversion between HBQ, halosemiquinone (HSQ), and halohydroquinone (HHQ). Although HBQs share

many chemical properties with BQ, a key distinctive feature of HBQs is the halogen and/or alkyl and hydroxyl substitution groups mentioned above. The substitution groups of HBQs define many of their chemical properties and have been shown to affect electron distribution (polarity) and half-wave potential, as well as equilibrium acid-base (pK$_a$) and redox (E^0) properties.[48] Generally, the presence of electron-withdrawing groups, such as halo groups, increases the one-electron reduction potential, decreases the pK$_a$, and elevates the E^0 (BQ/HQ). In contrast, electron-donating groups, such as methyl groups, induce opposite trends. As a result, these distinct chemical properties have become the basis of the analytical characterization of HBQs. Table 1.1 summarizes some of the main chemical properties of four HBQs. Conventional analytical techniques, such as UV-vis spectroscopy, electron spin resonance, and GC-MS, and the more advanced analytical techniques, LC-MS, are discussed below.

1.4.4 Early analytical techniques for HBQ analysis

1.4.4.1 UV-visible spectroscopy

BQ exhibits unique UV-vis absorption from two types of well-defined transitions: 1) an n-π* transition which results in a low intensity absorption at 476 nm; and 2) the π -π* transitions at 278 nm and 244 nm. The absorption at 244 nm is strong and is selected as the characteristic absorption wavelength (λ_{max}), while the transition at 278 nm is forbidden by the symmetry of the structure.[49] Derivatives with electron donors in the quinoid ring cause a hypochromatic shift because of charge migration from the donor group into the quinoid ring.[50] Accordingly, electron-withdrawing groups in the quinoid ring cause bathochromic displacements. The characteristic absorption of hydroxyl-benzoquinone is at 520 nm because of the bathochromic effect of its hydroxyl

substituent.[51] The λ_{max} of HBQs are summarized in Table 1.2. It is important to note that slight changes in the absorption maxima can occur due to variations in pH or in the ionic strength of the media. UV-visible absorption spectroscopy has been used to monitor the transformation of HBQs, and the interactions of HBQs with other molecules in real time.[52–56]

1.4.4.2 Electron spin resonance (ESR)

An important chemical feature of quinones is the ability to undergo reversible redox cycling without breaking the quinone/quinol ring. Halosemiquinone free radicals ($HSQ^{\bullet-}$) are formed as a relatively stable intermediate in the HBQ/halohydroquinone (HH_2Q) inter-conversion.[52,55] Electron spin resonance (ESR), also called electron paramagnetic resonance (EPR), has been used to identify $HSQ^{\bullet-}$ by measuring the g value (the splitting factor), hyperfine structure, and line shape of HBQs. The g value for a free electron is 2.00232 and most radicals have g values very close to 2.0. To confirm the formation of a free radical, the hyperfine structure and line shapes of ESR spectra representing the magnetic effects of atomic nuclei to the unpaired electron are used. Table 1.3 presents the characteristics of the ESR spectra of HBQs in various solvents.[57]

Formation of hydroxyl ($OH^{\bullet}$) free radicals in HBQ-involved reactions has been reported, and their formation is considered to be an important mechanism of HBQ carcinogenicity.[58–61] ESR spin trapping uses a trap reagent to capture reactive radicals and produce a long-lived radical for detection. The transient $OH^{\bullet}$ radicals are captured by 5,5-dimethyl-1-pyrroline N-oxide (DMPO). More conclusive evidence for $OH^{\bullet-}$ production is necessary by the inhibition of the DMPO/$OH^{\bullet}$ signal in the presence of known signal quenchers, dimethyl sulfoxide (DMSO) or formate.[62] Rapid freeze quenching (RFQ)

generates the radicals in a frozen transparent solid matrix to prevent collision or reaction of $HSQ^{\bullet}$ and $OH^{\bullet}$ radicals with other species, thus it can increase the opportunity to hunt transient radicals.[55]

1.4.4.3 Gas chromatography–mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) with electron ionization (EI) and chemical ionization (CI) methods have been applied in the determination of HBQs.[63-66] The positive-ion EI mass spectra of BQs show two characteristic features: 1) the stepwise loss of two molecules of carbon monoxide, generating $[M-CO]^{+\bullet}$ and $[M-2CO]^{+\bullet}$ and 2) the occurrence of both $[M]^{+\bullet}$ and $[M+2]^{+\bullet}$ ions.[49]

Some HBQs are nonvolatile or thermally unstable, and are therefore not amenable to direct GC-MS analysis. Hence, derivatization of these HBQs is required prior to GC-MS analysis. HBQ analogues (tert-butylhydroquinone, 2-tert-butyl-1,4-benzoquinone, 2-tert-butyl-5 or 6-hydroxy-1,4-benzoquinone) were converted to tert-butyldimethylsilyl derivatives by addition of N-methyl-N-(tert-butyldimethysilyl)-trifluorocetamide (MTBSTFA).[63,64] Other reagents have also been reported. For example, 1,2-phenylenediamine has also been used to derivate 1,4-quinones to phenazine derivatives;[67,68] heptafluorobutyrylimidazole (HFBI) is used to derivate HBQs and HBQ-protein adducts to heptafluorobutyryl derivatives[65,66,69-71] or converted 1-4 ring quinones to diacetyl derivatives.[72] The identification of HBQs by GC-MS is based on identical retention time and selective ion monitoring (SIM) with synthesized standards. Quantification is based on peak area relative to the isotopically-labelled internal standards[61,65] or their analogues.[72] The measured SIM ions, derivatives, and internal standards for GC-MS analysis of HBQs are listed in Table 1.4.

To date, the GC-MS analytical techniques have limited application in analysis of few HBQs as the accessory of phenol compounds. For example, Heasley et al. found the formation of 2,6-DCBQ and other products after chloramination of phenol and m-cresol using GC-MS.[73] Although GC-MS has been shown to be a promising technique for analyzing some HBQs in drinking water, its direct use is limited to (semi-)volatile, thermally-stable, non-ionic, and non-polar HBQs. Derivatization of the analytes could help overcome these obstacles; however, this makes the complicated procedures time-consuming and may introduce background interference, limiting quantification. Furthermore, the low resolution of GC-EI-MS also limits the application of GC-MS to identify new HBQ DBPs.[74]

1.4.5 LC-ESI-MS characterization and determination of HBQs

Due to the limited applicable scope of GC-MS analysis mentioned above, high performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) has become the analytical technique of choice for ——HBQs. HPLC-MS/MS techniques have been shown to be highly sensitive and accurate. Electrospray ionization (ESI) is a soft-ionization technique that can be used for thermally labile compounds and stable compounds. It is currently one of the most efficient ionization techniques being used for HPLC-MS/MS, enabling the detection of analytes of a wide range of molecular weights and polarity.[75] Here, we will discuss the HPLC-ESI-MS/MS methods developed for analysis of HBQ DBPs.

1.4.5.1 Electrospray ionization (ESI) of HBQs

HBQs have shown distinctive ESI spectra, which is related to the unique electrochemical properties of BQ-HQ couples. Hence, understanding ESI pathways is essential to

developing a reliable LC-MS method for HBQ analysis. Both ESI and atmospheric pressure chemical ionization (APCI) in either positive or negative modes were examined for HBQ analysis. This study identified that ESI-MS in negative mode can provide better sensitivity and more reproducible signals.[39] The four HBQs in this initial study were 2,6-DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ.[39] Unlike what is commonly observed with ESI-MS ionizations, the $[M+1]^+$ ions in positive mode and $[M-1]^-$ ions in negative mode were not reproducible and had weak intensity; instead, stable $[M+H]^-$ ions were obtained under negative ESI when the solutions were supplemented with 0.25% formic acid. Because the pK_a of the four HBQs are estimated to be between 5.8 and 6.3, the predominant species of HBQs in acidic solution are neutral molecules. Hence, under negative ESI conditions, both C=O groups were reduced to C-OH, producing the [M+2H] intermediate, which was followed by the rapid deprotonation of [M+2H] to produce $[M+H]^-$. This is consistent with the electrochemistry of the quinone couples: quinone + $2H^+$ + 2e = dihydroquinone (0.70 V).

To confirm the identity of the $[M+H]^-$ species, quadrupole time-of-flight (QToF) spectrometry was used to measure the accurate mass of the parent and product ions of these HBQs. Accurate mass measurements of the most abundant ions were consistent with the theoretical values of $[M+H]^-$. Tandem mass spectra showed that the major product ions for the four HBQs were $[M+H-HX]^-$, $[M+H-HX-CO]^-$, $[M+H-CO]^-$, and/or X^- (where X represents Cl or Br), supporting the identification of $[M+H]^-$.[40]

1.4.5.2 HPLC separation

To facilitate ESI-MS detection, HBQs are separated using reverse-phase chromatography. Octadecyl carbon chain (C18) bonded silica has been most commonly applied to separate

HBQs in several studies.[39,40,53,58,76–80] In addition to C18 columns, columns packed with octa carbon chain-bonded (C8) silica, phenyl-bonded silica, or graphite carbon, have also been examined to separate four HBQs (2,6-DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ). Among these columns, the C18 column provided the best separation.[39] Mobile phase composition included either methanol/water[40,76] or acetonitrile/water.[77,78,79] Formic acid is often added in the mobile phase to stabilize the HBQs and to enhance ionization.[39,40,76-79]

1.4.5.3 MS analysis

MS/MS coupled to HPLC is commonly used to continuously acquire mass-to-charge ratio (m/z) and fragment data for the determination of eluent composition in real time. Multiple reaction monitoring (MRM) or selective reaction monitoring (SRM) methods measure specific ion transitions of a compound instead of scanning an m/z range. Hence, these MRM and SRM methods spend more time on acquiring signals of specific ion transitions of HBQs and eliminate background, resulting in enhancement of their signals and thus sensitivity of HBQ quantitation. For each compound, the two most stable and abundant ion transitions are measured. The instrument parameters, including declustering potential (DP), entrance potential (EP), and collision energy (CE), are optimized for each ion transition. Identification of a compound is based on the consistent relative ratio of the two ion transitions as well as identical retention times. Quantification is based on the peak area of the most intense ion transition. MRM methods can also largely reduce isobaric interference, and enhance selectivity, sensitivity, and reproducibility. In 2010, Li's group developed MRM methods for four HBQs (2,6-DCBQ, DCMBQ, TriCBQ and 2,6-DBBQ) using triple quadrupole ion-trap (QTrap) MS/MS.[40] The standard addition method was used to rectify matrix effects, as no isotope-labeled internal standards are

available for these HBQs. The detection limits of the HPLC-MS/MS methods for the sixteen compounds were in the range of 0.5 to 1.9 ng/mL.

1.4.6 Analysis of drinking water using LC-MS/MS

1.4.6.1 Sample preparation

Because HBQs are unstable in water, it is essential to stabilize water samples immediately after collection. To this end, formic acid and ascorbic acid, two commonly used preservatives for drinking water samples, are added to both quench free chlorine in drinking water and to stabilize HBQs.[76] Formic acid (0.25%, v/v) was found to be a superior preservative, and could maintain the stability of HBQs in treated drinking water for at least 5 days.[39]

Because HBQ DBPs are typically detected at ng/L levels, Qin and Zhao used solid phase extraction (SPE) using Oasis HLB cartridges to extract HBQs in drinking water.[39] In this study, methanol showed a 5-time higher elution efficiency of HBQs than acetyl acetate, and the optimized washing solvent was determined to be 10 mL of water and methanol (v/v, 50/50). By coupling the HPLC-MS/MS method with SPE to concentrate the HBQs in 500 mL of drinking water into a 0.5 mL final solution, the quantification limits (LOQ) were between 1 and 6 ng/L and the recovery was in the range of 69 to 84% for the four tested HBQs.[40]

1.4.6.2 Occurrence of HBQs in drinking water

Analysis of HBQs in both raw water and drinking water is essential to confirm their occurrence frequency and concentrations as DBPs, and to this end, LC-MS techniques have been valuable. Qin et al. tested the occurrence of four HBQs in drinking water samples from six locations in two drinking water treatment plants (DWTPs) and drinking

water distribution systems (DWDSs) that both disinfected water using chloramination and UV irradiation.[39] Only 2,6-DCBQ was confirmed as a DBP, which occurred exclusively in the drinking water after disinfection. The concentration of 2,6-DCBQ in these drinking water samples was between 14.3–54.6 ng/L in Plant 1 and 5.3–14.3 ng/L in Plant 2. In both DWDSs, the concentration of 2,6-DCBQ decreased with increasing distance from the treatment plant. Zhao et al. further analyzed raw and treated water samples from a third DWTP that used chlorination as the disinfection method.[40] In addition to 2,6-DCBQ, which was found at high concentrations (165 ng/L), the other three tested HBQs (DCMBQ, TriCBQ, and 2,6-DBBQ) were also identified as new DBPs at relatively low concentrations ranging from 0.5 to 9.1 ng/L.

1.5 RATIONALE AND SCOPE OF THESIS

Water disinfection is necessary for killing or inactivating pathogens. However, it can create an unintended chemical risk through the formation of DBPs. Epidemiological studies have shown a consistent association between the consumption of chlorinated water and an increased risk of bladder cancer. To date, it is not known what specific chemicals in the disinfected water may contribute to the possible adverse health effects. The regulated DBPs, THMs and HAAs, are safely excluded as the culprit after more than 40 years of research on DBPs. This signifies the importance of the identification of toxicologically relevant DBPs.

HBQs were predicted to be potential DBPs of toxicity relevance based on their chemical structure. At the beginning of my PhD study, the LC-MS/MS method for analysis of HBQs in drinking water was limited to only four compounds. The four HBQs were discovered to be DBPs in drinking water, and confirmed to be highly cytotoxic

through the formation of ROS and dysfunction of the antioxidant system. My hypothesis is that there are more HBQs produced from chlorination of water. To confirm my hypothesis, I proposed to develop a LC-MS/MS method for analysis of more HBQs. The method can be used to identify more HBQs in disinfected water, and to demonstrate the wide occurrence of HBQs. In addition, it is unclear how HBQs are formed and transformed. I proposed to examine the formation and transformation mechanism of HBQs in laboratory experiments and field studies of drinking water treatment plants and distribution systems. Furthermore, I will apply the results from these studies to evaluate the effects of water treatment processes on the control of HBQs in drinking water. Finally, to understand the toxicological relevance, I will study the interaction of HBQs with biomolecules (specifically GSH) because of preliminary observations of the effects of HBQs on GSH. The specific objectives of my thesis research were as follows:

1. Develop a sensitive solid phase extraction–liquid chromatography–tandem mass spectrometry (SPE-LC-MS/MS) to analyze more HBQ species, and use the method to identify new HBQs as DBPs in swimming pool water and drinking water (Chapter 2);

2. Identify precursors of HBQs by examining the formation of HBQs from NOM and anthropogenic material, including urine, body lotion, and sunscreens (Chapter 3);

3. Investigate the effects of drinking water treatment techniques, including coagulation, filtration, granular activated carbon, ozonation, and ultraviolet irradiation, on the removal of HBQ precursors (Chapter 4);

4. Explore the transformation pathways of HBQs both in laboratory-controlled experiments and drinking water distribution systems, and compare the cytotoxicity of HBQs and their transformation products (Chapter 5); and

5. Examine the conjugation of HBQs with GSH in aqueous solution and HepG2 cells (Chapter 6).

The anticipated outcomes of my research will be to provide new knowledge about HBQ DBPs, new analytical methodologies for monitoring of HBQs, and to provide possible approaches to control and eliminate HBQs in drinking water.

1.6 REFERENCES

1. World Health Organization (WHO). *Guidelines for drinking-water quality*,.4th ed.; 2011. http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/. Accessed: Jan 5, 2016.
2. World Health Organization (WHO). Progress on sanitation and drinking water - 2015 update and MDG assessment. New York, NY, 2015.
3. World Health Organization (WHO). Water-related diseases. http://www.who.int/water_sanitation_health/diseases/en/. Accessed: September 15, 2015.
4. Hrudey, S. E., Backer, L. C., Humpage, A. R., Krasner, S. W., Michaud, D. S., Moore, L. E., Singer, P. C., Stanford, B. D. Evaluating evidence for association of human bladder cancer with drinking-water chlorination disinfection by-products. *J Toxicol Environ Health B Criti Rev* **2015**, *18* (5), 213–241.
5. King, W. D.; Marrett, L. D.; Woolcott, C. G. Case-control study of colon and rectal cancers and chlorination by-products in treated water. *Cancer Epidemiol Biomarkers Prev* **2000**, *9* (8), 813–818.
6. Villanueva, C. M.; Cantor, K. P.; Grimalt, J. O.; Malats, N.; Silverman, D.; Tardon, A.; Garcia-Closas, R.; Serra, C.; Carrato, A.; Castano-Vinyals, G.; Marcos, R.; Rothman, N.; Real, F. X.; Dosemeci, M.; Kogevinas, M. Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *Am J Epidemiol* **2007**, *165* (2), 148–156.

7. Hrudey, S. E. Chlorination disinfection by-products, public health risk tradeoffs and me. *Water Res* **2009,** *43* (8), 2057–2092.
8. Waller, K.; Swan, S. H.; DeLorenze, G.; Hopkins, B. Trihalomethanes in drinking water and spontaneous abortion. *Epidemiology* **1998**, *9* (2), 134–140.
9. Hunter, E. S.; Rogers, E. H.; Schmid, J. E.; Richard, A. Comparative effects of haloacetic acids in whole embryo culture. *Teratology* **1996**, *54* (2), 57–64.
10. Nieuwenhuijsen, M. J.; Toledano, M. B.; Eaton, N. E.; Fawell, J.; Elliott, P. Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review. *Occup Environ Med* **2000**, *57* (2), 73–85.
11. Colman, J.; Rice, G. E.; Wright, J. M.; Hunter, E. S.; Teuschler, L. K.; Lipscomb, J. C.; Hertzberg, R. C.; Simmons, J. E.; Fransen, M.; Osier, M.; Narotsky, M. G. Identification of developmentally toxic drinking water disinfection byproducts and evaluation of data relevant to mode of action. *Toxicol Appl Pharmacol* **2011**, *254* (2), 100–126.
12. Mitch, W. A.; Sedlak, D. L. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environ Sci Technol* **2002**, *36* (4), 588–595.
13. Liu, H. Z.; Korshin, G. V.; Ferguson, J. F. Interactions of Pb(II)/Pb(IV) solid phases with chlorine and their effects on lead release. *Environ Sci Technol* **2009**, *43* (9), 3278–3284.
14. Sedlak, D. L.; von Gunten, U. The chlorine dilemma. *Science* **2011**, *331* (6013), 42–43.
15. Huber, M. M.; Korhonen, S.; Ternes, T. A.; von Gunten, U. Oxidation of pharmaceuticals during water treatment with chlorine dioxide. *Water Res* **2005**, *39* (15), 3607–3617.
16. Condie, L. W., Toxicological problems associated with chlorine dioxide. *J Am Water Works Assoc* **1986**, *78* (6), 73–78.
17. von Gunten, U. Ozonation of drinking water: Part I. Oxidation kinetics and product formation. *Water Res* **2003**, *37* (7), 1443–1467.
18. Hijnen, W. A. M.; Beerendonk, E. F.; Medema, G. J. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Res* **2006**, *40* (1), 3–22.

19. Canonica, S.; Meunier, L.; Von Gunten, U. Phototransformation of selected pharmaceuticals during UV treatment of drinking water. *Water Res* **2008**, *42* (1-2), 121–128.
20. Collins, M. R.; Amy, G. L.; Steelink, C. Molecular-weight distribution, carboxylic acidity, and humic substances content of aquatic organic-matter–implications for removal during water-treatment. *Environ Sci Technol* **1986**, *20* (10), 1028–1032.
21. Chen, W.; Duan, L.; Zhu, D. Q. Adsorption of polar and nonpolar organic chemicals to carbon nanotubes. *Environ Sci Technol* **2007**, *41* (24), 8295–8300.
22. Farre, M. J.; Reungoat, J.; Argaud, F. X.; Rattier, M.; Keller, J.; Gernjak, W. Fate of N-nitrosodimethylamine, trihalomethane and haloacetic acid precursors in tertiary treatment including biofiltration. *Water Res* **2011**, *45* (17), 5695–5704.
23. Jacangelo, J. G.; Demarco, J.; Owen, D. M.; Randtke, S. J. Selected processes for removing NOM–an overview. *J Am Water Works Assoc* **1995**, *87* (1), 64–77.
24. Matilainen, A.; Vepsalainen, M.; Sillanpaa, M. Natural organic matter removal by coagulation during drinking water treatment: a review. *Adv Colloid Interfac* **2010**, *159* (2), 189–197.
25. Rook, J. J. Formation of haloforms during chlorination of natural waters. *J Water Treat Exam* **1974**, *23*, 234–243.
26. Bellar, T. A.; Lichtenberg, J. J.; Kroner, R. C. Occurrence of organohalides in chlorinated drinking waters. *J Am Water Works Assoc* **1974**, *66* (12), 703–706.
27. Symons, J. M.; Bellar, T. A.; Carswell, J. K.; Demarco, J.; Kropp, K. L.; Robeck, G. G.; Seeger, D. R.; Slocum, C. J.; Smith, B. L.; Stevens, A. A. National organics reconnaissance survey for halogenated organics. *J Am Water Works Assoc* **1975**, *67* (11), 634–647.
28. Quimby, B. D.; Delaney, M. F.; Uden, P. C.; Barnes, R. M. Determination of the aqueous chlorination products of humic substances by gas-chromatography with microwave emission detection. *Anal Chem* **1980**, *52* (2), 259–263.
29. Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.; Sclimenti, M. J.; Onstad, G. D.; Thruston, A. D. Occurrence of a new generation of disinfection byproducts. *Environ Sci Technol* **2006**, *40* (23), 7175–7185.

30. Itoh, S.; Gordon, B. A.; Callan, P.; Bartram, J. Regulations and perspectives on disinfection by-products: importance of estimting overall toxicity. *J Water Supply Res T* **2011**, *60* (5), 261–274.
31. Hrudey, S. E.; Charrois, J. W. A. *Disinfection by-Products: Relevance to Human Health,* IWA Publishing: London, 2012.
32. United States Environmental Protection Agency (US EPA). *National Primary Drinking Water Regulations: Stage 2 Disinfectants and Disinfection Byproducts Rule; Final Rule*. 2006; Vol. 71, pp 388–493.
33. Health Canada. *Guideline for Canadian Drinking Water Quality*, Ontario, *Canada,* 6th ed; 1996. http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/sum_guide-res_recom/index-eng.php. Accessed: Jan 5, 2016.
34. Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; DeMarini, D. M. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat Res-Rev Mutat Res* **2007**, *636* (1-3), 178–242.
35. Bull, R. J.; Reckhow, D. A.; Rotello, V.; Bull, O. M.; Kim, J. *Use of Toxicological and Chemical Models to Prioritize DBP Research*; American Water Works Association Research Foundation and American Water Works Association: Denver, CO, 2006.
36. Richardson, S. D.; Ternes, T. A. Water analysis: emerging contaminants and current issues. *Anal Chem* **2014**, *86* (6), 2813–2848.
37. Richardson, S. D.; Ternes, T. A. Water analysis: emerging contaminants and current issues. *Anal Chem* **2011**, *83* (12), 4614–4648.
38. Bull, R. J.; Reckhow, D. A.; Li, X.-F.; Humpage, A. R.; Joll, C.; Hrudey, S. E. Potential carcinogenic hazards of non-regulated disinfection by-products: haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology* **2011**, *286* (1-3), 1–19.
39. Qin, F.; Zhao, Y. Y.; Zhao, Y. L.; Boyd, J. M.; Zhou, W. J.; Li, X.-F. A toxic disinfection by-product, 2,6-dichloro-1,4-benzoquinone, identified in drinking water. *Angew Chem Int Ed* **2010**, *49* (4), 790–792.

40. Zhao, Y. L.; Qin, F.; Boyd, J. M.; Anichina, J.; Li, X. F. Characterization and determination of chloro- and bromo-benzoquinones as new chlorination disinfection byproducts in drinking water. *Anal Chem* **2010**, *82* (11), 4599–4605.
41. Du, H. Y.; Li, J. H.; Moe, B.; McGuigan, C. F.; Shen, S. W.; Li, X. F. Cytotoxicity and oxidative damage induced by halobenzoquinones to T24 bladder cancer cells. *Environ Sci Technol* **2013**, *47* (6), 2823–2830.
42. Li, J. H.; Wang, W.; Zhang, H. Q.; Le, X. C.; Li, X. F. Glutathione-mediated detoxification of halobenzoquinone drinking water disinfection byproducts in T24 cells. *Toxicol Sci* **2014**, *141* (2), 335–343.
43. Xiong, R.; Siegel, D.; Ross, D. Quinone-induced protein handling changes: implications for major protein handling systems in quinone-mediated toxicity. *Toxicol Appl Pharmacol* **2014**, *280* (2), 285–295.
44. El-Najjar, N.; Gali-Muhtasib, H.; Ketola, R. A.; Vuorela, P.; Urtti, A.; Vuorela, H. The chemical and biological activities of quinones: overview and implications in analytical detection. *Phytochem Rev* **2011**, *10* (3), 353–370.
45. Gunner, M. R.; Madeo, J.; Zhu, Z. Modification of quinone electrochemistry by the proteins in the biological electron transfer chains: examples from photosynthetic reaction centers. *J Bioenerg Biomembr* **2008**, *40* (5), 509–519.
46. Uchimiya, M.; Stone, A. T. Reversible redox chemistry of quinones: impact on biogeochemical cycles. *Chemosphere* **2009**, *77* (4), 451–458.
47. Brunmark, A.; Cadenas, E. Redox and addition chemistry of quinoid compounds and its biological implications. *Free Radic Biol Med* **1989**, *7* (4), 435–477.
48. Namazian, M.; Coote, M. L. Accurate calculation of absolute one-electron redox potentials of some para-quinone derivatives in acetonitrile. *J Phys Chem A* **2007**, *111* (30), 7227–7232.
49. Zeller, K.-P. Mass spectra of quinones. In *The Chemistry of Quinonoid Compounds*, Wiley Interscience: 1974; Vol. 1, pp 231.
50. Gan, D.; Jia, M.; Vaughan, P. P.; Falvey, D. E.; Blough, N. V. Aqueous photochemistry of methyl-benzoquinone. *J Phys Chem A* **2008**, *112* (13), 2803–2812.
51. Gorner, H. Photoprocesses of p-benzoquinones in aqueous solution. *J Phys Chem A* **2003,** *107* (51), 11587–11595.

52. Lafis, S.; Konidari, C. N.; Veltsistas, P. G.; Tzerpos, N.; Karayannis, M. I. Kinetic study of the fast step of the alkaline hydrolysis of *p*-chloranil using stopped flow technique. *Int. J. Chem. Kinet.* **1997**, *29* (5), 385–391.
53. Sarr, D. H.; Kazunga, C.; Charles, M. J.; Pavlovich, J. G.; Aitken, M. D. Decomposition of tetrachloro-1,4-benzoquinone (*p*-chloranil) in aqueous-solution. *Environ Sci Technol* **1995**, *29* (11), 2735–2740.
54. Kot, M.; Olech, Z. Influence of 2,5-dichloro-1,4-benzoquinone on jack bean urease activity. Inhibitory effect, total reducing capacity and DPPH radical scavenging activity. *Acta Biochim Pol* **2011**, *58* (4), 627–633.
55. Franzen, S.; Sasan, K.; Sturgeon, B. E.; Lyon, B. J.; Battenburg, B. J.; Gracz, H.; Dumariah, R.; Ghiladi, R. Nonphotochemical base-batalyzed hydroxylation of 2,6-dichloroquinone by H_2O_2 occurs by a radical mechanism. *J Phys Chem B* **2012**, *116* (5), 1666–1676.
56. Hu, M. Q.; Wang, Y.; Xiong, Z. G.; Bi, D. Q.; Zhang, Y. H.; Xu, Y. M. Iodine-sensitized degradation of 2,4,6-trichlorophenol under visible light. *Environ Sci Technol* **2012**, *46* (16), 9005–9011.
57. Pedersen, J. A. *Handbook of EPR Spectra from Quinones and Quinols*. Boca Raton, Fla.: CRC Press, 1985.
58. Zhu, B.-Z.; Kalyanaraman, B.; Jiang, G.-B. Molecular mechanism for metal-independent production of hydroxyl radicals by hydrogen peroxide and halogenated quinones. *Proc Natl Acad Sci USA* **2007**, *104* (45), 17575–17578.
59. Zhu, B.-Z.; Shan, G.-Q. Potential mechanism for pentachlorophenol-induced carcinogenicity: a novel mechanism for metal-independent production of hydroxyl radicals. *Chem Res Toxicol* **2009**, *22* (6), 969–977.
60. Song, Y.; Buettner, G. R. Thermodynamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide. *Free Radical Bio Med* **2010**, *49* (6), 919–962.
61. Lin, P. H.; Waidyanatha, S.; Pollack, G. M.; Swenberg, J. A.; Rappaport, S. M. Dose-specific production of chlorinated quinone and semiquinone adducts in rodent livers following administration of pentachlorophenol. *Toxicol Sci* **1999**, *47* (1), 126–133.

62. Qin, H.; Huang, C.-H.; Mao, L.; Xia, H.-Y.; Kalyanaraman, B.; Shao, J.; Shan, G.-Q.; Zhu, B.-Z. Molecular mechanism of metal-independent decomposition of lipid hydroperoxide 13-HPODE by halogenated quinoid carcinogens. *Free Radical Bio Med* **2013**, *63*, 459–466.
63. Rodil, R.; Quintana, J. B.; Basaglia, G.; Pietrogrande, M. C.; Cela, R. Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography-mass spectrometry. *J Chromatogr A* **2010**, *1217* (41), 6428–6435.
64. Rodil, R.; Quintana, J. B.; Cela, R. Oxidation of synthetic phenolic antioxidants during water chlorination. *J Hazard Mater* **2012**, *199*, 73–81.
65. Lin, P. H.; Waidyanatha, S.; Rappaport, S. M. Investigation of liver binding of pentachlorophenol based upon measurement of protein adducts. *Biomarkers* **1996**, *1* (4), 232–243.
66. Lin, P. H.; Waidyanatha, S.; Pollack, G. M.; Rappaport, S. M. Dosimetry of chlorinated quinone metabolites of pentachlorophenol in the livers of rats and mice based upon measurement of protein adducts. *Toxicol Appl Pharmacol* **1997**, *145* (2), 399–408.
67. Valli, K.; Wariishi, H.; Gold, M. H. Degradation of 2,7-dichlorodibenzo-para-dioxin by the lignin-degrading basidiomycete phanerochaete-chrysosporium. *J Bacteriol* **1992**, *174* (7), 2131–2137.
68. Joshi, D. K.; Gold, M. H. Degradation of 2,4,5-trichlorophenol by the lignin-degrading basidiomycete phanerochaete-chrysosporium. *Appl Environ Microbiol* **1993**, *59* (6), 1779–1785.
69. Waidyanatha, S.; Lin, P. H.; Rappaport, S. M. Characterization of chlorinated adducts of hemoglobin and albumin following administration of pentachlorophenol to rats. *Chem Res Toxicol* **1996**, *9* (3), 647–653.
70. Tsai, C. H.; Lin, P. H.; Troester, M. A.; Rappaport, S. M. Formation and removal of pentachlorophenol-derived protein adducts in rodent liver under acute, multiple, and chronic dosing regimens. *Toxicol Sci* **2003**, *73* (1), 26–35.

71. Tsai, C. H.; Lin, P. H.; Waidyanatha, S.; Rappaport, S. M. Characterization of metabolic activation of pentachlorophenol to quinones and semiquinones in rodent liver. *Chem Biol Interact* **2001**, *134* (1), 55–71.
72. Lim, D.; Ikeda, A.; Vu, K. K. T.; Yamaguchi, K. T.; Tyner, T. R.; Hasson, A. S. Method development for the measurement of quinone levels in urine. *J Chromatogr B* **2011**, *879* (30), 3592–3598.
73. Heasley, V. L.; Fisher, A. M.; Herman, E. E.; Jacobsen, F. E.; Miller, E. W.; Ramirez, A. M.; Royer, N. R.; Whisenand, J. M.; Zoetewey, D. L.; Shellhamer, D. F. Investigations of the reactions of monochloramine and dichloramine with selected phenols: examination of humic acid models and water contaminants. *Environ Sci Technol* **2004**, *38* (19), 5022–5029.
74. Richardson, S. D. The role of GC-MS and LC-MS in the discovery of drinking water disinfection by-products. *J Environ Monit* **2002**, *4* (1), 1–9.
75. Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. Electrospray ionization for mass-spectrometry of large biomolecules. *Science* **1989**, *246* (4926), 64–71.
76. Zhao, Y.; Anichina, J.; Lu, X.; Bull, R. J.; Krasner, S. W.; Hrudey, S. E.; Li, X.-F. Occurrence and formation of chloro- and bromo-benzoquinones during drinking water disinfection. *Water Res* **2012**, *46* (14), 4351–4360.
77. Pan, Y.; Zhang, X. Four groups of new aromatic halogenated disinfection byproducts: effect of bromide concentration on their formation and speciation in chlorinated drinking water. *Environ Sci Technol* **2013**, *47* (3), 1265–1273.
78. Zhai, H.; Zhang, X.; Zhu, X.; Liu, J.; Ji, M. Formation of brominated disinfection byproducts during chloramination of drinking water: new polar species and overall kinetics. *Environ Sci Technol* **2014**, *48* (5), 2579–2588.
79. Zhai, H.; Zhang, X. Formation and decomposition of new and unknown polar brominated disinfection byproducts during chlorination. *Environ Sci Technol* **2011**, *45* (6), 2194–2201.
80. Olkowski, A. A.; Aranda-Osorio, G.; McKinnon, J. Rapid HPLC method for measurement of vitamin D-3 and 25(OH)D-3 in blood plasma. *Int J Vitam Nutr Res* **2003**, *73* (1), 15–18.

81. von Sonntag, J.; Mvula, E.; Hildenbrand, K.; von Sonntag, C., Photohydroxylation of 1,4-benzoquinone in aqueous solution revisited. *Chem-Eur J* **2004**, *10* (2), 440–451.
82. Zhu, B.-Z.; Zhu, J.-G.; Mao, L.; Kalyanaraman, B.; Shan, G.-Q. Detoxifying carcinogenic polyhalogenated quinones by hydroxamic acids via an unusual double Lossen rearrangement mechanism. *Proc Natl Acad Sci USA* **2010**, *107* (48), 20686–20690.
83. Zhu, B. Z.; Har-El, R.; Kitrossky, N.; Chevion, M. New modes of action of desferrioxamine: scavenging of semiquinone radical and stimulation of hydrolysis of tetrachlorohydroquinone. *Free Radic Biol Med* **1998**, *24* (2), 360–369.

Table 1.1 Chemical properties of HBQs

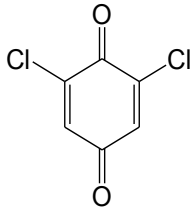
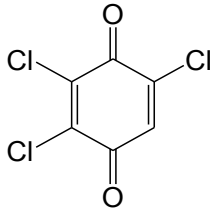
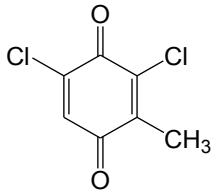
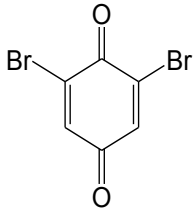
HBQs	Structure	Molecular formula	CAS number	MW	pK_a
2,6-dichloro-1,4-benzoquinone[1,2] (2,6-DCBQ)		$C_6H_2O_2Cl_2$	697-91-6	176.98	6.3[76]
2,3,6-trichloro-1,4-benzoquinone[1,2] (TriCBQ)		$C_6HCl_3O_2$	634-85-5	211.43	5.7[76]
2,6-dichloro-3-methyl-benzoquinone[1,2] (DCMBQ)		$C_7H_4Cl_2O_2$	40100-98-9	191.01	5.9[76]
2,6-dibromo-1,4-benzoquinone[1,2] (2,6-DBBQ)		$C_6H_2Br_2O_2$	19643-45-9	263.84	6.0[76]

Table 1.2 The characteristic absorption wavelength (λ_{max}) of HBQs

Analytes	λ_{max} (nm)	References
Benzoquinone	244	49
hydroxyl-benzoquinone	520	51
Hydroquinone	280	81
mBQ	250	50
2,5-dichloro-3,4-hydroxy-1,4-benzoquinone	530	54
2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone	332	82
Tetrachloro-1,4-benzoquinone (TetraCBQ)	292	57
Tetrachloro-1,4-hydroquinone (TetraCHQ)	326	57
tetrachlorosemiquinone (TCSQ)	455	59, 83
Trichloro-hydroxyl-benzoquinone (OH-TriCBQ)	295, 535	59, 83
2,6-DCBQ	273	55
OH-2,6-DCBQ	524	55

Table 1.3 Characteristics of the ESR spectra of HBQs

Substitution group	Splitting constants/Gauss				g-factor	Solvent
	R2	R3	R5	R6		
2-trifluoromethyl	CF3 2.66	H 3.37	H 1.62	H 2.19		MeCN
2-fluoro	F 3.50	H 1.85	H 3.15	H 1.95		MeCN
2-chloro	Cl	H 2.147 2.208 2.30	H 2.459 2.453 2.30	H 2.241 2.208 2.30		Water/EtOH[1] EtOH/Water MeOH
2-bromo	Br	H 2.159	H 2.367	H 2.355		Water/EtOH
2-bromo-5-*t*-butyl	Br	H 2.9	-CMe_3	H 1.4		Water/MeOH
2,3-dichloro	Cl	Cl	H 2.319	H 2.319		EtOH/Water
2,5-difluoro	F 5.24 5.35	H 1.42 1.41	F 5.24 5.35	H 1.42 1.41		DME/MeCN MeCN
2,5-dichloro	Cl	H 2.030 2.15 2.16 1.98	Cl	H 2.030 2.15 2.16 1.98	 2.0055	 EtOH/Water DMSO MeCN C_6H_6
2,6-dichloro	Cl	H 2.320 2.35 2.38	H 2.320 2.35 2.38	Cl	 2.0053	 EtOH/Water DMSO C_6H_6
2,6-diiodo	I	H 2.30	H 2.30	I	 2.0061	 Water/BuOH

Substitution group	Splitting constants/Gauss				g-factor	Solvent
	R2	R3	R5	R6		
trichloro	Cl	Cl	Cl	H		
				2.163		EtOH/Water
tetrafluoro	F	F	F	F		
	4.14	4.14	4.14	4.14	2.0048	Water/EtOH
tetrachloro	Cl	Cl	Cl	Cl	2.005819	DME
					2.005859	DMSO
					2.0061	C_6H_6
tetrabromo	Br	Br	Br	Br	2.008433	DMSO
tetraiodo	I	I	I	I	2.011399	DMSO

[1] Data are from Reference 57: Pedersen, J. A. *Handbook of EPR Spectra from Quinones and Quinols*. Boca Raton, Fla.: CRC Press, 1985.

[2.] MeCN: acetrontrile; EtOH: ethanol; DMSO: dimethyl sulfoxide; DME: dimethoxyethane; BuOH: butanol; MeOH: methanol.

Table 1.4 The measured ions in GC-MS SIM method for HQ and HHQs

Compound	MW	Measured Ions	Derivative	Internal standard	Measured Ions	References
Hydroquinone (HQ)	110	305	HFBI	[^{13}C6]HQ	311	70
Monochlorohydroquinone (MCHQ)	144	339	HFBI	[^{13}C6]MCHQ	345	70
Dichlorohydroquinone (DCHQ)	177	373	HFBI	[^{13}C6]DCHQ	379	70
Trichlorohydroquinone (TriCHQ)	212	409	HFBI	[^{13}C6]TriCHQ	415	70
Tetrachlorohydroquinone (TCHQ)	247	441	HFBI	[^{13}C6]TCHQ	451[1]	70
2,5-dichloro-1,4-benzoquinone (2,5-DCBQ)	176	176, 113	Phenazine	No[2]	No	68
2,5-dichloro-1,4-hydroquinone (2,5-DCHQ)	177	178, 114	Phenazine	No[2]	No	68
4-chlro-1,2-benzoquinone (4-MC-1,2-BQ)	142	214, 216	Phenazine	No[2]	No	67

[1] 451 was chosen instead of 447 or 449 due to the interference from chlorine isotope.

[2] No internal standard was used. The compound was quantified using external calibration curve.

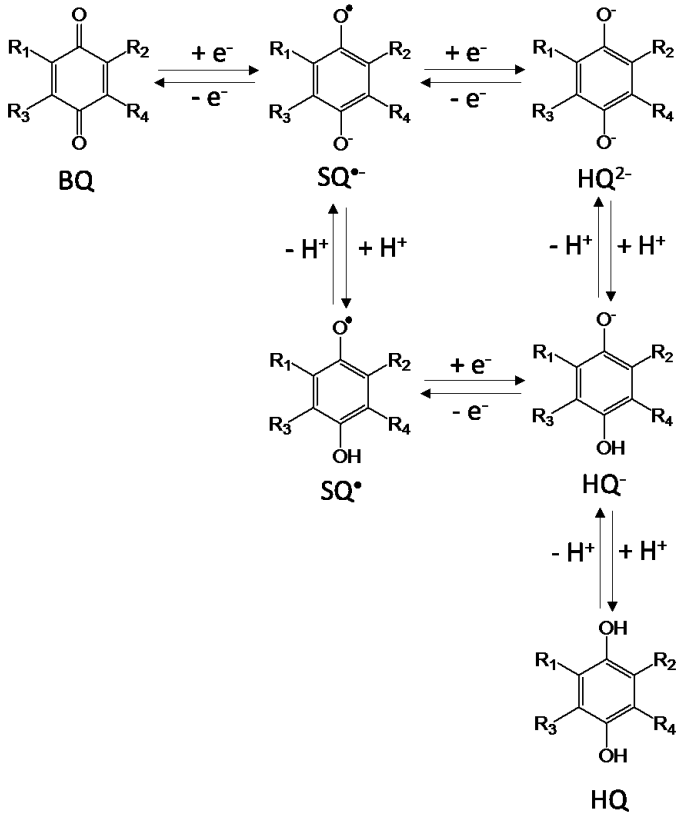


Figure 1.1 The basic structure and reversible redox reaction between benzoquinone (BQ), semiquinone radical (SQ), and hydroquinone (HQ).
R1, R2, R3 and R4 are hydrogen.

2 DISCOVERY OF NEW HALOBENZOQUINONES IN SWIMMING POOL WATERS

2.1 INTRODUCTION

Swimming is one of the most popular leisure activities around the world because of its health benefit and fun. To prevent waterborne diseases, the input or recycled water in swimming pools must be disinfected to inactivate pathogens. Common disinfection methods used in public swimming pools include chlorination, bromination, ozonation, and ultraviolet (UV) irradiation as well as combinations of these treatments.[1,2] Chlorination is used most often because it is cost-effective and convenient.[3] According to the World Health Organization (WHO) guidelines, the concentration of free chlorine should be maintained at 1–3 mg/L in swimming pools and 2–5 mg/L in hot tubs.[4] UV irradiation is often utilized as a secondary disinfection after chlorination to inactivate chlorine-resistant microbial pathogens.[5,6] Lower free chlorine concentration (0.5 mg/L or less) may be kept in the pools treated with a combination of chlorination and UV irradiation.[4]

DBPs are produced from the reactions of NOM and/or anthropogenic organic compounds in water with the disinfectants such as chlorine. The dissolved organic carbon (DOC) and chlorine dose are the key factors affecting their formation. Because of the high DOC and chlorine doses of swimming pools, more HBQs could be produced there than in the tap water. Therefore, my first aim is to discover what HBQs are formed in swimming pool waters.

Disinfection of water is essential to inactivate pathogens. However, it also produces byproducts resulting from unavoidable reactions between disinfectants and

organic/inorganic matter in water.[7] Epidemiological studies have observed an association of swimming in chlorinated water with an increased risk of bladder cancer, but we don't know if this association is casual.[3,8] Common disinfection byproducts (DBPs), such as trihalomethanes (THMs) and trihaloacetic acids (HAAs), cannot account for the epidemiologically observed bladder cancer risk.[9] Recent research has identified halobenzoquinones (HBQs) as one of five DBP classes of toxicological relevance.[10] HBQs are likely to be carcinogenic based on the toxicological evidence of benzoquinone and related compounds.[11,12] Computational modeling predicted that the lowest observed adverse effect levels (LOAEL) of HBQs may be as much as 10,000 times lower than the regulated DBPs such as chloroform.[13,14] Four HBQs have been identified as DBPs in disinfected drinking water at low ng/L concentrations.[15,16] They are 2,6-dichloro-(1,4)benzoquinone (2,6-DCBQ), 2,3,6-trichloro-(1,4)benzoquinone (TriCBQ), 2,6-dichloro-3-methyl-(1,4)benzoquinone (DCMBQ), and 2,6-dibromo-(1,4)benzoquinone (2,6-DBBQ). To date, no study has investigated the presence of HBQs in swimming pool water.

DBP formation in swimming pools is complex. While various types of organic matter remain in the input tap water,[17,18] the shared use of swimming pool water by a large number of swimmers may introduce additional contaminants, such as biological fluids (urine and sweat) and personal care products (cosmetics, lotions, and sunscreen).[19,20] For example, it has been estimated that an average urine release per swimmer could be approximately 25–77 mL.[21,22] Octocrylene (OC, a UV filter) has been detected in pools with concentrations ranging from 3 ng/L to 12 μg/L depending on the season.[23,24] Avobenzone (a UV filter) was identified in pools with concentration lower

than 24 ng/L.[24,25] Terasaki et al. identified a dichlorinated byproduct of methylparaben (a UV filter) at concentrations lower than 10 ng/L.[26] These contaminants can accumulate in swimming pools over time, leading to an increased concentration of organic matter. Several surveys have consistently shown that the total organic carbon (TOC) in swimming pool water is significantly higher than in the input tap water.[27] The complex components of organic matter exhibit diverse reactivity toward HBQ formation.

In the present study, we aim to address a few important questions concerning HBQs in swimming pools: 1) what species and concentrations of HBQs are present and 2) what factors contribute to HBQ formation? The results of this study are important to assess human exposure to HBQs through various routes and understand their potential health risks.

2.2 EXPERIMENTAL SECTION

2.2.1 Chemicals and solvents

2,6-dibromo-(1,4)benzoquinone (2,6-DBBQ) was purchased from Indofine Chemical (Hillsborough, NJ). 2,6-dichloro-3-methyl-(1,4)benzoquinone (DCMBQ) and 2,3,6-trichloro-(1,4)benzoquinone (TriCBQ) were synthesized by Shanghai Acana Pharmtech (Shanghai, China). Other chemicals, including 2,6-dichloro-(1,4)benzoquinone (2,6-DCBQ), 2,5-dibromo-(1,4)benzoquinone (2,5-DBBQ), 2,3-dibromo-5,6-dimethyl-(1,4)benzoquinone (DBDMBQ), 2,3,5,6-tetrabromo-(1,4)benzoquinone (TetraB-1,4-BQ), 3,4,5,6-tetrabromo-(1,2)benzoquinone (TetraB-1,2-BQ), sodium hypochlorite solution (reagent grade, available chlorine 10–15%) and formic acid (FA, analytical grade) were obtained from Sigma-Aldrich (Oakville, ON). Water (Optima LC/MS grade), methanol (Optima LC/MS grade), and hydrochloric acid (HCl, ACS grade) were purchased from

Fisher Scientific (Nepean, ON). Synthetic urine was purchased from Ricca Chemical Company (Arlington, TX).

2.2.2 Extraction of water samples

The water samples were extracted for HBQs using solid phase extraction (SPE). The SPE extraction of HBQs in swimming pool water was modified from the procedure used for drinking water.[14] Considering that swimming pool water has high salinity and complex matrices, we increased the volume of washing solvent. Briefly, HLB cartridges (6 mL, 200 mg per cartridge; Waters, Milford, MA) were pre-conditioned with 12 mL acidified methanol (0.25% FA) and 12 mL acidified water (0.25% FA). A vacuum system (-30 kpa) was then used to draw the 500-mL water sample through the cartridge at a flow rate of 2–3 mL/min. The loaded cartridge was sequentially washed with 18 mL of acidified water (0.25% FA) and 6 mL of methanol/water (0.25% FA, 50/50 vol/vol). The HBQs captured on the cartridge were eluted with 12 mL acidified methanol (0.25% FA). This eluent was collected and condensed down to 100 μL under a high purity nitrogen stream in a 40 ^{o}C water bath and then reconstituted to 500 μL with acidified water (0.25% FA). The extracts were stored at 4 ^{o}C and analyzed by LC-MS/MS within 24 h.

2.2.3 LC-MS/MS anlysis of HBQs

Separation of eight HBQs was achieved using a Luna C18(2) column (100 × 2.0 mm i.d., 3 μm; Phenomenex, Torrance, CA) and an Agilent 1100 HPLC (Santa Clara, CA) system. Solvent A was acidified water (0.25% FA) and solvent B was acidified methanol (0.25% FA). The mobile phase gradient program consisted of linearly increasing solvent B from 20% to 60% in 22 min, then increasing to 90% of B over 7 min, keeping B at 90% for 7 min, returning B back to 30% in 0.1 min, followed by a 15-min re-equilibration step prior

to the next sample injection. The flow rate was 170 μL/min, and the sample injection volume was 30 μL.

A triple quadrupole tandem mass spectrometer (QTRAP 5000, AB Sciex, Concord, ON, Canada) with an electrospray ionization (ESI) source was coupled to the HPLC system to quantify HBQ concentrations. The HBQ analysis was performed at negative ESI mode and with multiple reaction monitoring (MRM). The mass instrument parameters were set as follows: ionspray voltage, -4500 V; temperature, 450 ^{o}C; curtain gas, 30.0 psi; collision gas, medium (9.0 psi); gas 1 (nebulizer gas), 50.0 psi; gas 2 (heater gas), 60.0 psi; entrance potential, -10.0 psi; accumulation time, 200 ms. The MRM ion pairs and the optimized values of declustering potential (DP), collision energy (CE) and cell exit potential (CXP) for every analyte are described in Table 2.1.

The HBQs were identified based on retention time and relative abundance of the two ion pairs, and quantified using the peak area of the most abundant ion. To reduce the matrix effect, the standard addition method was used to generate calibration curves for each sample. Each extract was first analyzed to estimate the concentrations of the HBQs, based on which the concentrations of the standards for spiking were determined to establish calibration curves for each HBQs. Four aliquots of 90 μL from the 500-μL SPE extracts were added to 10-μL mixed standards of different concentrations. The concentrations of HBQs were determined based on the calibration curves which presented MS peak area as a function of added standard concentrations.

2.2.4 Collection of pool water samples

We collected water samples from 10 indoor swimming pools in Edmonton, Canada, from March 2011 to October 2011. These pools were treated with chlorine alone or chlorine

combined with UV irradiation, and the input water was the city tap water. Table 2.2 lists the disinfection conditions and basic water parameters of the 10 swimming pools and input tap water. The water samples were collected and stored in pre-cleaned 4-L amber bottles. To stop further reaction by quenching chlorine and to stabilize HBQs by adjusting the pH to 2.5, an aliquot of 20 mL FA was added to every sample immediately after collection.[28,29] The samples were transported to the laboratory within 1 hour and analyzed immediately.

A travel-blank sample (500-mL Optima water, 0.25% FA) was prepared and transported along with the swimming pool water samples in each sampling trip. Similarly, a SPE-blank sample (500-mL Optima water, 0.25% FA) was included in each batch of SPE extraction. The HBQ concentrations of the travel blank and SPE blank were analyzed to ensure no contamination occurred during sampling and pretreatment. To detect and avoid any carryover during sequential LC-MS/MS analyses, the analysis-blank sample consisting of the mobile phase (500 μL, 20% methanol, 80% water, 0.25% FA) was injected into the LC-MS/MS after every five samples. No HBQs were detected in any of the blank samples, confirming no HBQ contamination occurred during sample transportation, pretreatment, and analysis processes in this study. Free chlorine was determined using DOC was determined by a modified method based on U.S. EPA Method 415.1 using a Shimadzu TOC-5000A Total Organic Carbon Analyzer, at the Biogeochemical Analytical Service Laboratory of the University of Alberta. Free chlorine was determined using DPD colorimetry (Hach Odyssey D5/250kit).

2.3 RESULTS AND DISCUSSION

We have developed a new method to investigate whether other HBQs are produced in swimming pools in addition to the four HBQs previously detected in tap water. Figure 2.1 shows typical extracted ion chromatograms obtained from analysis of the eight HBQs (Table 2.1) using LC-MS/MS, indicating the complete separation of eight HBQs. The identification of an HBQ was based on the criteria that retention times are identical for two ion transitions of the specific HBQ and the relative intensity ratio of these two ion transitions detected in the samples is consistent with that in the standard solutions. When a HBQ was identified, the ion transition with higher abundance was used for quantification. The LC-MS/MS method was validated for analysis of the eight HBQs in swimming pool waters. Table 2.3 summarizes the reproducibility of retention times with relative standard deviations (RSD) less than 0.2%, the method detection limits (LODs) ranging from 0.03 to 1.12 ng/L, the SPE recovery and precision ranging from 67±2 to 102±3%, and the matrix effects ranging from 73±2 to 105±5% for the eight HBQs.

Having established a sensitive and reliable method, we used it to analyze HBQs in 10 swimming pools and their input tap waters. Table 2.4 presents the concentrations of the HBQs determined in the 10 pools. All samples from the swimming pools and the input water contained 2,6-DCBQ. The average concentration of 2,6-DCBQ in the input tap water samples was 3.1±1.9 ng/L, while the concentration of 2,6-DCBQ ranged from 26.6±3.4 to 299.0±7.1 ng/L in the pool waters. Therefore, greater 2,6-DCBQ concentration by a factor of 5–100 was produced in pool water than in the input tap water. Three additional HBQs, TriCBQ, DMDBBQ, and 2,6-DBBQ, were detected in pool water but not input tap water. TriCBQ was detected in four pools with an average

concentration of 9.0±1.8 ng/L, while DMDBBQ and 2,6-DBBQ were detected in Pools #5 and #8 with an average concentration of 0.6±0.1 and 2.7±1.6 ng/L, respectively. This is the first time that DMDBBQ has been detected in any water samples.

The higher concentrations of 2,6-DCBQ and the formation of other HBQs in the pools over the input water led to further investigation into the potential factors contributing to the elevated HBQ formation. Water temperature may be a factor contributing to the increased levels of HBQs in the pools.[30,31] The 10 tested indoor swimming pools can be separated into two groups based on their temperatures, 25 ºC or 35 ºC, as shown in Table 2.2. The temperature of their input tap water was much lower, generally around 10 ºC. We observed 15-fold greater 2,6-DCBQ median levels in the 25 ºC pools (47.0 ng/L median, 65.8 ng/L maximum, n=6) compared to the input tap water (3.0 ng/L median, 5.6 ng/L maximum, n=10). The 2,6-DCBQ concentrations in the 35 ºC pools were 101.4 ng/L median and 299.0 ng/L maximum (n=4). The average concentration of 2,6-DCBQ in the 35 ºC pools was approximately 52-fold greater than in the input tap water, and 3.6-fold greater than in the 25 ºC pools. The average concentration of 2,6-DCBQ in the 35 ºC pools was statistically higher than that in the tap waters and the 25 ºC pools (ANOVA, $p<0.001$), as shown in Figure 2.2(A). Also, Dunn's statistical analysis showed that 2,6-DCBQ concentrations were significantly different ($p<0.05$) for the group pairings: 25 ºC pools > tap waters, 35 ºC pools > tap waters, and 35 ºC pools > 25 ºC pools.

The chlorine dose is known to impact the formation of halogenated DBPs in water.[32,33] Concentrations of the residual free chlorine in the pools were determined to be an average of 1.2 mg/L (median=1.2 mg/L), significantly higher than those in the input

tap waters (<0.1 mg/L). This suggested that the higher chlorine doses in swimming pools may promote 2,6-DCBQ formation. To confirm this, we added free chlorine to the tap water and determined the concentrations of HBQs before and after addition of free chlorine. Only 2,6-DCBQ was observed after the chlorination of tap water. The concentration of 2,6-DCBQ after addition of 5 mg/L free chlorine for 36 h was 71.0±0.9 ng/L, significantly increased from 3.5±0.7 ng/L in the tap water without addition of free chlorine (Table 2.5). The results support the hypothesis that a higher chlorine dose in the pool contributes to the enhancement of 2,6-DCBQ formation.

The disinfection method may play a key role in 2,6-DCBQ formation. Five of the ten swimming pools were treated by chlorine alone, and the other five were treated by chlorine combined with UV (Table 2.2). Figure 2.2(B) compares the HBQ concentrations in the pools treated with chlorine alone and with the combined chlorine and UV irradiation. Based on ANOVA analysis, the mean concentration of 2,6-DCBQ in the chlorinated pools is not statistically different than that in the pools treated with both chlorine and UV. The statistical outlier is Pool #5 (label A in the Figure). This may be due to its higher temperature (35 ^{o}C) and higher DOC (10.7 mg/L, the highest of the ten swimming pools).

We further conducted linear correlation analysis of the potential relationship between the HBQ concentrations with pH, free chlorine, total chlorine, DOC, and TOC. The formula and R-square values are summarized in Table 2.6. The pooled data show no linear correlations between the HBQ concentrations and these parameters except DOC. HBQ formation is statistically correlated with DOC (R^2=0.65).

2.4 CONCLUSION

This study developed a reproducible method combining SPE with LC-MS/MS for determination of eight HBQs, enabling the comprehensive investigation of HBQ formation in swimming pools. Because of the potential toxic effects of HBQs, the results of this study provide useful information guiding the management of swimming pool disinfection and public education on personal hygiene and practice to maintain the high quality and safety of swimming pool water. High chlorine dose could enhance the microbiological security; in turn, however, it may increase the formation of DBPs. Warm water could provide a comfortable environment for swimmers, and are mainly used for children. However, it may also the accelerate chlorination reaction. The results are useful to the assessment of human exposure through various routes. This also demonstrates the analytical methodology development and its application to address environmental health issues.

2.5 REFERENCES

1. Richardson, S. D. Environmental mass spectrometry: emerging contaminants and current issues. *Anal Chem* **2012**, *84* (2), 747–778.
2. Zwiener, C.; Richardson, S. D.; De Marini, D. M.; Grummt, T.; Glauner, T.; Frimmel, F. H. Drowning in disinfection byproducts? Assessing swimming pool water. *Environ Sci Technol* **2007**, *41* (5), 363–372.
3. Lakind, J. S.; Richardson, S. D.; Blount, B. C. The good, the bad, and the volatile: can we have both healthy pools and healthy people? *Environ Sci Technol* **2010**, *44* (9), 3205–3210.
4. World Health Organization (WHO). *Guidelines for Safe Recreational Water Environments. volume 2, Swimming Pools and Similar Environments*; WHO Press: Geneva, 2006.

5. Hijnen, W. A. M.; Beerendonk, E. F.; Medema, G. J. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Res* **2006**, *40* (1), 3–22.
6. Craik, S. A.; Weldon, D.; Finch, G. R.; Bolton, J. R.; Belosevic, M. Inactivation of Cryptosporidium parvum oocysts using medium- and low-pressure ultraviolet radiation. *Water Res* **2001**, *35* (6), 1387–1398.
7. Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; DeMarini, D. M. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research. *Mutat. Res-Rev Mutat* **2007**, *636* (1-3), 178–242.
8. Villanueva, C. M.; Cantor, K. P.; Grimalt, J. O.; Malats, N.; Silverman, D.; Tardon, A.; Garcia-Closas, R.; Serra, C.; Carrato, A.; Castano-Vinyals, G.; Marcos, R.; Rothman, N.; Real, F. X.; Dosemeci, M.; Kogevinas, M. Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *Am J Epidemiol* **2007**, *165* (2), 148–156.
9. Hrudey, S. E.; Charrois, J. W. *Disinfection By-Products and Human Health*. IWA Publishing: London, 2012.
10. Bull, R. J.; Reckhow, D. A.; Rotello, V.; Bull, O. M.; Kim, J. *Use of Toxicological and Chemical Models to Prioritize DBP Research*; American Water Works Association Research Foundation and American Water Works Association: Denver, CO, 2006.
11. Anichina, J.; Zhao, Y.; Hrudey, S. E.; Le, X. C.; Li, X.-F. Electrospray ionization mass spectrometry characterization of interactions of newly identified water disinfection byproducts halobenzoquinones with oligodeoxynucleotides. *Environ Sci Technol* **2010**, *44* (24), 9557–9563.
12. Song, Y.; Wagner, B. A.; Witmer, J. R.; Lehmler, H. J.; Buettner, G. R. Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones. *Proc Natl Acad Sci USA* **2009**, *106* (24), 9725–9730.
13. Bull, R. J.; Reckhow, D. A.; Li, X.-F.; Humpage, A. R.; Joll, C.; Hrudey, S. E. Potential carcinogenic hazards of non-regulated disinfection by-products: haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology* **2011**, *286* (1-3), 1–19.

14. Anichina, J.; Zhao, Y.; Hrudey, S. E.; Schreiber, A.; Li, X.-F. Electrospray ionization tandem mass spectrometry analysis of the reactivity of structurally related bromo-methyl-benzoquinones toward oligonucleotides. *Anal Chem* **2011**, *83* (21), 8145–8151.
15. Qin, F.; Zhao, Y.; Zhao, Y.; Boyd, J. M.; Zhou, W.; Li, X.-F. A Toxic Disinfection By-product, 2,6-Dichloro-1,4-benzoquinone, identified in Drinking Water. *Angew Chem Int Ed* **2010**, *49* (4), 790–792.
16. Zhao, Y.; Anichina, J.; Lu, X. F.; Bull, R. J.; Krasner, S. W.; Hrudey, S. E.; Li, X.-F. Occurrence and formation of chloro- and bromo-benzoquinones during drinking water disinfection. *Water Res* **2012**, *46* (14), 4351–4360.
17. Hu, J. Y.; Aizawa, T.; Ookubo, S. Products of aqueous chlorination of bisphenol A and their estrogenic activity. *Environ Sci Technol* **2002**, *36* (9), 1980–1987.
18. Mitch, W. A.; Sedlak, D. L. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environ Sci Technol* **2002**, *36* (4), 588–595.
19. Esbenshade, J. L.; Cardoso, J. C.; Zanoni, M. V. B. Removal of sunscreen compounds from swimming pool water using self-organized TiO_2 nanotubular array electrodes. *J Photochem Photobio A* **2010**, *214* (2-3), 257–263.
20. Brausch, J. M.; Rand, G. M. A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. *Chemosphere* **2011**, *82* (11), 1518–1532.
21. Weng, S. C.; Li, J.; Blatchley, E. R. Effects of UV_{254} irradiation on residual chlorine and DBPs in chlorination of model organic-N precursors in swimming pools. *Water Res* **2012**, *46* (8), 2674–2682.
22. De Laat, J.; Feng, W. T.; Freyfer, D. A.; Dossier-Berne, F. Concentration levels of urea in swimming pool water and reactivity of chlorine with urea. *Water Res* **2011**, *45* (3), 1139–1146.
23. Balmer, M. E.; Buser, H. R.; Muller, M. D.; Poiger, T. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. *Environ Sci Technol* **2005**, *39* (4), 953–962.
24. Poiger, T.; Buser, H. R.; Balmer, M. E.; Bergqvist, P. A.; Muller, M. D. Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. *Chemosphere* **2004**, *55* (7), 951–963.

25. Giokas, D. L.; Slvador, A; Chisvert, A. UV filters: From sunscreens to human body and the environment. *Trends Anal Chem* **2007**, *26* (5), 360-374.
26. Terasaki, M.; Makino, M. Determination of chlorinated by-products of parabens in swimming pool water. *Int J Environ An Ch* **2008**, *88* (13), 911–922.
27. Kanan, A.; Karanfil, T. Formation of disinfection by-products in indoor swimming pool water: the contribution from filling water natural organic matter and swimmer body fluids. *Water Res* **2011**, *45* (2), 926–932.
28. Hoq, M. F.; Indu, B.; Ernst, W. R.; Neumann, H. M. Kinetics of the reaction of chlorine with formic acid in aqueous sulfuric acid. *J Phys Chem* **1991**, *95* (2), 681–683.
29. Yu, H. G.; Francisco, J. S.; Muckerman, J. T. Ab initio and direct dynamics study of the reaction of Cl atoms with HOCO. *J Chem Phys* **2008**, *129* (6), 1890–1896.
30. Roccaro, P.; Chang, H. S.; Vagliasindi, F. G. A.; Korshin, G. V. *Water Res* **2008**, *42* (8-9), 1879–1888.
31. Ribes, A.; Grimalt, J. O.; Garcia, C. J. T.; Cuevas, E. Temperature and organic matter dependence of the distribution of organochlorine compounds in mountain soils from the subtropical Atlantic (Teide, Tenerife Island). *Environ Sci Technol* **2002**, *36* (9), 1879–1885.
32. Zhou, W.; Boyd, J. M.; Qin, F.; Hrudey, S. E.; Li, X.-F. Formation of N-nitrosodiphenylamine and two new N-containing disinfection byproducts from chloramination of water containing diphenylamine. *Environ Sci Technol* **2009**, *43* (21), 8443–8448.
33. Gallard, H.; Von Gunten, U. Chlorination of phenols: Kinetics and formation of chloroform. *Environ Sci Technol* **2002**, *36* (5), 884–890.
34. Diemert, S.; Wang, W.; Andrews, R. C.; Li, X-F. Removal of halobenzoquinone (emerging disinfection by-product) precursor material from three surface waters using coagulation. *Water Res* **2013**, *47* (5), 1773–1782.

Table 2.1 The transaction ion pairs and optimized declustering potential (DP), collision energy (CE), and cell exit potential (CXP) of eight HBQs.

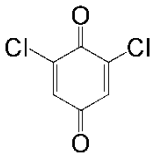
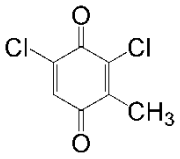
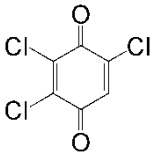
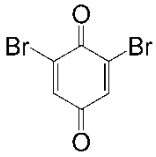
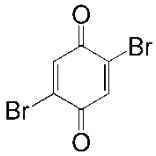
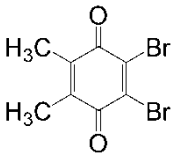
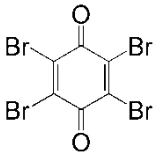
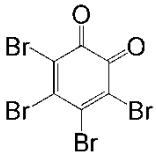
HBQ /Molecular formula	Chemical structure	Parent ion (m/z)	Product ion (m/z)	DP	CE	CXP
2,6-DCBQ		177	113	-100	-24	-13
$C_6H_2Cl_2O_2$		177	141	-100	-20	-13
DCMBQ		191	127	-85	-24	-11
$C_7H_4Cl_2O_2$		191	155	-85	-20	-11
TriCBQ		211	175	-80	-18	-11
$C_6HCl_3O_2$		211	147	-80	-20	-11
2,6-DBBQ		267	79	-100	-50	-11
$C_6H_2Br_2O_2$		267	81	-100	-50	-11
2,5-DBBQ		267	79	-100	-50	-10
$C_6H_2Br_2O_2$		267	81	-100	-50	-10
DMDBBQ		294	79	-75	-40	-11
$C_8H_6Br_2O_2$		294	81	-75	-40	-11
TetraB-1,4-BQ		423	79	-50	-70	-11
$C_6Br_4O_2$		424	81	-50	-70	-11
TetraB-1,2-BQ		423	79	-50	-70	-11
$C_6Br_4O_2$		424	81	-50	-70	-11

Table 2.2 Disinfection methods and water parameters of the swimming pools studied.

Pool No.	Treatment	Temperature (°C)	pH	Free chlorine (mg/L)	Total chlorine (mg/L)	DOC (mg/L)	TOC (mg/L)
1	Cl	25	7.28	0.60±0.02	1.84±0.33	8.1	15.3
2	Cl	25	7.10	0.91±0.05	2.16±0.21	8.1	15.9
3	Cl	25	7.12	1.33±0.04	2.46±0.15	6.5	12.3
4	Cl	25	7.56	1.82±0.04	3.11±0.30	5.9	10.7
5	Cl	35	7.14	1.72±0.03	3.41±0.11	10.7	11.6
6	Cl+UV	25	7.49	0.91±0.01	1.63±0.15	4.9	10.5
7	Cl+UV	25	7.44	0.74±0.03	1.03±0.10	5.5	11.7
8	Cl+UV	35	7.44	1.55±0.07	2.10±0.24	8.4	11.7
9	Cl+UV	35	7.51	1.34±0.05	2.56±0.11	8.1	11.9
10	Cl+UV	35	7.36	1.52±0.02	2.96±0.13	9.5	14.8
tap	Chloramine*	10	7.82	<0.1	1.68±0.09	2.2	2.5

*Tap water in Edmonton was chlorinated followed by UV irradiation. Chloramine was used as the secondary disinfectant for the water distribution system.

Table 2.3 Performance of the SPE-HPLC-MS/MS method for the eight HBQs.

Analyte	HPLC Retention time (min) (RSD%)	SPE-HPLC-MS/MS LOD (ng/L)	Recovery and precision (Mean±SD%, n=3)	Matrix effect (%, n=3)
2,6-DCBQ	22.70±0.01 (0.04%)	0.19	82±5	75±6
DCMBQ	29.68±0.02 (0.06%)	0.06	67±2	73±2
TriCBQ	30.27±0.02 (0.06%)	0.10	96±3	87±2
2,6-DBBQ	25.40±0.01 (0.04%)	0.05	102±3	105±5
2,5-DBBQ	35.65±0.03 (0.11%)	0.12	93±10	97±4
DMDBBQ	34.67±0.05 (0.14%)	0.05	69±7	77±5
TB-1,4-BQ	26.37±0.05 (0.19%)	1.12	77±5	88±3
TB-1,2-BQ	37.37±0.03 (0.08%)	0.03	79±5	89±2

Table 2.4 HBQ concentrations (ng/L) in 10 swimming pools and the input tap water.

	Swimming pool water				Input tap water
Pool No.	2,6-DCBQ	TriCBQ	DMDBBQ	2,6-DBBQ	2,6-DCBQ
1	55.9±0.2	<0.1	<0.05	<0.05	1.2±0.1
2	65.9±8.3	<0.1	<0.05	<0.05	1.2±0.1
3	34.9±4.0	<0.1	<0.05	<0.05	5.6±1.3
4	26.6±3.4	<0.1	<0.05	<0.05	4.3±0.2
5	299.0±7.1	8.0±3.9	0.7±0.1	1.6±0.3	2.6±0.1
6	47.0±4.6	<0.1	<0.05	<0.05	1.1±0.1
7	18.9±3.4	<0.1	<0.05	<0.05	2.0±0.0
8	90.8±14.7	7.3±0.7	0.6±0.1	3.8±0.2	3.1±0.3
9	97.3±5.8	11.3±1.5	<0.05	<0.05	3.7±0.2
10	105.4±9.1	9.4±2.2	<0.05	<0.05	3.2±0.2

Table 2.5 2,6-DCBQ concentration after further disinfection of tap water.

Water sample	2,6-DCBQ contents (ng/L)
Optima water	< 0.03
Tap water	3.5 ± 0.7
Tap water + chlorination	71.0 ± 0.9

Table 2.6 Linear correlation between pH, free chlorine, total chlorine, DOC, TOC against HBQ concentrations in swimming pools.

Parameter	Formula	R^2
pH	y = -176x + 1377.7	0.14
Free chlorine	y = 61.3x + 11.3	0.16
Total chlorine	y = 68.2x - 74.4	0.37
DOC	y = 35.7x - 185.9	0.65
TOC	y = -1.72x + 106.0	0.00

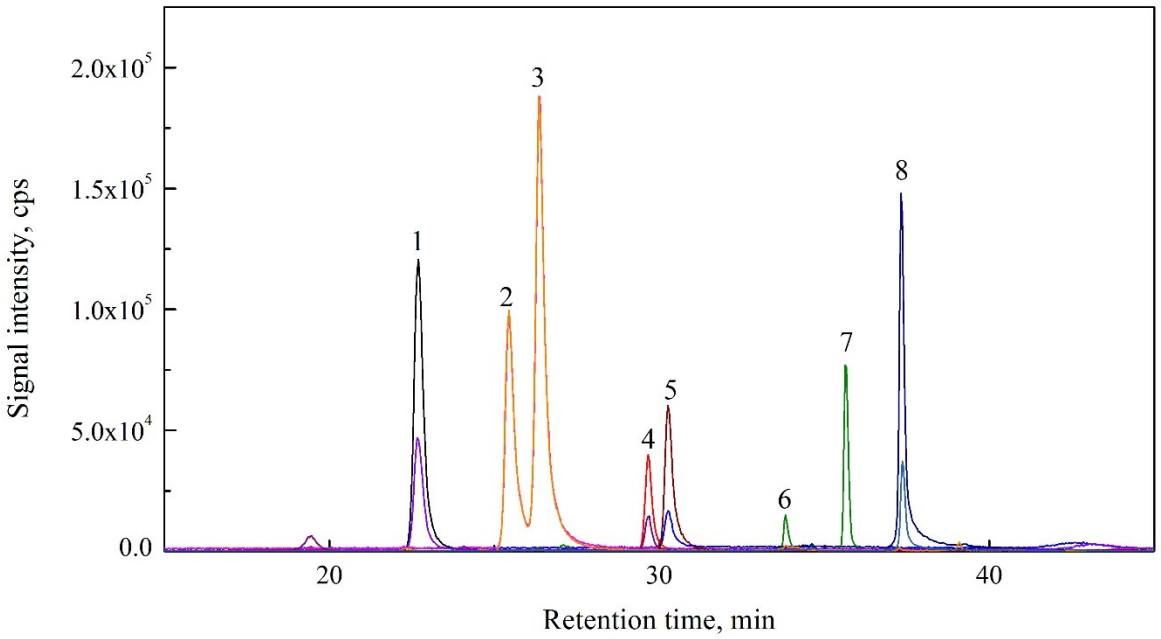


Figure 2.1 Separation of eight HBQs using LC-MS/MS, MRM mode.

1: 2,6-DCBQ; 2: 2,6-DBBQ; 3: 2,5-DBBQ; 4: DCMBQ; 5: TriCBQ; 6: TetraB-1,4-BQ; 7: DMDBBQ; 8: TetraB-1,2-BQ

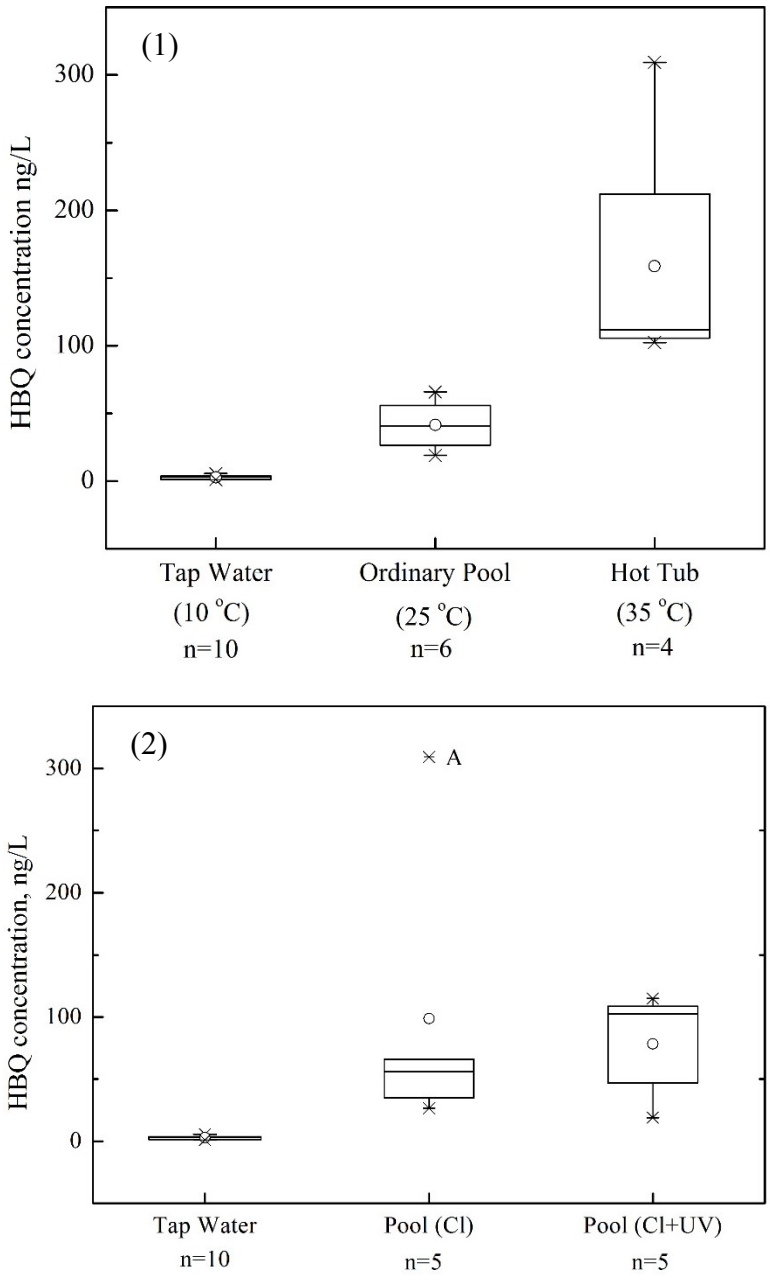


Figure 2.2 Aqueous concentrations of 2,6-DCBQ in swimming pools and the input tap waters.

The central lines, ends, and error bars of the box define the medians, 25th and 75th percentiles, and the 99% confidence intervals of the mean, respectively.

3 FORMATION OF HALOBENZOQUINONES FROM NATURAL ORGANIC MATTER AND ANTHROPOGENIC MATERIAL

3.1 INTRODUCTION

The presence of various HBQs in swimming pool water and input water led to the question of what precursors result in the formation of the HBQs. Natural organic matter (NOM) from source water is widely recognized as the dominant source of DBP precursors.[1,2] NOM is a complex mixture of ill-defined organic materials which vary both spatially and temporally.[3] Every source water of different location is different in terms of precursor composition, thus the formation of HBQs may be regional after water treatment. We collaborated with Dr. R. Andrews' group at the University of Toronto to address whether NOM or specific components of NOM can contribute to HBQ formation. Three representative source waters in Canada (Lake Ontario, Otonabee River, and Grand River) were collected, coagulated, and chlorinated to compare the NOM composition and HBQ formation.

Because of its complicated composition, several techniques have been developed to characterize the NOM based on some specific parameters or properties, such as molecular weight, UV absorption, and hydrophilicity/hydrophobility. One emerging analytical technique to characterize the composition of NOM is liquid chromatography–organic carbon detection–organic nitrogen detection (LC-OCD-OND). By this method, the dissolved organic carbon (DOC) portion of NOM is separated into fractions using size-exclusion chromatography (SEC), and measured via an organic carbon detector and

an organic nitrogen detector.[4] The method could separate NOM as hydrophobic DOC, biopolymers, humic substance, building blocks, low molecular weight acids, and low molecular weight neutrals (Figure 3.1). LC-OCD-OND has been used previously to track NOM removal through different steps in water treatment processes, including coagulation.[5] The production of regulated DBPs (THMs and HAAs) has previously been shown to be highly correlated to the humic substance and biopolymer NOM fractions in a coagulation study of Otonabee River water.[6] Therefore, LC-OCD can be used to assess potential association of the NOM fractions that contribute to the formation of HBQs, in order to identify the precursors of HBQs.

The detection of three additional HBQs in the swimming pools but not in the input water led to the investigation into what organic compounds in swimming pools may contribute to the formation of HBQs. The average DOC in the pool waters was 7.5 mg/L, while the average DOC in the input tap waters was 2.2 mg/L. Addition of free chlorine to the tap water only increased the concentration of 2,6-DCBQ but did not result in the formation of other HBQs. This suggested that other organic precursors of HBQs may be present in the pools but not in the tap water.

While various types of organic matter remain in the input tap water,[7,8] the shared use of swimming pool water by a large number of swimmers may introduce additional contaminants, such as biological fluids (urine and sweat), and personal care products (cosmetics, lotions, and sunscreen).[9,10] For example, it has been estimated that an average urine release per swimmer could be approximately 25–77 mL.[11,12] Octocrylene (OC, a UV filter) has been detected in pools with concentrations ranging from 3 ng/L to 12 μg/L depending on the season.[13,14] Avobenzone (a UV filter) was identified in pools with

concentration lower than 24 ng/L.[15,16] Terasaki et al. identified a dichlorinated byproduct of methylparaben (a UV filter) at concentrations lower than 10 ng/L.[26] These contaminants can accumulate in swimming pools over time, leading to an increased concentration of organic matter. Several surveys have consistently shown that the total organic carbon (TOC) in swimming pool water is significantly higher than in the input tap water.[17] The complex components of organic matter exhibit diverse reactivity toward HBQ formation.

The aims of this study were to 1) examine what NOM fractions (as detected via LC-OCD-OND) in three different surface waters are associated with the formation of HBQs after coagulation and chlorination, and 2) identify anthropogenic materials as precursors of HBQs in swimming pool water.

3.2 EXPERIMENTAL SECTION

3.2.1 Reagents

Aluminum sulfate (alum) was obtained from General Chemical (Parsippany, NJ). Sodium hydroxide (50% NaOH) for pH adjustment, sodium hypochlorite (10-15% chlorine) for chlorination, and Optima LC–MS grade formic acid were purchased from Sigma Aldrich (St. Louis, MO). DPD colorimetry packets were purchased from Hach (Dusseldorf, Germany). Optima LC–MS grade water and methanol were used as solvents and were obtained from Fisher Scientific (Fair Lawn, NJ). 2,6-dibromo-(1,4)benzoquinone (2,6-DBBQ) was purchased from Indofine Chemical (Hillsborough, NJ). 2,6-dichloro-3-methyl-(1,4)benzoquinone (2,6-DCMBQ) and 2,3,6-trichloro-(1,4)benzoquinone (2,3,6-TriCBQ) were synthesized by Shanghai Acana Pharmtech (Shanghai, China); 2,6-dichloro-(1,4)benzoquinone (2,6-DCBQ) was purchased from Sigma Aldrich.

3.2.2 Coagulation and chlorination of source water

3.2.2.1 Source water collection and characterization

Raw, untreated water was collected between October and December 2011 from three water utilities in Ontario, Canada: Lake Ontario water from the Ajax Water Supply Plant (Ajax, ON), Otonabee River water from the Peterborough Water Treatment Plant (Peterborough, ON), and Grand River water from the Mannheim Water Treatment Plant (Waterloo, ON). The three waters are all representative drinking water sources in Ontario, Canada. Water quality characteristics are listed in Table 3.1. Following collection, water samples were stored at 4 ^{o}C prior to bench-scale experiments.

3.2.2.2 Coagulation experiment

For bench-scale jar testing, six 2-L jars (B-ker2 square jars; Phipps and Bird, Richmond, VA, USA) were filled with water (raw surface water and one system blank consisting of Milli-Q^{TM}) and placed in a bench scale stirrer (Phipps and Bird). Alum was added as the coagulant using an Eppendorf pipette. Alum doses to achieve DOC reduction targets were determined via preliminary jar tests (described in Section 3.2.2.3); two additional doses were chosen ±40% from the targeted dose to provide a range of coagulant concentrations.

Samples were subjected to 90 s of rapid mixing (100 rpm), 15 min of slow mixing (30 rpm), and 30 min of sedimentation.[18] Settled water samples were collected from each jar: two 500 mL samples with no headspace were collected in pre-cleaned amber bottles with Teflon^{TM} caps (acid washed, rinsed with distilled water and baked at 300 ^{o}C for 6 h, and soaked in a dilute sodium hypochlorite solution for 24 h). For LC-OCD-OND analysis, 100 mL water was collected and filtered using vacuum filtration with 0.45 mm filters (Pall Corporation, Ann Arbor, MI).

3.2.2.3 Determination of alum doses

To determine the target alum doses, preliminary jar tests were conducted. Using water from the same collection date as the EDC, PhAC, and NOM removal tests, six 2-L jars (B-ker^2 square jars; Phipps and Bird) were filled with water (raw surface water and one system blank of Milli-Q®) and placed in a bench scale stirrer (Phipps and Bird). Alum was added as the coagulant using an Eppendorf pipette. A range of alum doses was applied and DOC reduction was calculated.

A narrow range of alum doses (32–42 mg/L) was used for Otonabee River water, as jar tests performed prior to the preliminary tests (Sept 18, 2011) indicated that the optimal dose was approximately 36 mg/L, and raw Otonabee River water had maintained similar NOM fraction concentrations between this testing date and the preliminary test date (Oct. 4, 2011). A wider range of alum doses were used for Lake Ontario and Grand River waters; these waters had varied considerably in composition by the dates of their preliminary tests (Nov. 22 and Dec. 6, 2011 respectively) compared to previous testing in September, and as such a larger range was desired in order to capture the DOC reduction targets.

DOC reduction targets were selected according to the USEPA Enhanced Coagulation guidelines (Table 3.1), and were calculated according to raw water DOC and alkalinity values (listed in Table 3.1). Otonabee River required 35% reduction, while Lake Ontario and Grand River water required 25% reduction. Results of preliminary jar tests are shown in Tables 3.2–3.4 and Figures 3.2–3.4. Asterisks indicate the target alum dose required to achieve desired DOC reductions.

3.2.2.4 Chlorination experiment

For the HBQ formation potential experiments, raw and coagulated water samples were adjusted to a pH of 7.0±0.2 using 50% NaOH. Sodium hypochlorite doses were determined through preliminary chlorine demand experiments (described in Section 3.2.2.5). These doses were applied such that residuals after 40 h of reaction time would reflect targets in standard reaction conditions, i.e., between 3 and 5 mg/L free chlorine.[19] The free chlorine concentration of the sodium hypochlorite dosing solution (Sigma-Aldrich, 10-15% chlorine diluted to 2 mg Cl_2/L with Milli-Q™ water) and residuals were quantified using DPD colorimetry (Hach Odyssey D5/250 kit). Buffers were not added in order to avoid interference with LC–MS/MS analysis, but pH varied less than 0.2 units over the chlorination reaction period.[19] After 40 h, Optima LC–MS grade formic acid (1.25 mL per 500 mL water sample) was added to sample jars for HBQ preservation.[20] Formic acid could serve as a quenching agent to remove residual free chlorine in less than 0.1 s, and also stabilize HBQs in water samples.[21–23] Samples, including a travel blank (Milli-Q™), were shipped on ice from the University of Toronto (Toronto, ON, Canada) to the University of Alberta (Edmonton, AB, Canada) for HBQ analysis. Laboratory experiments confirmed that HBQs were stable after one week of storage when formic acid was added.

3.2.2.5 Determination of chlorine demand

To determine chlorine demand for different coagulation-treated waters, two "chlorine demand" jar tests were run using identical water source and alum doses as determined in the preliminary jar tests. Two 500 mL samples were collected from each jar and filtered using a 0.45 μm filter (Supor-450 47mm filters; Pall Corporation, Ann Arbor, MI). The

pH was measured before the addition of chlorine. The chlorine dose chosen for the test was approximately 10 mg/L Cl_2; this is a common dose used to test chlorine demand of waters with DOC <10 mg/L. 2.2 mL of a NaOCl spiking solution (2.31×10^2 mg/L Cl_2) was added to each of the 500 mL samples. The excess volume of water was discarded in an effort to maintain a total volume of 500 mL with no headspace in the bottles.

Immediately after chlorine addition, the pH was adjusted to 7.0 using HCl. The samples were then held for 40 h of reaction time. After 40 h, free chlorine residual was measured using a Hach kit (Odyssey D5/250 spectrophotometer) and a colorimetric test (using DPD free chlorine powder pillows and detection at 530 nm).

Chlorine demand is determined as:

$$D = C_o - R, \quad (1)$$

where D = chlorine demand, C_o = initial chlorine concentration, R = residual chlorine. Once chlorine demand had been determined, the initial chlorine doses could be calculated to reach the target residual (R_{target} = 4 mg/L Cl_2; although 3-5 mg/L Cl_2 was considered appropriate for residuals):

$$C_o = D + R_{target} \quad (2)$$

3.2.3 Chlorination of water samples containing PCPs

3.2.3.1 Preparation of water samples containing PCPs

Four body lotions and four sunscreens were selected as representative personal care products (PCPs). The basic compositions of L1 and L4 are similar, and the difference is that L4 does not contain perfume. The basic compositions of L2 and L3 are similar, and the difference is that L3 contains plant extracts from cucumber and aloe, whereas L2 does not include the plant extracts. S1 and S2 contain the combination of organic and

inorganic UV filters; S3 contains only inorganic UV filters; and S4 contains only organic UV filters. The SPF values of the four sunscreens S1, S2, S3, and S4 are 30, 50, 50 and 40, respectively. The DOC of swimming pool water was around 5–10 mg/L, and the DOC of tap water was around 2 mg/L. Therefore, we controlled the DOC of the PCP solutions at 5 mg/L. To determine the appropriate spiking doses of PCPs, we prepared a set of water samples containing 25, 50, and 100 μg of individual PCPs in 400 mL. The PCP water samples were dissolved in an ultrasound bath. We obtained a calibration curve of the PCP doses vs. DOC (Figure 3.5) to determine the required amount of each PCP to prepare a 400 mL water sample of DOC 5 mg/L. Table 3.5 summarizes the amounts of PCPs used in the water samples.

3.2.3.2 Preparation of chlorine stock solution

A chlorine stock solution (20 mg/mL free chlorine) was prepared from the commercial sodium hypochlorite solution (12.1 g/mL free chlorine). The concentration of free chlorine was measured by a chlorine amperometric titrator (Autocat 9000, Hach). The 20 mg/mL free chlorine stock solution was used for the chlorination experiments.

3.2.3.3 Initial chlorine dose

We chose 3 mg/L as the concentration of residual chlorine based on the WHO guideline of free chlorine in swimming pools.[24] A chlorine demand experiment was conducted to determine an appropriate initial chlorine dose. Two bottles of 400 mL samples were prepared and the pH was measured before the addition of chlorine. The initial dose of free chlorine was determined to be 10 or 15 mg/L of free chlorine. Immediately after the addition of chlorine, the pH was adjusted to 7.0 with 0.1 mol/L HCl. After reaction for 36 h, the free chlorine residual was measured using the chlorine amperometric titrator.

Chlorine demand was calculated using Equation (1) in Section 3.2.2.5. C_o is 10 or 15 mg/L. After chlorine demand was determined, the initial dose was calculated using Equation (2) in Section 3.2.2.5. Table 3.5 summarizes the chlorine demand for each sample.

3.2.3.4 Chlorination reaction

The chlorination reactions were conducted in 500-mL amber glass bottles with PTFE screw caps. Each reaction mixture contained a specific amount of chlorine solution and PCPs in 400 mL water, as described above. The pH was adjusted to 7.0±0.1 with 0.1 mol/L HCl immediately after the sample preparation. The reaction solutions were kept in the dark at 24 °C for 36 h. FA was added into the solution at the end of the experiment to quench residual chlorine and stabilize HBQs. The reaction mixtures were analyzed for HBQs by the LC-MS/MS method.

3.2.4 Determination of chlorination reaction time

To determine the appropriate chlorination reaction time, we monitored the variations of HBQ concentrations in one swimming pool over one chlorination cycle (from the addition of chlorine to the next addition). We chose Pool #5 for investigation, because of its relatively consistent use (approximately five swimmers per day). Its pH, free chlorine, total chlorine, and TOC are similar with these of other pools (analyzed by q-test, $q<q_{10,0.99}=0.57$). Based on this and the sampling access to Pool #5 for entire chlorination cycles, we studied the dynamic variation of HBQs in Pool #5. A commercial bleach containing sodium hypochlorite was used to chlorinate the pool following the standard practice. Samples were collected immediately following addition of chlorine, followed by sampling every 12 h. In Pool #5, water temperature, pH, DOC and TOC are stable during

the study period, supported by q-test ($q<q_{7,\,0.99}=0.68$). Free and total chlorine increased quickly after chlorine addition to the pool, and gradually decreased after 12 h addition of chlorine. The concentration of 2,6-DCBQ increased steadily and peaked at 36 h (Figure 3.6). This was consistent with the results from our previous study that the maximal HBQ concentration was obtained after a 36-h reaction of chlorine with phenol.[1] TriCBQ and DMDBBQ were also detectable in all samples, but their concentrations were very low and did not show any obvious trend. Therefore, we selected 36–40 h as the reaction time for the chlorination experiment.

3.3 RESULTS AND DISCUSSION

3.3.1 Formation of HBQs after chlorination of natural organic matter

Of the four HBQs studied, only one, 2,6-DCBQ, was detected after chlorination of source water samples with or without coagulation. This is in agreement with previous measurements of HBQs in treated water, which indicated 2,6-DCBQ concentrations were considerably higher than those of 2,6-DCMBQ, 2,3,6-TriCBQ, and 2,6-DBBQ.[20,21] 2,6-DCBQ concentrations appear to follow a similar trend to the humic substance and biopolymer concentrations with respect to alum dose in the higher DOC waters: formation decreases with increased alum doses until a plateau is reached at the target alum doses (38 and 25 mg/L alum for Otonabee and Grand River waters, respectively). A small decrease in 2,6-DCBQ formation can be noted for Lake Ontario water, but it exhibits a weaker linear correlation with alum dose when compared to the river waters (Table 3.6). It appears that at bench-scale, coagulation with alum is linked with a reduction in post-chlorination 2,6-DCBQ production. No HBQs were identified in the unchlorinated raw waters; this supports the assumption that 2,6-DCBQ was formed

following chlorination as a DBP. Also no HBQs were identified in travel blanks or alum blanks (coagulant in high purity water), indicating no contribution from shipping or alum addition.

Linear correlations were developed for different water parameters with 2,6-DCBQ formation, both within the individual waters and with all water data pooled (Table 3.7). As shown in Figure 3.7, the humic substance concentration, UV_{254} (for filtered water and for humic OC peak only), and humic SUVA values tend to show moderate correlation ($R^2 > 0.65$) with 2,6-DCBQ formation for individual waters (with the exception of humic substances in Lake Ontario water, likely due to low variation in humic concentration following coagulation at different doses). It has been previously postulated that the most likely source of the HBQs would be the humic fraction – since these generally contain quinone or phenolic moieties,[25] and reaction pathways have been proposed between these compounds and chlorine which lead to the formation of various HBQs.[26] Similarly, the UV_{254} value reflects the extent of conjugation in the dissolved compounds; many π-π^* transitions within conjugated molecules occur close to this wavelength.[27] Quinones, which exhibit strong UV absorption properties in this region, would be expected to be derived from compounds with similar levels of unsaturation (such as phenolic compounds).[21] These results indicate that the majority of 2,6-DCBQ precursors may be found within the humic region (UV-active and larger molecular weight compounds), which is amenable to removal by coagulation. However, humic-indicator values alone are not sufficient to assess the 2,6-DCBQ formation potential of the waters; for example, the humic concentration in Grand River water coagulated with 25 mg/L alum (3.3 mg/L) is very similar to the humic levels in raw Otonabee River water (3.2 mg/L), but the 2,6-

DCBQ formation potentials are very different (32 ± 5 ng/L and 184 ± 30 ng/L, respectively). Indeed, this supports the hypothesis that the larger humics (which are removed in coagulation) may be acting as 2,6-DCBQ precursors while other humic material has lower activity to HBQ formation with chlorination.

Interestingly, while correlations within each water type are strong for the humic-related indicators, the pooled data shows poor overall correlations among all three waters. In contrast, the biopolymer fraction appears to show lower correlations ($R^2 = 0.56–0.74$) with 2,6-DCBQ formation in individual waters, but is the strongest overall predictor of 2,6-DCBQ formation when data is pooled between the three waters ($R^2 = 0.78$; Table 3.7 and Figure 3.8). Additionally, the biopolymer fraction was determined to be the most significant factor for 2,6-DCBQ formation (for pooled data in the three waters) using multiple linear regression with SPSS (Version 13, IBM Corporation). Initially, independent variables with simple linear regression significance $p \leq 0.20$ (Table 3.8) were selected. A forward stepwise method was used to select relevant independent variables during multiple linear regression. This allows for optimization of the regression according to statistical significance (determined by an F statistic, defined as the regression sum of squares divided by the mean squared error) while allowing for the smallest number of variables to be used.[28] The most significant model formula was found to be a simple linear equation, containing only biopolymers as an independent variable ($p < 0.001$, $R^2 = 0.78$): 2,6-DCBQ [ng/L] = 464 ×(Biopolymers [mg/L]) – 40.4.

Biopolymers initially were not hypothesized as major contributors to 2,6-DCBQ formation, due to the types of compounds eluted in this peak. The biopolymer fraction, which elutes first in the LC-OCD, is assumed to consist of high molecular weight

compounds (>10 kDa) such as polysaccharides, proteins, and some amino sugars.[4] According to the chromatographically-separated UV spectra (produced along with the organic carbon chromatograms in the LC-OCD), the biopolymer fraction shows little UV absorbance at 254nm (Figure 3.9); this indicates a lack of aromatic and conjugated groups in the compounds, and thus a lack of unsaturated precursors previously considered necessary to form haloquinones. Additionally, the small ON peak suggests that organic nitrogen (potentially within proteins) is present in these compounds. While biopolymers comprise a small proportion of NOM present in untreated surface waters, they make up the bulk of soluble microbial products (SMP) which are organic compounds that are produced during metabolism processes by biomass. Chemical analogues of the SMP portions of NOM (including glucose, starch, and albumin) have been previously shown to form structurally smaller DBPs such as haloacetic acids (HAAs) and trihalomethanes (THMs).[29,30] However, these DBPs lack the more complex, aromatic structure of the quinones. One possible interpretation could be that the biopolymers may not be directly contributing to the formation of the HBQs; instead, the presence of the biopolymers may improve the removal of small concentrations of HBQ precursors (which are potentially scattered among different NOM fractions) by enhancing the coagulation process. Organic polymers, including synthetic compounds like polyDADMAC[31] or naturally-derived compounds like chitosan[32] which are chemically similar to the biopolymeric NOM fraction have been used in drinking water treatment, either as primary coagulants or in conjunction with metal salts. Mechanistically, they act through adsorption to surfaces of contaminants and by forming bridges between particles[33]. Therefore in the present study, as the biopolymers are removed through coagulation in metal hydroxide flocs, they may

be interacting with HBQ precursors through particle bridges and co-removing these compounds. As such, the biopolymers are likely not the sole NOM fraction which contributes to DBCQ formation.

In both river waters, however, even after coagulation-flocculation at the highest alum doses, a small amount of 2,6-DCBQ is formed and detected after chlorination: 42 ± 10 ng/L in Otonabee River water and 24 ± 5 ng/L in Grand River water (2,6-DCBQ concentrations after 35 mg/L alum coagulation in Lake Ontario water averaged at 3 ± 3 ng/L, due to measurements found below detection limit). Therefore, some portions of NOM which are recalcitrant to coagulation appear to contribute to 2,6-DCBQ formation. These precursors could include smaller molecular weight humics, or LMW neutral organics (for example, phenol, a known precursor to 2,6-DCBQ, is postulated to elute within these regions). NOM fractions with smaller molecular weight (building blocks, LMW acids and neutrals, and hydrophobic DOC) are poorly removed during coagulation; as such, their concentrations vary little over the study and their contributions to HBQ formation cannot be clearly assessed.

3.3.2 Formation of HBQs after chlorination of anthropogenic materials

Detection of three additional HBQs in the swimming pools but not in the input water led to the investigation into what organic compounds in swimming pools may contribute to the formation of HBQs. Average DOC in the pools was 7.5 mg/L, while the average DOC in the input tap waters was 2.2 mg/L. Addition of free chlorine to the tap water only increased the concentration of 2,6-DCBQ but did not result in the formation of other HBQs (Table 2.5). This suggested that other organic precursors for HBQs may be present in the pools but not in the tap water.

Previous studies on DBPs in swimming pools have suggested that human fluids such as urine are an important source of precursors.[34,35] To examine the formation of HBQs from urine, we collected and mixed urine samples from twenty healthy individuals. Mixed urine samples and commercial synthetic urine were chlorinated and analyzed for HBQs. No HBQs were detected in the urine samples with or without chlorination, which reveals that urine is not the main source of HBQ formation detected in the pools. PCPs are also a possible source of HBQ precursors in swimming pool water. Considering that swimmers may have PCPs on their skin, we decided to focus on lotions and sunscreens that are likely to contain organic compounds consisting of benzoquinone/phenol structures.[36] We chose four body lotions and four sunscreens commonly available from supermarkets. Before testing the formation of HBQs from these PCPs, we performed experiments to determine control parameters for comparison. We determined DOC and UV_{254} absorbance of each samples when 0.01, 0.025, 0.05, and 0.1 g of PCPs were dissolved in 400 mL of pure water. As shown in Figure 3.5, the DOC and UV_{254} of each PCP are linearly correlated with the spiked dose of the PCP from 0.01 to 0.1 g in 400 mL of Optima water. These results suggest that the PCPs were well dissolved in the water within the concentrations studied. Table 3.5 summarizes the chlorine demand at a given dose and the measurements of pH before and after the chlorination reaction.

To compare the HBQ formation potential of different PCPs from chlorination, we prepared samples containing the same DOC content of 5 mg/L and maintained the same free chlorine residual at 3.0±0.1 mg/L. Figure 3.10 shows the concentrations of HBQs produced from the chlorination of samples (at DOC 5 mg/L) of lotions, sunscreens, or

urine, compared to those in tap water. All chlorinated samples of lotions and sunscreens produced 2,6-DCBQ. Sunscreen #4 (S4) generated the highest 2,6-DCBQ concentration (5420±373 ng/L). The 2,6-DCBQ concentrations produced from the other lotions and sunscreens were at similar levels (18±6 to 194±6 ng/L). This result indicated that lotions and sunscreens introduced by swimmers may increase the formation of 2,6-DCBQ. Besides 2,6-DCBQ, some of these chlorinated PCP samples contained other HBQ species. DCMBQ was detected in all four lotion samples and two sunscreen samples, ranging from <0.1 to 4.0±0.6 ng/L. TetraB-1,4-BQ was detected in all four sunscreen samples, ranging from 0.9±1.0 to1.6±1.4 ng/L, but it was not detectable in any lotion samples. TriCBQ was found in two sunscreen samples (S2: 3.5±0.5 ng/L, S4: 30.7±7.6 ng/L). These results support that lotions and sunscreens are precursors for HBQs other than 2,6-DCBQ, such as TriCBQ in swimming pool water.

The difference in HBQ formation from the PCPs tested is possibly due to differences in the active ingredients containing aromatic structures in each PCP. Previous studies demonstrated that phenols and quinones are likely to be the precursors of HBQs.[20,37,38] Some common ingredients of lotions, such as benzyl alcohol, lecithin, parabens, perfume, and some plant extracts containing vitamins, amino acids, peptides, and proteins may act as HBQ precursors. UV filters in sunscreens, such as avobenzone, octocrylene, and terephthalilidene dicamphor sulfonic acid may also be HBQ precursors. Based on the information on the labels, the ingredients that may serve as HBQ precursors in the tested PCPs are summarized in Table 3.9. Specific differences in PCPs are noted here. S4 contains only organic UV filters and no inorganic UV filter. Its active ingredients are octinoxate, bisoctrizole, and bemotrizinol, completely different from the other three

sunscreens. The higher content of these aromatic compounds may be the reason for the higher formation of HBQs from S4.

Using the method of SPE with LC-MS/MS for determination of eight HBQs described in Chapter 2, we have successfully studied HBQ formation in swimming pools. Some unique HBQs in swimming pools can be produced from the PCPs, demonstrating potential human contribution to DBPs in swimming pools. Higher DOC requires higher chlorine dose to disinfect microorganisms, which may increase the formation of HBQs. Warm water providing a comfortable environment for swimmers may accelerate the chlorination reaction to produce more HBQs. The results are useful to the management of swimming pool disinfection practice and the assessment of human exposure through various routes.

3.4 CONCLUSION

In the current study, 2,6-DCBQ was formed after bench-scale chlorination of three raw surface waters with or without coagulation. Several measurements of humic contents showed moderate correlation with 2,6-DCBQ formation within individual waters, including humic substance concentration (except in low DOC Lake Ontario water), UV absorption of filtered water and humic portion only, and humic SUVA as detected via LC-OCD-OND. The biopolymeric fraction of NOM was moderately correlated to 2,6-DCBQ formation in pooled data between all waters; this indicated that biopolymeric NOM may be an important source of 2,6-DCBQ precursors. In addition, we discovered that personal care products (PCPs) such as lotions and sunscreens can serve as precursors to form additional HBQs after chlorination, such as TriCBQ, DCMBQ, and TetraB-1,4-BQ. The

results explain why some HBQs were formed in swimming pools but not in the input water.

3.5 REFERENCES

1. Bond, T.; Heniriet, O.; Goslan, E. H.; Parsons, S. A.; Jefferson, B. Disinfection byproduct formation and fraction behavior of natural organic matter surrogates. *Environ Sci Technol* **2009**, *43* (15), 5982–5989.
2. Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.; Sclimenti, M. J.; Onstad, G. D.; Thruston Jr., A. D. Occurrence of a new generation of disinfection byproducts. *Environ Sci Technol* **2006**, *40* (23), 7175–7185.
3. Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; MacCarthy, P. An introduction to humic substances in soil, sediment and water. In: *Humic Substances in Soil, Sediment and Water;* John Wiley and Sons: New York, NY, 1985; pp 1.
4. Huber, S. A.; Balz, A.; Abert, M.; Pronk, W. Characterization of aquatic humic and non-humic matter with size-exclusion chromatography – organic carbon detection – organic nitrogen detection (LC-OCD-OND). *Water Res* **2011**, *45* (2), 879–885.
5. Baghoth, S. A.; Dignum, M.; Grefte, A.; Kroesbergen, J.; Amy, G.L. Characterization of NOM in a drinking water treatment process train with no disinfectant residual. *Wat Sci Technol: Wat Supply* **2009**, *9* (4), 379–386.
6. Wassink, J.K.; Andrews, R.C.; Peiris, R.H.; Legge, R.L. Evaluation of fluorescence excitation-emission and LC-OCD as methods of detecting removal of NOM and DBP precursors by enhanced coagulation. *Wat Sci Technol: Wat Supply* **2011**, *11* (5), 621–630.
7. Hu, J. Y.; Aizawa, T.; Ookubo, S. Products of aqueous chlorination of bisphenol A and their estrogenic activity. *Environ Sci Technol* **2002**, *36* (9), 1980–1987.
8. Mitch, W. A.; Sedlak, D. L. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environ Sci Technol* **2002**, *36* (4), 588–595.
9. Esbenshade, J. L.; Cardoso, J. C.; Zanoni, M. V. B. Removal of sunscreen compounds from swimming pool water using self-organized TiO_2 nanotubular array electrodes. *J Photochem Photobio A* **2010**, *214* (2-3), 257–263.

10. Brausch, J. M.; Rand, G. M. A review of personal care products in the aquatic environment: environmental concentrations and toxicity. *Chemosphere* **2011**, *82* (11), 1518–1532.
11. Weng, S. C.; Li, J.; Blatchley, E. R. Effects of UV_{254} irradiation on residual chlorine and DBPs in chlorination of model organic-N precursors in swimming pools. *Water Res* **2012**, *46* (8), 2674–2682.
12. De Laat, J.; Feng, W. T.; Freyfer, D. A.; Dossier-Berne, F. Concentration levels of urea in swimming pool water and reactivity of chlorine with urea. *Water Res* **2011**, *45* (3), 1139–1146.
13. Balmer, M. E.; Buser, H. R.; Muller, M. D.; Poiger, T. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. *Environ Sci Technol* **2005**, *39* (4), 953–962.
14. Poiger, T.; Buser, H. R.; Balmer, M. E.; Bergqvist, P. A.; Muller, M. D. Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. *Chemosphere* **2004**, *55* (7), 951–963.
15. Giokas, D. L.; Slvador, A; Chisvert, A. UV filters: From sunscreens to human body and the environment. *Trends Anal Chem* **2007**, *26* (5), 360–374.
16. Terasaki, M.; Makino, M. Determination of chlorinated by-products of parabens in swimming pool water. *Int J Environ An Ch* **2008**, *88* (13), 911–922.
17. Kanan, A.; Karanfil, T. Formation of disinfection by-products in indoor swimming pool water: the contribution from filling water natural organic matter and swimmer body fluids. *Water Res* **2011**, *45* (2), 926–932.
18. Wassink, J.K.; Andrews, R.C.; Peiris, R.H.; Legge, R.L. Evaluation of fluorescence excitation-emission and LC-OCD as methods of detecting removal of NOM and DBP precursors by enhanced coagulation. *Wat Sci Technol: Wat Supply* **2011**, *11* (5), 621–630.
19. American Public Health Association (APHA); American Water Works Association (AWWA); Water Environment Federation (WEF). *Standard Methods for the Examination of Water and Wastewater*, 21st ed. Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg. A.E., Eds. Washington, USA, 2005.

20. Zhao, Y.; Anichina, J.; Lu, X. F.; Bull, R. J.; Krasner, S. W.; Hrudey, S. E.; Li, X.-F. Occurrence and formation of chloro- and bromo-benzoquinones during drinking water disinfection. *Water Res* **2012**, *46* (14), 4351–4360.
21. Zhao, Y., Qin, F., Boyd, J.M., Anichina, J.; Li, X.-F. Characterization and determination of chloro- and bromo-benzoquinones as new chlorination disinfection by-products in drinking water. *Anal Chem* **2010**, *82* (11), 4599–4605.
22. Li, Q.; Osborne, M.C.; Smith, I.W.M. Rate constants for the reactions of Cl atoms with HCOOH and with HOCO radicals. *Int J Chem Kinet* **2000**, *32* (2), 85–91.
23. Yu, H.-G.; Francisco, J.S.; Muckerman, J.T. Ab initio and direct dynamics study of the reaction of Cl atoms with HOCO. *J Chem Phys* **2008**, *129* (064301), 1–7.
24. World Health Organization. *Guidelines for Safe Recreational Water Environments. volume 2, Swimming Pools and Similar Environments*; WHO Press: Geneva, 2006.
25. Tan, K.H. *Humic Matter is Soil and the Environment*. Marcel Dekker: New York, NY, 2003.
26. Bull, R.J.; Reckhow, D.A.; Li, X.-F.; Humpage, A.R.; Joll, C.; Hrudey, S.E. Potential carcinogenic hazards of non-regulated disinfection by-products: haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology* **2011**, *286* (1-3), 1-19.
27. Workman, J. Jr. *The Handbook of Organic Compounds: NIR, IR, Raman, and UV-Vis Spectra Featuring Polymers and Surfactants*. Academic Press: San Diego, CA, 2001.
28. Neter, J.; Wasserman, W.; Kutner, M. H. *Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs*, 3rd ed.; Richard, D., Ed.; Irwin Inc.: Homewood, IL. 1990.
29. Liu, J.-L.; Li, X.-Y. Biodegradation and biotransformation of wastewater organics as precursors of disinfection by-products in water. *Chemosphere* **2010**, *81* (9), 1075–1083.
30. Wei, Y.-Y.; Liu, Y.; Zhang, Y.; Dai, R.-H.; Liu, X.; Wu, J.-J.; Zhang, Q. Influence of soluble microbial products (SMP) on wastewater disinfection by-products: trihalomethanes and haloacetic acid species from the chlorination of SMP. *Environ Sci Pollut Res* **2011**, *18* (1), 46–50.

31. Treguer, R.; Couvert, A.; Wolbert, D.; Suty, H.; Randon, G. Particulate products and new polymers for a more efficient removal of dissolved organic matter in drinking water resources. *Environ Technol* **2007**, *28* (8), 861–869.
32. Brown, T. J.; Emelko, M. B. Chitosan and metal salt coagulant impacts on *Cryptosporidium* and microsphere removal by filtration. *Water Res* **2009**, *43* (2), 311–338.
33. O'Melia, C. R. Coagulation and flocculation. In *Physicochemical Processes for Water Treatment Control*; W. J. Weber, Ed.; Wiley: New York 1972; 61–109.
34. Blatchley, E. R.; Cheng, M. M. Reaction mechanism for chlorination of urea. *Environ Sci Technol* **2010**, *44* (22), 8529–8534.
35. Xiao, F.; Zhang, X. R.; Zhai, H. Y.; Lo, I. M. C.; Tipoe, G. L.; Yang, M. T.; Pan, Y.; Chen, G. H. New Halogenated disinfection byproducts in swimming pool water and their Permeability across skin. *Environ Sci Technol* **2012**, *46* (13), 7112–7119.
36. Brausch, J. M.; Rand, G. M. A review of personal care products in the aquatic environment: Environmental concentrations and toxicity. *Chemosphere* **2011**, *82* (11), 1518–1532.
37. Heasley, V. L.; Fisher, A. M.; Herman, E. E.; Jacobsen, F. E.; Miller, E. W.; Ramirez, A. M.; Royer, N. R.; Whisenand, J. M.; Zoetewey, D. L.; Shellhamer, D. F. Investigations of the reactions of monochloramine and dichloramine with selected phenols: examination of humic acid models and water contaminants. *Environ Sci Technol* **2004**, *38* (19), 5022–5029.
38. Kutyrev, A. A. Nucleophilic reactions of quinones. *Tetrahedron* **1991**, *47* (38), 8043–8065.

Table 3.1 Raw water characteristics.

Parameter	Otonabee River	Grand River	Lake Ontario
pH	8.0	8.1	8.2
DOC (mg/L)	6.42	6.95	1.89
Alkalinity (mg/L as $CaCO_3$)	100	260	116
UV_{254} (cm^{-1})	0.129	0.213	0.022
SUVA (L/mg C/m)	2.01	3.06	1.16
Hydrophobic DOC (mg/L)	1.11	0.52	0.32
Biopolymers (mg/L)	0.48	0.40	0.16
Humic substances (mg/L)	3.21	4.23	0.70
Building blocks (mg/L)	0.85	0.91	0.46
Low molecular weight acids (mg/L)	0.22	0.17	0.08
Low molecular weight neutrals (mg/L)	0.55	0.71	0.18
UV_{254} for humics (cm^{-1})	0.114	0.175	0.012
Humic SUVA (L/mg C/m)[a]	3.56	4.13	1.72
Humic molecular weight (g/mol)	608	633	590
USEPA Enhanced Coagulation TOC reduction target (1999)[b]	35%	25%	25%[c]
Alum dose required to meet target (mg/L)	38.0	25.0	25.0

[a] Calculated using LC-OCD software as UV_{254} absorbance for the humic fraction, multiplied by 100 and divided by the concentration of humic substances.

[b] DOC was used instead of TOC for increased analytical precision and correlation with LC-OCD, which requires filtration.

[c] Lake Ontario water has a low organic carbon content and SUVA (<2mg/L and <2, respectively), thus it is exempt from USEPA guidelines. For inclusion in the experiments, it was approximated to the nearest appropriate treatment category based on alkalinity.

Table 3.2 Preliminary jar test results for Otonabee River water + alum.

Alum dose (mg/L)	DOC (mg/L)	% reduction DOC
0	6.50	-
32	4.34	33%
34	4.34	33%
36	4.44	32%
38[a]	4.18	36%
40	4.09	37%
42	3.99	39%

[a] Dose required to attain 35% reduction in DOC

Table 3.3 Preliminary jar test results for Lake Ontario + alum.

Alum dose (mg/L)	DOC (mg/L)	% reduction DOC
0	1.97	-
10	1.74	12%
20[a]	1.51	24%
30[b]	1.46	26%
40	1.45	27%
50	1.38	30%
60	1.31	34%

[a, b] Dose required to attain 25% DOC reduction falls between these doses

Table 3.4 Preliminary jar test results for Grand River water + alum.

Alum dose (mg/L)	DOC (mg/L)	% reduction DOC
0	7.29	-
10	6.42	12%
20[a]	5.83	20%
30[b]	5.28	28%
40	4.68	36%
50	4.43	39%
60	4.29	41%

[a, b] Dose required to attain 25% DOC reduction falls between these dose

Table 3.5 Experiment parameters during the chlorination of personal care products.

Sample	Dose mg/L	DOC mg/L	UV_{254}	Cl demand mg/L	pH of PCP solution	pH before chlorination	pH after chlorination
L1	74.4	5.0	0.041	2.32	5.01	7.00	6.38
L2	125	5.0	0.071	6.18	5.12	7.07	6.56
L3	167	5.0	0.120	6.05	5.25	7.01	7.52
L4	53.	5.0	0.033	2.14	5.30	7.10	6.89
S1	36.5	5.0	0.006	2.65	5.31	7.01	7.02
S2	48.2	5.0	0.011	1.58	5.50	7.05	7.02
S3	42.5	5.0	0.009	2.69	5.15	7.07	7.02
S4	43.1	5.0	0.026	2.49	5.31	7.01	7.01
Blank	0	0.5	0	0	4.96	7.03	7.03

We first established a linear calibration curve of DOC versus the dose of PCP for each PCP. Based on the calibration curve, we calculated the dose of PCP required providing DOC of 5 mg/L. Chlorine demand is tested to determine an appropriate initial chlorine dose and to maintain the free chlorine residual at 3.0±0.1 mg/L. pH was adjusted to 7.0 with 0.1 mol/L HCl.

Table 3.6 R-squared values for linear correlations between applied alum dose (independent variable) and various measured water parameters in different water types (n = 13 to 16)

Parameter	Otonabee River	Grand River	Lake Ontario
UV	0.93	0.87	0.85
SUVA	0.87	0.93	0.40
Humic UV	0.93	0.71	0.77
Humic SUVA	0.92	0.98	0.86
Total DOC	0.93	0.68	0.88
Hydrophobic	0.68	0.95	0.07
Biopolymer	0.89	0.93	0.89
Humic substances	0.93	0.03	0.29
Building blocks	0.03	0.03	0.38
LMW acids	0.03	0.43	0.07
LMW neutrals	0.43	0.83	0.00
2,6-DCBQ	0.83	0.92	0.56

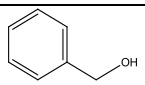
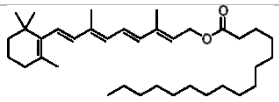
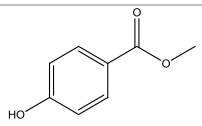
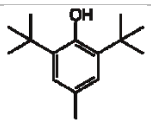
Table 3.7 R-squared values for linear correlations between 2,6-DCBQ formation (dependent variable) and NOM measurements (set as independent variables) for three waters (n = 13 to 16) and pooled data (n = 42).

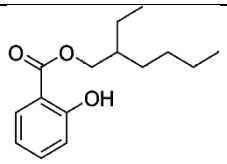
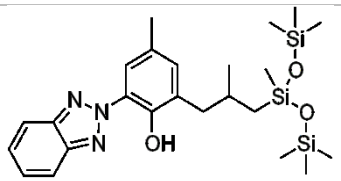
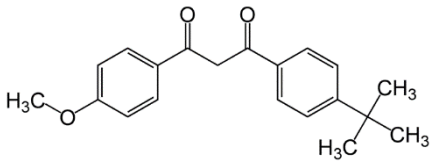
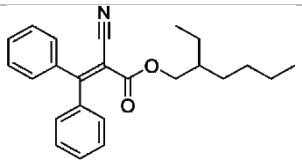
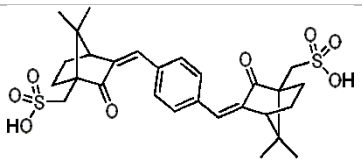
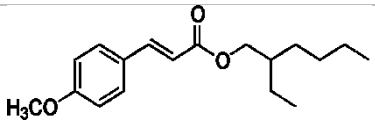
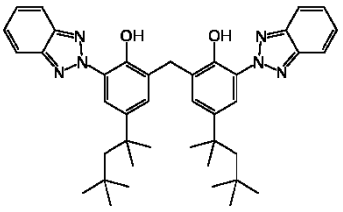
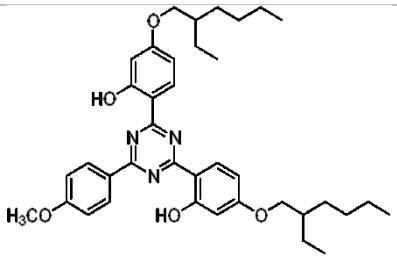
Parameter	Otonabee River	Grand River	Lake Ontario	All waters
UV	0.78	0.85	0.70	0.21
SUVA	0.72	0.53	0.58	0.13
Humic UV	0.77	0.72	0.66	0.20
Humic SUVA	0.89	0.55	0.75	0.26
Total DOC	0.79	0.53	0.43	0.39
Hydrophobic	0.60	0.18	0.03	0.42
Biopolymer	0.74	0.57	0.56	0.78
Humic substances	0.75	0.69	0.10	0.25
Building blocks	0.00	0.27	0.19	0.35
LMW acids	0.02	0.14	0.15	0.37
LMW neutrals	0.42	0.25	0.02	0.20

Table 3.8 Linear regression of related parameters to 2,6-DCBQ formation.

Parameter	Formula	P value	R^2
UV	y = 431.8x + 20.0	0.002	0.207
SUVA	y = 29.93x + 3.0	0.017	0.133
DOC	y = 19.11x – 20.2	<0.001	0.388
Hydrophobic DOC	y = 130.8x – 11.0	<0.001	0.417
Biopolymers	y = 464.9x – 40.4	<0.001	0.778
Humic substances	y = 23.69x + 11.3	<0.001	0.254
Building blocks	y = 145.1x – 50.9	<0.001	0.354
LMW acids	y = 630.5x – 45.5	<0.001	0.372
LMW neutrals	y = 123.3x – 1.8	0.003	0.204

Table 3.9 Ingredients in tested personal care products that could be HBQ precursors

Name	Formula/Structure	Function	Source
Benzyl alcohol			L1,L4
Lecithin	Mixture of fatty substances	Emulsifier, lubricant and surfactant	L2,L3
Perfume	Mixture of fragrant essential oils or aroma compounds, fixatives and solvents	Produce a pleasant scent	L1,L2,L3
Retinyl palmitate		Vitamin A supplement	L2,L3
Methylparaben		Antioxidant	L2,L3
Butylated hydroxytoluene			L2
Collagen amino acids	Mixture of amino acids	Lubricate dry skin and have an emollient texture	L2,L3
Aloe barbadensis	Mixture: may contain amino acids, polysaccharide, Vitamin C, Vitamin E		L3
Helianthus annuus (sunflower) seed oil	Mixture: mainly a triglyceride		L2,L3
Glycine soja (soybean) sterols	Mixture		L2,L3
Sativus (cucumber) extract	Mixture: may contain amino acids, proteins, lipids, vitamin C		L3

Name	Formula/Structure	Function	Source
Octisalate		UV filter	S2
Drometrizole trisiloxane			S1,S2,S3
Avobenzone (4-tert-butyl-4-methoxydibenzoyl methane, BDM)			S1,S2,S3
Octocrylene (OC)			S1,S2,S3
Terephthalylidene dicamphor sulfonic acid (TDSA)			S1,S2,S3
Octinoxate			S4
Bisoctrizole			S4
Bemotrizinol			S4

The ingredients are according to the composition lists of the products

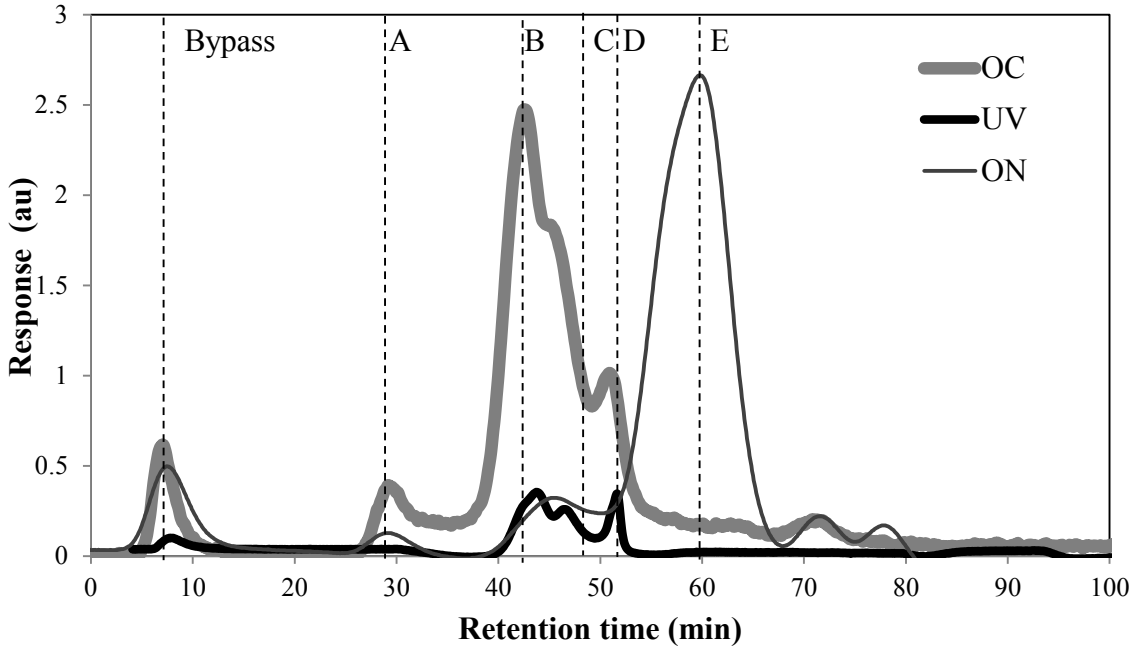


Figure 3.1 Spectrum of LC-OCD-UVD detection.

OC: organic carbon content; UV: ultraviolet absorbance; ON: organic nitrogen content; A: biopolymers; B: humic substances; C: building blocks; D: low-molecular-weight acids; E: low-molecular-weight neutrals

The fraction A to E is defined according to retention time and divided by peak boundary or peak slope.

Biopolymers: polysaccharides with some contribution from nitrogen-containing material such as proteins or amino sugars. The fraction elutes close to the exclusion volume of the SEC column indicating it is a hydrophobic fraction with a molecular weight of 10 kDa or higher.

Humic substances: the end product of decaying organic matter. Its retention time is 45 min and showed strong UV absorbance and high organic carbon content.

Building blocks: Breakdown products of humic substance, namely, humic-substance-like material of lower molecular weight. The left boundary of this fraction is defined by the right slope of the HS-fraction, the right boundary is defined by the low molecular weight acids fraction.

Low-molecular-weight acids: Anions at the neutral pH of the buffer. The left boundary of this fraction is defined by the right slope of the building blocks fraction, the right boundary is defined by a vertical line to the baseline at the maximum curvature of Low-molecular-weight neutrals fraction.

Low-molecular-weight neutrals: alcohols, aldehydes, ketones, sugars and amino acids, which are characterized by low molecular weight and low ion density. The left boundary of this fraction is defined by the vertical line to the baseline at the maximum curvature of the LMW-acid peak, the right boundary is defined as the baseline once it is reached after completion of analysis.

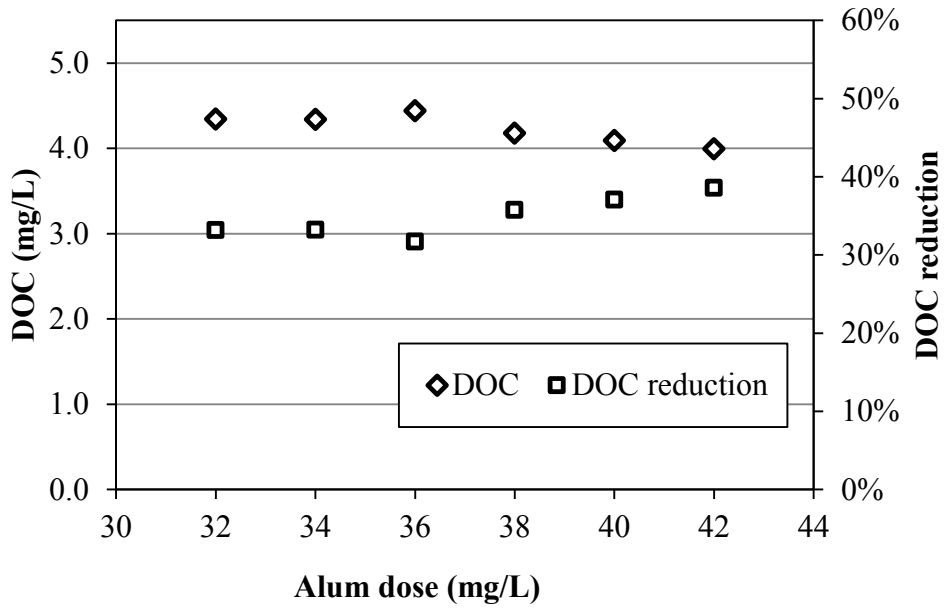


Figure 3.2 Preliminary jar test plot for Otonabee River water + alum.

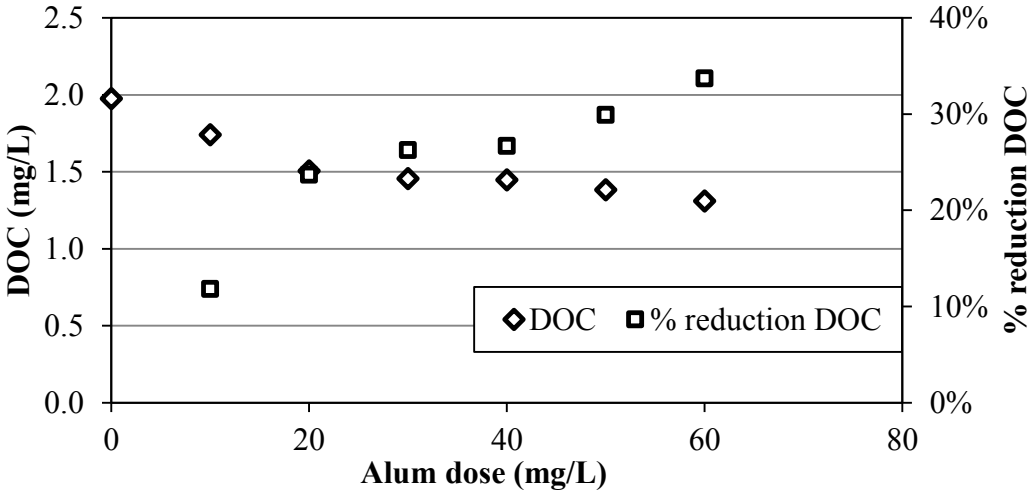


Figure 3.3 Preliminary jar test plot for Lake Ontario water + alum.

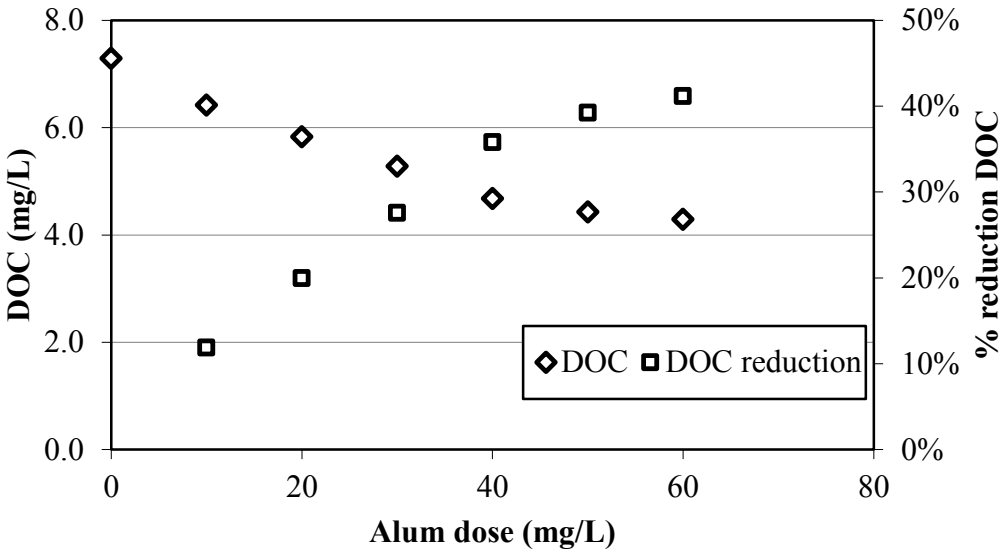


Figure 3.4 Preliminary jar test plot for Grand River water + alum.

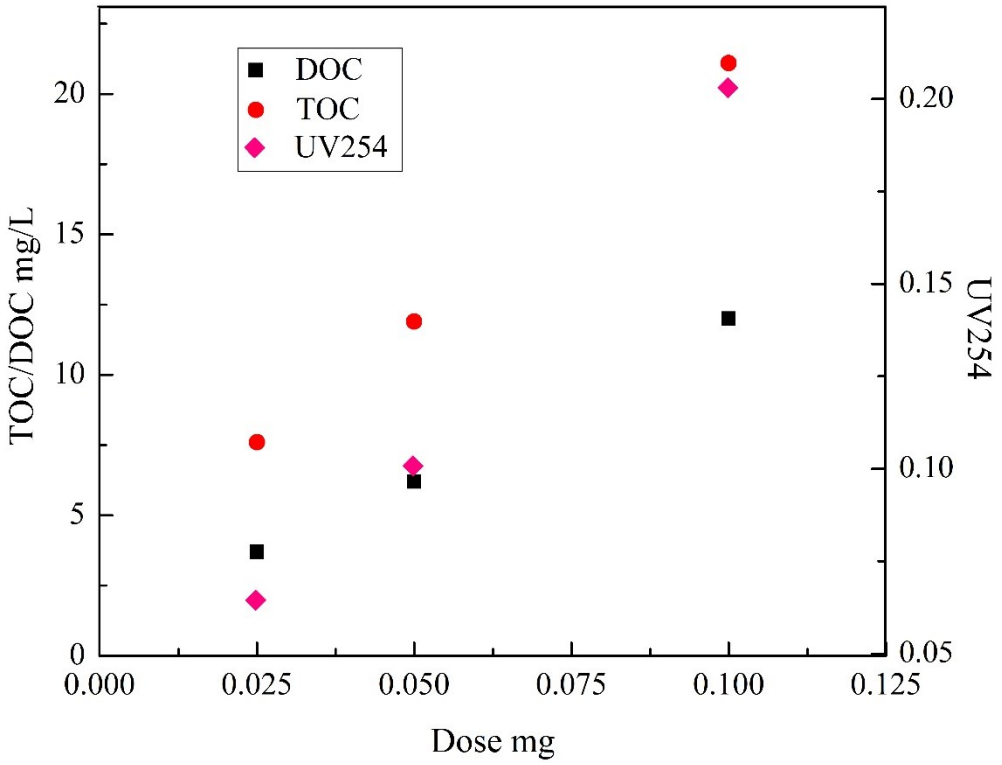


Figure 3.5 TOC, DOC and UV254 as a function of spiking dose of Lotion #1 (L1).

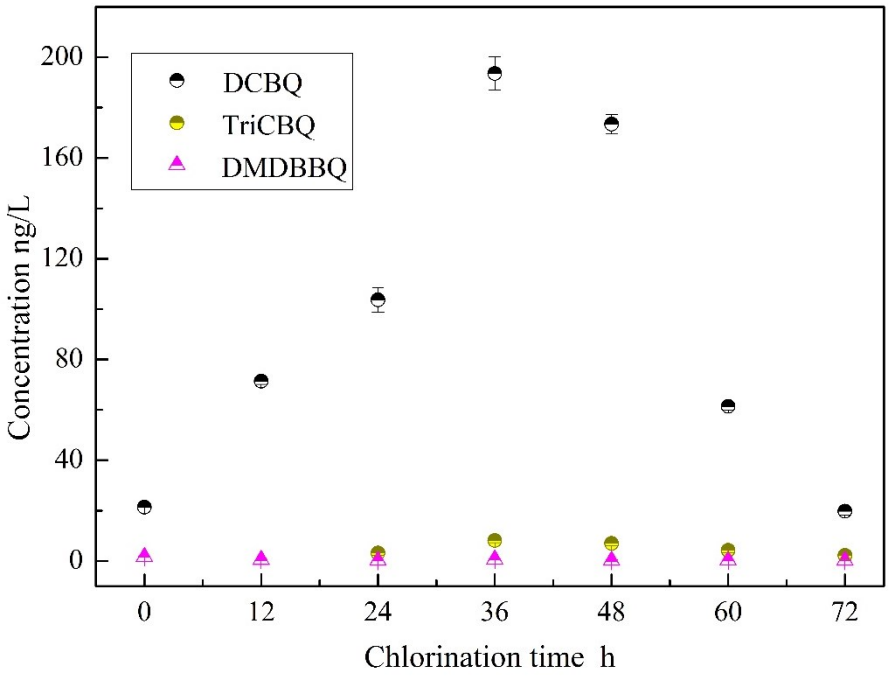
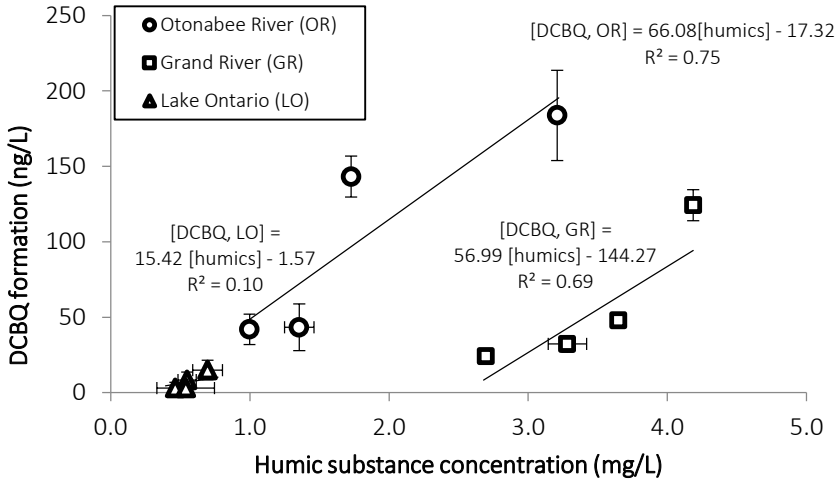
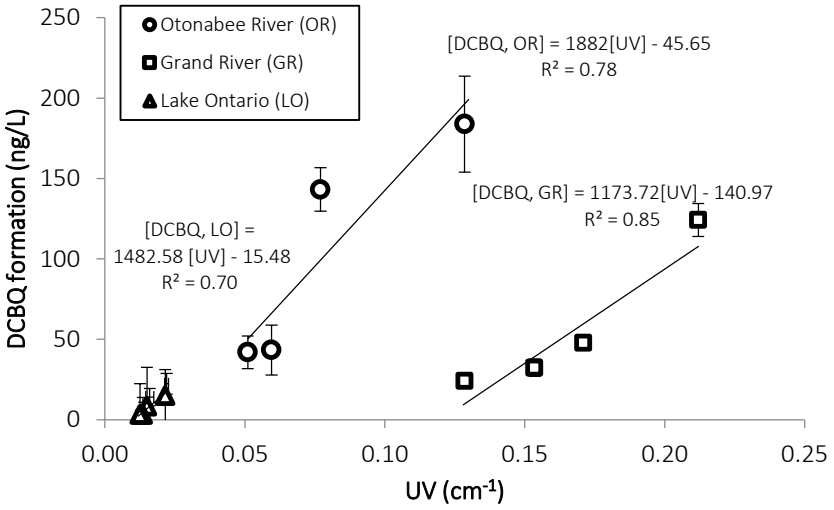
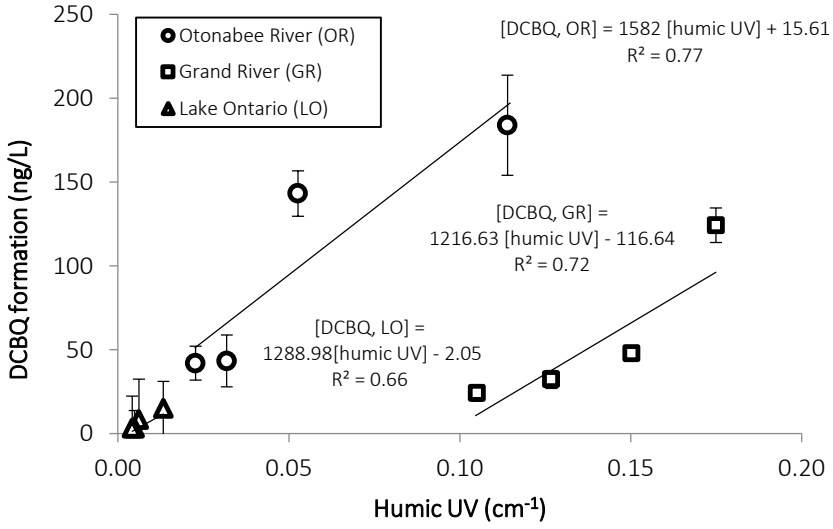


Figure 3.6 HBQ formation during a chlorination cycle in swimming pool #5.
Pool #5 is disinfected by chlorine alone. The time of pumping the bleach to the pool is determined according to the result of special test strips. We studied the pool for a period of 72 h. The first sample (0 h) was before the bleach was added. Additional samples were taken every 12 hours. A second dose of bleach was added to the pool after the 60 h sample had been collected.


Otonabee River (OR)
Grand River (GR)
Lake Ontario (LO)
[DCBQ, OR] = 66.08[humics] - 17.32
R² = 0.75
[DCBQ, LO] =
15.42 [humics] - 1.57
R² = 0.10
[DCBQ, GR] =
56.99 [humics] - 144.27
R² = 0.69
DCBQ formation (ng/L)
Humic substance concentration (mg/L)


Otonabee River (OR)
Grand River (GR)
Lake Ontario (LO)
[DCBQ, OR] = 1882[UV] - 45.65
R² = 0.78
[DCBQ, GR] = 1173.72[UV] - 140.97
R² = 0.85
[DCBQ, LO] =
1482.58 [UV] - 15.48
R² = 0.70
DCBQ formation (ng/L)
UV (cm-1)


Otonabee River (OR)
Grand River (GR)
Lake Ontario (LO)
[DCBQ, OR] = 1582 [humic UV] + 15.61
R² = 0.77
[DCBQ, GR] =
1216.63 [humic UV] - 116.64
R² = 0.72
[DCBQ, LO] =
1288.98[humic UV] - 2.05
R² = 0.66
DCBQ formation (ng/L)
Humic UV (cm-1)

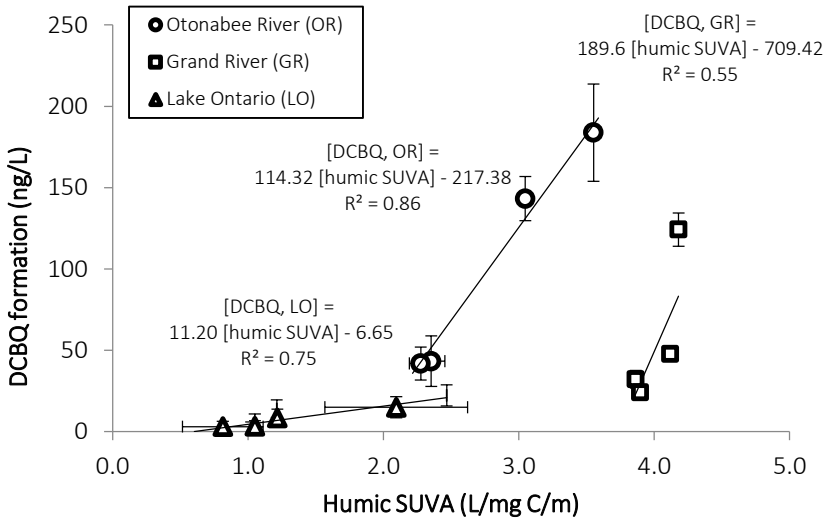


Figure 3.7 Linear correlations between NOM measurements against 2,6-DCBQ formation in three test waters.

a) humic substance concentration, b) UV, c) UV for humic substances and d) SUVA for humics (from LC-OCD-UVD). Vertical error bars are standard deviation of 2,6-DCBQ measurements for replicate jar tests, with two samples from each jar; horizontal error bars are standard deviation of water quality parameters, on sample from each jar.

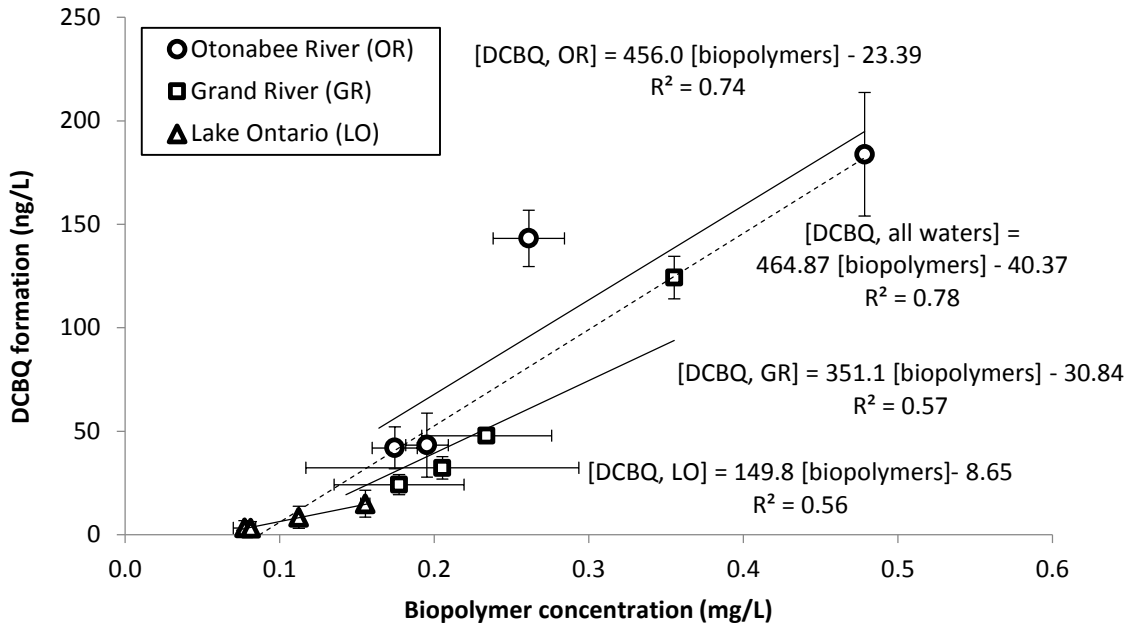


Figure 3.8 Linear correlations between biopolymer concentration against 2,6-DCBQ formation in three test waters.

Vertical error bars are standard deviation of 2,6-DCBQ measurements for replicate jar tests, with two samples from each jar; horizontal error bars are standard deviation of water quality parameters, on sample from each jar.

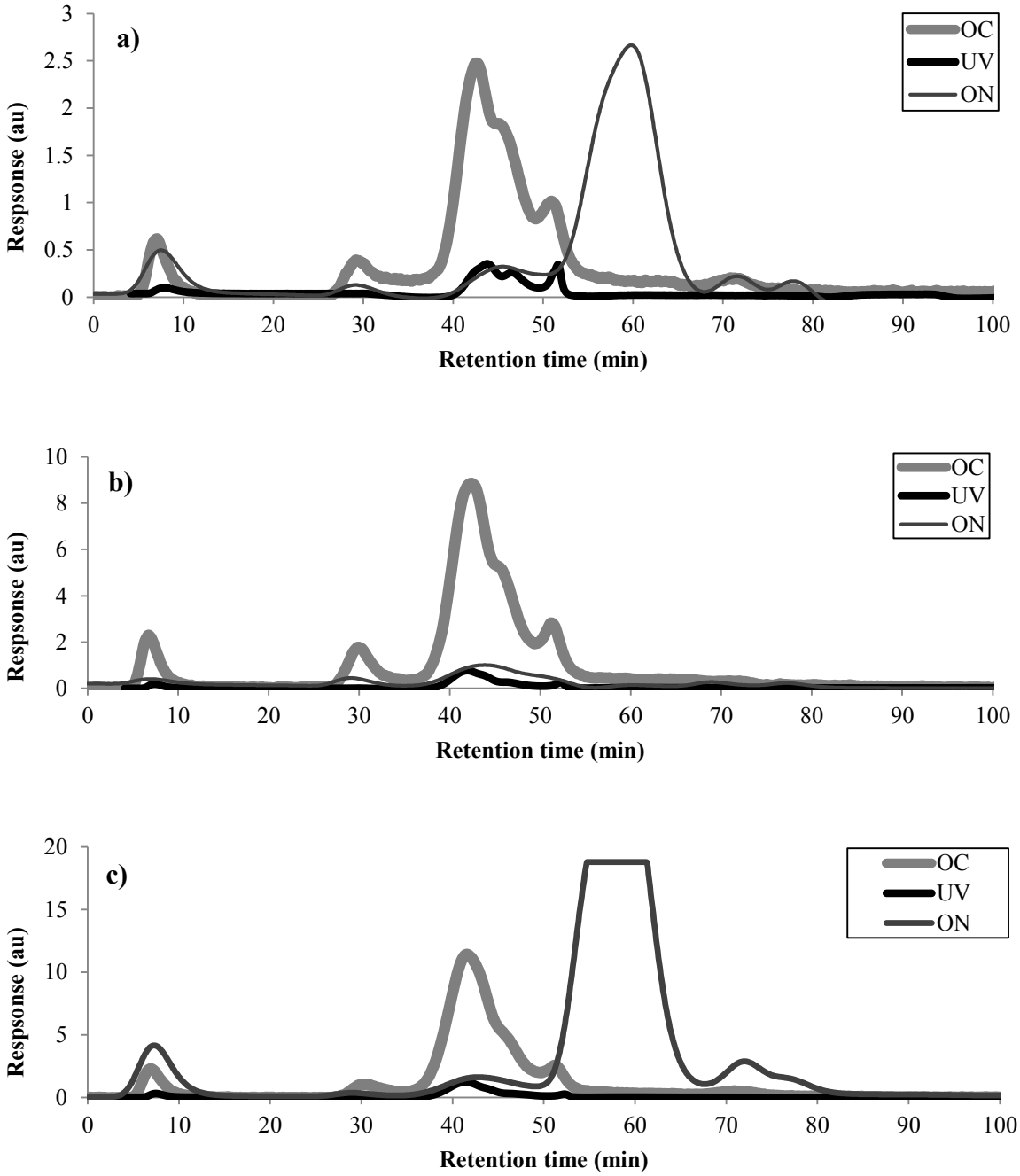


Figure 3.9 LC-OCD-UV-OND chromatograms for three raw waters.

a) Lake Ontario, b) Otonabee River and c) Grand River raw water. Note the different y-axis scales (adjusted to show peak detail). Retention times have been adjusted such that bypass peaks align at approximately 6 min.

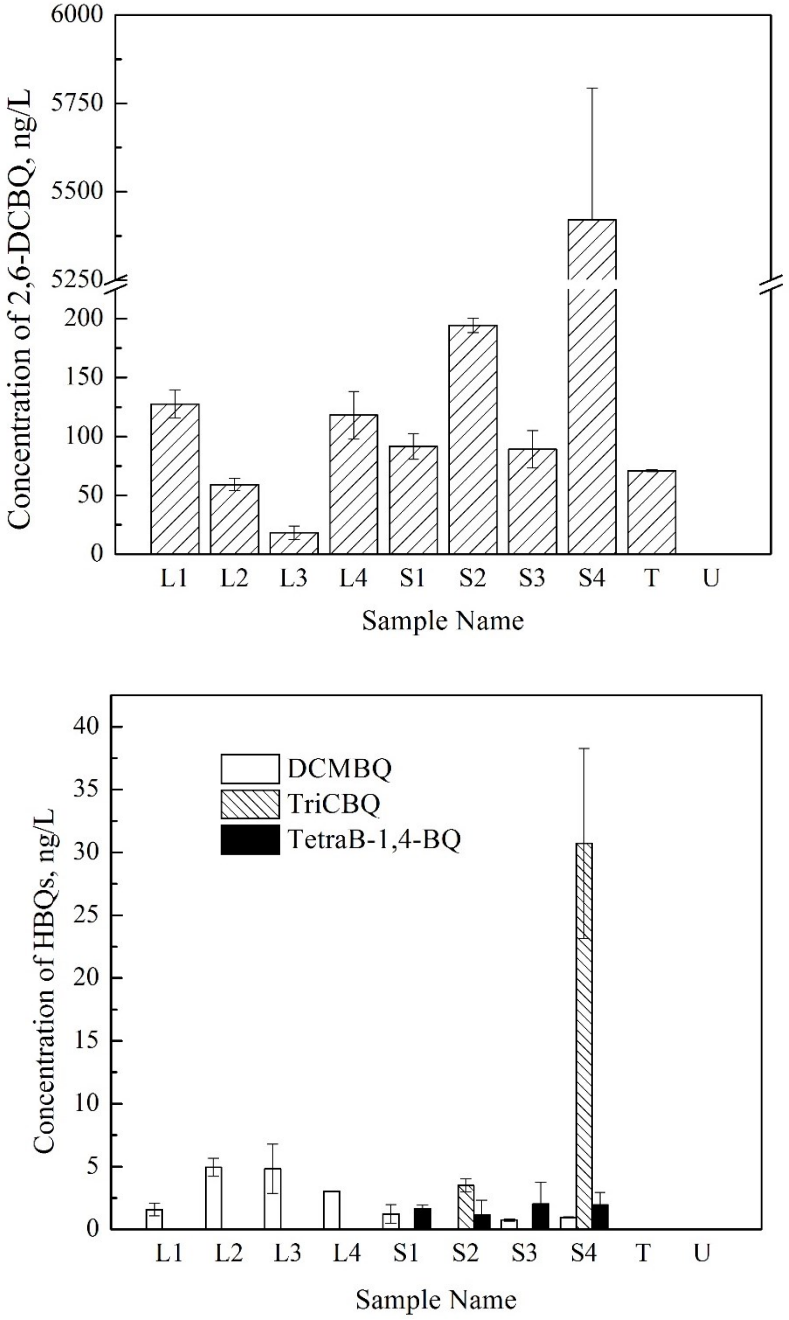


Figure 3.10 Formation of HBQs after chlorination of water containing lotion (L), sunscreen (S), urine (U) or tap water (T).

The samples of lotion, sunscreens, and urine had the same DOC level of 5 mg/L, and tap water had DOC of 2 mg/L.

4 REMOVAL OF HALOBENZOQUINONE PRECURSORS DURING WATER TREATMENT STEPS

4.1 INTRODUCTION

Other studies and my work described in Chapters 2 and 3 have found four HBQs, 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ), 2,3,6-trichloro-1,4-benzoquinone (TriCBQ), and 2,6-dibromo-1,4-benzoquinone (2,6-DBBQ) occurring commonly in drinking water treatment plant effluents at ng/L levels.[1,2] 2,6-DCBQ was detected most frequently and was the most abundant (up to 275 ng/L) in low-bromide waters among the four HBQs.[1,2] The predicted chronic lowest adverse effect levels (LOAELs) for HBQs were 10,000 times lower (i.e., more toxic) than the regulated DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs).[3] The chronic cytotoxicity (IC_{50} at 72 h) of HBQs on Chinese hamster ovary (CHO) cells was significantly higher than that of the regulated DBPs.[2,4] Because of the occurrence and high toxicity of HBQs, there is a need to understand the precursors and their fate through drinking water treatment processes.

In Chapter 3, I demonstrated that a portion of natural organic matter (NOM) (e.g., humic substances) may serve as precursors of 2,6-DCBQ.[5] Phenol, a common organic compound of natural and anthropogenic origin,[6] can react with chlorine or chloramines to form 2,6-DCBQ, TriCBQ, and 2,6-DBBQ (in the presence of bromide).[1] However, no study has examined the occurrence of HBQ precursors in source waters. In addition, a better understanding of the fate of HBQ precursors is necessary to develop strategies to minimize HBQ formation.

Currently, an effective strategy to limit the formation of DBPs in DWTPs is to destroy/remove their precursors during water treatment processes before chlorine is added. Coagulation preferentially removes humic substances and high-molecular-weight NOM, and can remove the precursors for halogenated DBPs such as THMs and HAAs.[7,8] In bench-scale experiments, coagulation using alum was shown to partially remove the precursors of 2,6-DCBQ and the removal efficiency increased with alum dose until it reached a plateau.[5] This is the only study that has reported the impact of a unit water treatment process on HBQ precursors in laboratory experiments. Ozonation can transform humic substances to non-humic substances and can convert high-molecular-weight NOM to low-molecular weight NOM.[9] The formation of THMs was found to be reduced[10] following preozonation in different studies, but no results are available for the effect of ozonation on the formation of HBQs. Moreover, ozonation can cause a shift to the formation of bromine-containing DBPs during subsequent chlorination,[11-14] which may potentially increase the formation of 2,6-DBBQ. Granular activated carbon (GAC) adsorption can remove the precursors of halogenated and non-halogenated DBPs.[15,16] GAC can effectively absorb/remove aromatic compounds (e.g., phenol, polycyclic aromatic hydrocarbons, bisphenol A) from water [17,18] and the adsorption of aromatic compounds on carbon materials was stronger than that of aliphatic compounds.[19] The surface and macropores of GAC can also host microbial communities, where biodegradation may become the dominant mechanism.[13,20] Ultraviolet (UV) disinfection has no impact on the formation of regulated DBPs in water, but recent studies have reported enhanced formation of certain DBPs (e.g., chloropicrin) in water treated with UV, in particular moderate-pressure UV, or UV combined with hydrogen peroxide.[21-23]

In Chapter 4, I investigated precursors of 2,6-DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ using formation potential (FP) tests. Plant influents and effluents after each treatment step prior to chlorination were collected at nine DWTPs in Canada. The HBQ FP results across the different treatment processes were used to evaluate the impact of each treatment process on HBQ precursors. It is important to note that the organic precursors for 2,6-DBBQ should be the same as the precursors for 2,6-DCBQ, when the presence of bromide in source waters results in some 2,6-DBBQ formation.

4.2 EXPERIMENTAL SECTION

4.2.1 Chemicals

2,6-DCBQ was purchased from Sigma-Aldrich (St. Louis, MO); 2,6-DBBQ was obtained from Indofine Chemical Company (Hillsborough, NJ); DCMBQ and TriCBQ were synthesized by Shanghai Acana Pharmtech (Shanghai, China). The water and methanol used in this study were Optima LC–MS grade and were purchased from Fisher Scientific (Fair Lawn, NJ, USA). LC–MS grade formic acid (FA, 49-51%) and sodium hypochlorite (reagent grade, available chlorine 10-15%) were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals used were of analytical grade.

4.2.2 Water sample collection

Water samples were collected from nine DWTPs across Canada in 2013 and 2014. Plant influents and effluents after each treatment step (post coagulation, post ozonation, post filtration, post UV irradiation, and post GAC) were sampled using 2-L amber glass bottles. Treatment processes and the sampling points at the nine DWTPs are presented in Figure 4.1. All samples were collected before chlorination. A travel-blank sample (500

mL of Optima water, 0.25% formic acid) was prepared and transported with all of the samples. Water samples were filtered with a glass microfiber filter (47 mm x 1.5 μm, Waterman) and nylon membrane disc filter (47 mm x 0.45 μm, Pall Corporation) to remove insoluble particles. Samples were stored at 4 ºC prior to use.

4.2.3 FP conditions and procedures

The dissolved organic carbon (DOC) and ammonia concentration of the water samples were determined prior to the FP tests. The dosage of free chlorine was based on the levels of DOC and ammonia and was calculated based on eq (1) below developed by Krasner et al.[24] A free chlorine residual was achieved by adding sufficient chlorine to breakout the ammonia in the water samples. The pH of the samples was initially adjusted to 7 and maintained with addition of phosphate buffer solution (pH = 7). 2,6-DCBQ is stable at pH <6.8, but undergoes hydrolysis at pH 7.6.[1] The FP samples were kept in the dark at room temperature (25 ºC) for 72 h. The pH and free chlorine concentrations were determined immediately after the FP hold time was finished. Formic acid (0.25%, v/v) was added to the FP samples to quench the free chlorine residual and to stabilize the HBQs (lowered the pH to 2.6–2.8).[25] Two FP blank samples containing Optima water were included in each batch of FP tests. If high concentrations of HBQs were detected in FP blank samples, the entire batch of FP experiments from the reaction to the analysis should be rerun. In our experiments, HBQs were not detected in all FP blank samples.

Free chlorine dosage (mg/L as Cl_2) = 3 x DOC (mg/L) + 7.6 x NH_3-N (mg/L) + 10 Eq(1)

4.2.4 Analytical methods

The concentrations of the four HBQs were determined using a solid-phase extraction−high-performance liquid chromatography−tandem mass spectrometry (SPE-

HPLC-MS/MS) method, as described previously.[1] Briefly, HBQs were first extracted from 500 mL of water using Waters Oasis HLB cartridges (6 cc, 200 mg sorbent per cartridge; Milford, MA). The HBQs in the final extracts were separated using an Agilent 1290 liquid chromatography (LC) system (Waldbronn, Germany) with a Luna C18(2) column (100 mm × 2.0 mm i.d., 3 μm; Phenomenex, Torrance, CA). The mobile phase consisted of solvent A (0.1% FA in water) and solvent B (0.1% FA in methanol), and linearly increased B from 20 to 90% in 20 min. Flow rate was 0.17 mL/min, and sample injection volume was 20 μL. A triple quadrupole ion-trap tandem mass spectrometer (MS/MS) (AB SCIEX QTRAP 5500, Concord, ON, Canada) with multiple-reaction monitoring (MRM) methods (negative mode) was used to quantify the four HBQs. Optimized MS conditions and MRM ion pairs have been described previously.[1] To reduce/eliminate matrix effects on the LC-MS/MS accuracy, the standard addition method was applied to quantify the four HBQs. Analyst (1.6, AB Sciex) and MultiQuant (3.0, AB Sciex) software were employed for data acquisition and data analysis.

UV_{254} was measured with a UV/Vis spectrometer (Lambda 35, PerkinElmer) at 254 nm. DOC and ammonia concentrations were determined in the Biogeochemical Analytical Service Laboratory of the University of Alberta. Two sweeteners, sucralose (SUC) and acesulfame (ACE), that are used as indicators for wastewater impact,[26,27] were analyzed using the method described previously.[26]

4.2.5 Quality assurance and quality control

All glassware used in this study were rinsed with Optima methanol and baked at 180 °C overnight prior to use. An SPE blank and a spiked sample were included in each batch of SPE samples. Method blank samples consisting of Optima water/methanol (20/80) with

0.25% FA were analyzed to detect any contamination during LC-MS/MS analysis. Experiments were performed in duplicate or triplicate, and all of the mean values were reported with relative standard deviations less than 5%.

4.3 RESULTS AND DISCUSSION

4.3.1 Measurement of HBQ precursors in DWTP influents

None of the HBQs were detected in the plant influents from the nine DWTPs, which is consistent with the previous studies that suggested that HBQs were formed by chlorination.[1,2] To evaluate the concentration of HBQ precursors in the plant influents, we performed FP tests on the nine plant influents and measured the formation of four HBQs. Table 4.1 presents the results of HBQ FP testing in the nine plant influents. HBQ precursors were present in all of the plant influents. 2,6-DCBQ precursors had the most abundance; FP tests resulted in 2,6-DCBQ concentrations from 16 to 205 ng/L. The wide occurrence of 2,6-DCBQ precursors detected here is consistent with the prevalence of 2,6-DCBQ in DWTP effluents.[1,2] TriCBQ precursors were also present in all nine influents, but at much lower concentrations (3.6–48 ng/L) compared to those of 2,6-DCBQ. DCMBQ and 2,6-DBBQ were detected in 5 of the 9 influent FP samples at concentrations ranging from 1.6 to 4.1 ng/L for DCMBQ and from 1.8 to 8.7 ng/L for 2,6-DBBQ. These results agree with the previous findings that 2,6-DCBQ was the most frequently detected HBQ in low-bromide DWTP effluents and in swimming pool water.[1,2,28]

2,6-DBBQ was only detected in the FP samples of five plant influents where bromide was present (Table 4.1). Formation of TriCBQ and DCMBQ was limited by their precursor concentrations; whereas 2,6-DBBQ was limited by the content of bromide.

Figure 4.2 shows that 2,6-DBBQ formation in FP samples was linearly correlated with the bromide content in the five plant influents with bromide (R^2 of 0.85). This is consistent with previous laboratory disinfection experiments that 2,6-DBBQ formation was linearly correlated with the concentrations of bromide and the precursor phenol.[1] The result also supports the previous finding that Br-DBPs are limited by the initial bromide concentration, whereas the Cl-DBPs would be limited by the organic matter.[20,29] As 2,6-DCBQ and 2,6-DBBQ were formed in the FP tests with bromide-containing waters, then the bromochloro species would also be formed, although not measured in this study due to lack of a standard.

A previous study showed that phenol can react with hypochlorous acid (HOCl) or hypobromous acid (HOBr) to form 2,6-DCBQ or 2,6-DBBQ through similar formation pathways,[1] suggesting compounds with similar aromatic structures may serve as mutual precursors for 2,6-DCBQ and 2,6-DBBQ. The production of 2,6-DBBQ should increase when the source waters contain elevated bromide concentrations. The IC_{50} value of 2,6-DBBQ (19.8 μM) was much lower (more toxic) than most of the regulated DBPs (IC_{50} at mM levels),[2,3] thus it is important to pay attention to bromide concentrations in source waters when selecting disinfection processes. For example, many plants that treat high-bromide waters use chloramines to minimize the formation of halogenated DBPs.[11]

To characterize the nature of the source water NOM and possibly the HBQ precursors, we analyzed the influent samples for DOC, UV_{254}, and specific UV absorbance (SUVA) (Table 4.1). The concentration of DOC in the plant influents ranged from 1.3 to 17.0 mg/L, with a median concentration of 6.5 mg/L. These values are similar to the range of the total organic carbon (TOC) concentrations (3.0–13 [median = 5.8]

mg/L) in the plant influents in a U.S. nationwide survey.[11] The values of UV_{254} in the plant influents were 0.02–0.41 cm^{-1} (median = 0.16 cm^{-1}), and UV_{254} measurements showed a strong positive linear correlation (R^2 = 0.90, Figure 4.3) with DOC concentrations. The value of SUVA in the plant influents ranged from 1.2 to 2.9 L/mg-m, with a median value of 2.1 L/mg-m. These value are low compared to the values (1.9–3.9 [median = 2.9] L/mg-m) in the plant influents in a U.S. nationwide survey.[11] SUVA is an indicator of humic content, which suggests that the Canadian waters studied were lower in humic substances than many of the U.S. waters surveyed. Linear correlations between HBQ FP and DOC or UV_{254} (Figures 4.4 and 4.5) were observed to be fair to good for DCMBQ or 2,6-DBBQ FP (with R^2 of 0.5–0.8), whereas there were fair correlations (R^2 of 0.52 and 0.68, respectively) between 2,6-DCBQ or DCMBQ FP and SUVA (Figure 4.6). This suggests that humic substances (SUVA) may serve as the precursors for 2,6-DCBQ and DCMBQ. However, there was a high degree of scatter. Future studies should examine waters with a wider range of SUVA values. As expected, there was no correlation between 2,6-DBBQ FP and SUVA, because the formation is dependent on the concentration of bromide.

To estimate the impact of wastewater on the HBQ precursors, we analyzed two artificial sweeteners, SUC and ACE, in the plant influents as the markers of wastewater impact on source water.[26] In a U.S. survey, SUC was found to be present in all the source waters with known wastewater impact.[27] HBQ FPs were compared to the concentration of SUC, but there was no correlation (R^2 = 0.07).

4.3.2 Removal of HBQ precursors at various treatment steps

To evaluate the removal of HBQ precursors by coagulation, sand filtration, GAC, ozonation, and UV, we performed HBQ FP tests on the plant influents and the effluents of each treatment process used in the nine DWTPs. Table 4.2 presents the results of HBQ FPs in the plant influents and the effluents after each treatment process, showing the removal or destruction of HBQ precursors by these unit processes. The treatment processes at the nine DWTPs are summarized in Table 4.3. Note, none of the three plants that used GAC removed much DOC. Thus, these three plants were not set up for the adsorption of NOM by GAC. In general, these treatments reduced HBQ FPs to some extent, but none of them appreciably removed HBQ FPs. This indicates that HBQ precursors cannot be well removed with conventional treatment processes.

Coagulation was used at the nine DWTPs in this study. The HBQ FP results of the nine plant influents and the effluents after coagulation showed that coagulation removed on average 31% of the DOC (range = 0–56%, median = 40%) and 41% of UV_{254} (range = 0–79%, median = 43%). Low removal efficiencies of DOC and UV_{254} were observed at DWTPs 8 and 9, which applied low dosages of coagulants to waters that were low in DOC ($\leq$ 2.0 mg/L) and UV_{254} ($\leq$0.03 cm^{-1}) (Tables 4.1 and 4.3). Moreover, the source waters of DWTPs 8 and 9 were low in SUVA (1.2–1.8 L/mg-m), corresponding to low humic substances in these source waters. Coagulation at DWTPs 8 and 9 was not able to remove DOC because this unit process preferentially removes humic substances.

Coagulation was also somewhat effective in removing HBQ precursors (Table 4.2), and the removal efficiencies of 2,6-DCBQ FP, DCMBQ FP, TriCBQ FP, and 2,6-DBBQ FP, when detected, ranged from 4.7 to 39% (median = 19%, plants 1–9), 7.7 to

19% (median = 12%, plants 1–5), 0.9% to 24% (median = 12%, plants 1–9), and 18 to 45% (median = 21%, plants 1–5), respectively. However, as shown in Figure 4.7, the removal efficiencies of the HBQ FPs at most of the plants were lower than the removal of DOC or UV_{254}-absorbing NOM. On a median basis, the removal efficiency of coagulation was in the following order: UV_{254} ~ DOC >> 2,6-DCBQ ~ 2,6-DBBQ > DCMBQ ~ TriCBQ. This finding suggests that the organic matter (measured by DOC and UV_{254}) removed by coagulation had more non-precursor material than HBQ precursors.

The evidence can also be observed in Tables 4.4 and 4.5, where the HBQ production per unit DOC or UV_{254} increased after coagulation at most DWTPs. For DWTPs 1-5, after coagulation, the unit DOC yields showed an average increase of 31%, 41%, 36%, and 31% for 2,6-DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ, respectively. As for $UV_{254,}$ the average increase was even higher. (Note, the variations in DOC were very small at DWTPs 6-9, thus the calculated values of HBQ yields per unit DOC may not properly represent the variation trend.) Coagulation is recognized to be more effective for removing large molecular weight, hydrophobic NOM, i.e., humic substances.[9,31] Our results and others suggest that HBQ precursors may have fractions of low molecular weight (LMW) (e.g., LMW humic substances and LMW neutral organic compounds[5]) that may be not easily removed by coagulation.

The formation of 2,6-DCBQ was found to decrease with increased alum dose in a simulated bench-scale coagulation process.[5] In this study (Table 4.2), a moderate dosage of alum (30 mg/L as alum, Table 4.3) resulted in a high removal of DOC (from 7.8 to 3.4 mg/L) and 2,6-DCBQ precursors (from 196 to 145 ng/L) at DWTP 3. However, alum dose was not linearly correlated with the removal of HBQ precursors when all of the

DWTPs were evaluated. Coagulation efficiency is dependent on many factors, including the alum dose, the pH of coagulation, and the nature of the precursors. These factors will need to be systematically varied on a few representative waters to optimize the removal of HBQ precursors by coagulation.

The removal of the HBQ precursors by anthracite/sand filtration and GAC was also evaluated. Anthracite and sand filtration (DWTPs 1, 3, 5; Table 4.3) had essentially no impact on HBQ precursors; the removal efficiencies of the four HBQ precursors were ≤3% at these DWTPs (Figure 4.8A). Note, DOC and UV_{254} removal were also this low (Figure 4.8A). The low removal efficiency for anthracite and sand filtration was expected based on its mode of action. When GAC was combined with sand filtration at DWTP 2, removal of DOC, UV_{254}, and HBQ precursors was improved; for example, 8% of the DOC and 11% of the 2,6-DCBQ precursors could be removed by GAC combined with sand (DWTP 2, Figure 4.8B). Similar results were observed at DWTPs 1 and 4, where 10–20% of the four HBQ precursors were removed by GAC, whereas ≤5% of the DOC was removed (Figure 4.8B).

In contrast to the coagulation process, the reduction of HBQ FPs exceeded somewhat the removal of DOC and UV_{254} after GAC treatment. For example, as shown in Figure 4.8B, the removals of DOC and UV_{254} were small (almost unchanged, DWTP 4), yet HBQ precursors were removed (ranged from 8.9 to 15% at DWTP 4). This was also supported by the findings that HBQ yields per unit DOC or UV_{254} decreased by over 10% (in general) after GAC treatment at DWTPs 1 and 4 (Tables 4.4 and 4.5). The very low removals of GAC indicate that these plants were not being operated in a typical adsorption mode (e.g., for DOC or THM FP removal). At DWTP 4, GAC was

downstream of ozonation. It is likely that this and possibly the other GAC plants were operated with biodegradation. Future research should include plants that are operating with GAC for the removal of DOC and/or THM precursors. More research is necessary to quantify the adsorption versus biodegradation process to fully understand the removal mechanisms of GAC. However, in the current study, coagulation typically resulted in more removal of HBQ precursors than GAC.

Ozonation destroyed or transformed HBQ FPs; the HBQ FPs decreased 10–30% after ozonation treatment at DWTPs 4 and 5 (Figure 4.8C). From these tests, it is not possible to determine if there was a shift from chlorinated HBQs to brominated HBQs after ozonation. There was less destruction of 2,6-DBBQ precursors than that of 2,6-DCBQ (Figure 4.8C); however, that could reflect in part a shift in speciation. Nonetheless, a higher ozone dose at DWTP 4 than at DWTP 5 resulted in a higher removal of HBQ precursors. Further optimization of ozonation for this purpose will require evaluation of residual ozone concentration and contact time (CT values, mg/L x minutes) and pH of ozonation.

UV irradiation had no impact on the removal of HBQ precursors (Figure 4.8D). The removals were ±4%. These values are essentially negligible changes and simply reflect experimental and analytical variability.

4.4 CONCLUSION

In Chapter 4, we have investigated HBQ precursors in plant influents and their removal by each treatment process before chlorination in nine DWTPs. The levels of HBQ precursors were determined using formation potential (FP) tests for four HBQs: 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), 2,3,6-trichloro-1,4-benzoquinone (TriCBQ),

2,6-dichloro- 3-methyl-1,4-benzoquinone (DCMBQ), and 2,6-dibromo-1,4-benzoquinone (2,6-DBBQ). HBQ precursors were present in all of the plant influents. 2,6-DCBQ precursors were the most abundant (2,6-DCBQ FP up to 205 ng/L) in the low-bromide waters evaluated. Coagulation removed dissolved organic carbon (DOC) (up to 56%) and HBQ precursors (up to 39% for 2,6-DCBQ), suggesting that organic matter removed by coagulation had a high proportion of non-HBQ-precursor material. Granular activated carbon (GAC) decreased HBQ FPs by 10–20%, where DOC removal was only 0.2–4.7 %, suggesting that the GAC was not in the adsorption mode and that biodegradation may have been occurring. As expected, anthracite/sand filtration had little to no impact on the HBQ precursors. Ozonation destroyed or transformed HBQ FPs by 10–30%, whereas UV irradiation had no impact. The results demonstrated that the combined treatments partially removed, but did not substantially reduce HBQ precursor levels in water. Better understanding of HBQ precursors in source waters and evaluation of GAC in the adsorption mode may improve precursor removal and reduce HBQ formation in drinking water.

4.5 REFERENCES

1. Zhao, Y.; Anichina, J.; Lu, X.; Bull, R. J.; Krasner, S. W.; Hrudey, S. E.; Li, X.-F. Occurrence and formation of chloro- and bromobenzoquinones during drinking water disinfection. *Water Res* **2012**, *46* (14), 4351–4360.
2. Wang, W.; Qian, Y.; Li, J.; Moe, B.; Huang, R.; Zhang, H.; Hrudey, S. E.; Li, X.-F. Analytical and toxicity characterization of halo-hydroxylbenzoquinones as stable halobenzoquinone disinfection byproducts in treated water. *Anal Chem* **2014**, *86* (10), 4982–4988.
3. Bull, R. J.; Reckhow, D. A.; Li, X.-F.; Humpage, A. R.; Joll, C.; Hrudey, S. E. Potential carcinogenic hazards of non-regulated disinfection by-products:

haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology* **2011**, *286* (1-3), 1–19.

4. Plewa, M. J.; Simmons, J. E.; Richardson, S. D.; Wagner, E. D. Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products. *Environ Mol Mutagen* **2010**, *51* (8-9), 871–878.
5. Diemert, S.; Wang, W.; Andrews, R. C.; Li, X.-F. Removal of halobenzoquinone (emerging disinfection by-product) precursor material from three surface waters using coagulation. *Water Res* **2013**, *47* (5), 1773–1782.
6. Busca, G.; Berardinelli S.; Resini, C.; Arrighi L. Technologies for the removal of phenol from fluid streams: A short review of recent developments. *J Hazard Mater* **2008**, *160* (2-3), 265–288.
7. Collins, M. R.; Amy, G. L.; Steelink, C. Molecular weight distribution, carboxylic acidity, and humic substances content of aquatic organic matter: implications for removal during water treatment. *Environ Sci Technol* **1986**, *20* (10), 1028–1032.
8. Zhao, H.; Hu, C. Z.; Liu, H. J.; Zhao, X.; Qu, J. H. Role of aluminum speciation in the removal of disinfection byproduct precursors by a coagulation process. *Environ Sci Technol* **2008**, *42* (15), 5752–5758.
9. Amy, G. L.; Kuo, C. J.; Sierka, R. A. Ozonation of humic substances: effects on molecular weight distributions of organic carbon and trihalomethane formation potential. *Ozone- Sci Eng* 1988, *10* (1), 39–54.
10. Galapate, R. P.; Baes, A. U.; Okada, M. Transformation of dissolved organic matter during ozonation: effects on trihalomethane formation potential. *Water Res* **2001**, *35* (9), 2201–2206.
11. Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.; Sclimenti, M. J.; Onstad, G. D.; Thruston, A. D. Occurrence of a new generation of disinfection byproducts. *Environ Sci Technol* **2006**, *40* (23), 7175–7185.
12. Hu, J.; Song, H.; Karanfil, T. Comparative analysis of halonitromethane and trihalomethane formation and speciation in drinking water: the effects of disinfectants, pH, bromide, and nitrite. *Environ Sci Technol* **2010**, *44* (2):794–799.

13. Krasner, S. W.; Mitch, W. A.; Westerhoff, P.; Dotson, A. Formation and control of emerging C- and N-DBPs in drinking water. *J Am Water Works Assoc* **2012**, *104* (11), E582–E595.
14. Mao, Y.; Wang, X.; Yang, H.; Wang, H.; Xie, Y. F. Effects of ozonation on disinfection byproduct formation and speciation during subsequent chlorination. *Chemosphere* **2014**, *117*, 515–520
15. Chiu, C. A.; Westerhoff, P.; Ghosh, A. GAC removal of organic nitrogen and other DBP precursors. *J Am Water Works Assoc* **2012**, *104* (7), E406–E405.
16. Hanigan, D.; Zhang, J. W.; Herckes, P.; Krasner, S. W. Chen, C.; Westerhoff, P. Adsorption of N-nitrosodimethylamine precursors by powdered and granular activated carbon. *Environ Sci Technol* **2012**, *46* (22), 12630–12639.
17. Villacanas, F; Pereira, M. F. R.; Orfao, J. J. M.; Figueiredo, J. L. Adsorption of simple aromatic compounds on activated carbons. *J Colloid Interface Sci* **2006**, *293* (1), 128–136.
18. Kalmykova, Y.; Moona, N.; Stromvall, A. M.; Bjorklund, K. Sorption and degradation of petroleum hydrocarbons, polycyclic aromatic hydrocarbons, alkylphenols, bisphenol A and phthalates in landfill leachate using sand, activated carbon and peat filters. *Water Res* **2014**, *56* (16), 246–257.
19. Chen, W.; Duan, L.; Zhu, D. Adsorption of polar and nonpolar organic chemicals to carbon nanotubes. *Environ Sci Technol* **2007**, *41* (24), 8295–8300.
20. Farre, M. J.; Reungoat, J.; Argaud, F. X.; Rattier, M.; Keller, J.; Gernjak, W. Fate of N-nitrosodimethylamine, trihalomethane and haloacetic acid precursors in tertiary treatment including biofiltration. *Water Res* **2011**, *45* (17), 5695–5704.
21. Shah, A. D.; Dotson, A. D.; Linden, K. G.; Mitch, W. A. Impact of UV disinfection combined with chlorination/chloramination on the formation of halonitromethanes and haloacetonitriles in drinking water. *Environ Sci Technol* **2011**, *45* (8), 3657–3664.
22. Metz, D. H.; Meyer, M.; Dotson, A.; Beerendonk, E.; Dionysiou, D. D. The effect of UV/H_2O_2 treatment on disinfection by-product formation potential under simulated distribution system conditions. *Water Res* **2011**, *45* (13), 3969–3980.

23. Deng, L.; Huang, C. H.; Wang, Y. L. Effects of combined UV and chlorine treatment on the formation of trichloronitromethane from amine precursors. *Environ Sci Technol* **2014**, *48* (5), 2697–2705.
24. Krasner, S. W.; Sclimenti, M. J.; Guo, Y. C.; Hwang, C. J.; Westerhoff, P. Development of DBP and nitrosamine formation potential tests for treated wastewater, reclaimed water, and drinking water. *Proceedings of the 2004 AWWA Water Quality Technology Conference;* AWWA: Denver, CO, 2004.
25. Qin, F.; Zhao, Y. Y.; Zhao, Y.; Boyd, J. M.; Zhou, W.; Li, X.-F. A toxic disinfection by-product, 2,6-dichloro-1,4-benzoquinone, identified in drinking water. *Angew Chem Int Ed* **2010**, *49* (4), 790–792.
26. Wu, M. H.; Qian, Y. C.; Boyd, J. M.; Leavey, S.; Hrudey, S. E.; Krasner, S. W.; Li, X.-F. Identification of tobacco-specific nitrosamines as disinfection byproducts in chloraminated water. *Environ Sci Technol* **2014**, *48* (3), 1828–1834.
27. Mawhinney, D. B.; Young, R. B.; Vanderford, B. J.; Borch, T.; Snyder, S. A. Artificial sweetener sucralose in U.S. drinking water systems. *Environ Sci Technol* **2011**, *45* (20), 8716–8722.
28. Wang, W.; Qian, Y. C.; Boyd, J. M.; Wu, M. H.; Hrudey, S. E.; Li, X.-F. Halobenzoquinones in swimming pool waters and formation from personal care products. *Environ Sci Technol* **2013**, *47* (7), 3275–3282.
29. Hua, G.; Reckhow, D. A., Kim, J. Effect of bromide and iodide ions on the formation and speciation of disinfection byproducts during chlorination. *Environ Sci Technol* **2006**, *40* (9), 3050–3056.
30. Krasner, S. W.; Westerhoff, P.; Chen, B. Y.; Rittmann, B. E.; Nam, S. N.; Amy, G. Impact of wastewater treatment processes on organic carbon, organic nitrogen, and DBP precursors in effluent organic matter. *Environ Sci Technol* **2009**, *43* (8), 2911–2918.
31. Bolto, B.; Dixon, D.; Eldridge, R.; King, S. Removal of THM precursors by coagulation or ion exchange. *Water Res* **2002**, *36* (20), 5066–5073.

Table 4.1 Concentrations of the four HBQs in the FP samples and water quality of the nine plant influents.

Plant	Concentrations of HBQs in FP samples (ng/L)				DOC	UV_{254}	SUVA	Bromide	SUC	ACE
	2,6-DCBQ	DCMBQ	TriCBQ	2,6-DBBQ	(mg/L)	(cm^{-1})	(L/mg-m)	(mg/L)	(μg/L)	(μg/L)
1	76.7	3.6	7.5	6.7	17.0	0.41	2.4	0.089	1.4	0.8
2	97.9	1.6	6.2	8.7	14.6	0.30	2.0	0.112	0.3	0.2
3	195.6	4.1	13.6	3.2	7.8	0.28	2.7	0.022	ND	ND
4	204.6	2.6	20.0	4.5	6.5	0.16	2.9	0.086	0.8	0.5
5	61.8	2.9	11.6	1.8	9.2	0.23	2.8	0.016	0.5	0.3
6	57.8	ND	44.2	ND	1.3	0.02	1.5	<0.003	ND	ND
7	55.0	ND	48.2	ND	1.4	0.03	2.1	<0.003	ND	ND
8	15.5	ND	9.2	ND	1.8	0.03	1.8	<0.01	ND	ND
9	21.4	ND	3.6	ND	2.0	0.02	1.2	<0.01	ND	ND

ND: not detected; DOC: dissolved organic carbon; UV_{254}: UV absorbance at 254 nm; SUVA: specific UV absorbance, calculated as the UV absorbance (254 nm) multiplied by 100 and divided by the DOC value; SUC: sucralose (SUC); ACE: acesulfame.

Table 4.2 HBQ FPs of the plant influents and of the effluents of each treatment process, indicating the removal/destruction of HBQ precursors at the nine DWTPs.

DWTP	Sample location	DOC	UV_{254}	HBQs FP (ng/L)			
				2,6-DCBQ	DCMBQ	TriCBQ	DBBQ
1	Plant Inf	17.0	0.41	76.7	3.6	7.5	6.7
	Coag. Eff	8.6	0.14	55.8	2.9	6.0	5.3
	Filter Eff	8.6	0.14	55.1	2.8	6.0	5.3
	GAC Eff	7.8	0.12	46.3	2.1	5.1	4.3
	UV Eff	7.9	0.12	47.2	2.0	5.0	4.3
2	Plant Inf	14.6	0.30	97.9	1.6	6.2	8.7
	Coag. Eff	7.0	0.10	59.9	1.4	4.7	4.8
	GAC Eff	5.8	0.08	49.2	1.3	4.3	4.2
	UV Eff	6.3	0.08	51.7	1.3	4.4	4.1
3	Plant Inf	7.8	0.28	195.6	4.1	13.6	3.2
	Coag. Eff	3.4	0.06	145.4	3.6	11.2	2.7
	Filter Eff	3.4	0.06	139.0	3.5	11.2	2.7
4	Plant Inf	6.5	0.16	204.6	2.6	20.0	4.5
	Coag. Eff	3.9	0.09	177.4	2.4	15.4	3.7
	Ozone Eff	3.4	0.05	151.3	1.7	10.9	3.2
	GAC Eff	3.4	0.05	128.2	1.3	8.5	2.8
5	Plant Inf	9.2	0.14	61.8	2.9	11.6	1.8
	Coag. Eff	5.0	0.08	50.1	2.6	10.2	1.4
	Ozone Eff	4.9	0.06	45.1	2.3	9.4	1.3
	Filter Eff	4.5	0.06	44.8	2.3	9.3	1.3
	UV Eff	4.2	0.06	45.0	2.2	9.1	1.2
6	Plant Inf	1.3	0.015	57.2	ND	44.2	ND
	Coag. Eff	1.1	0.009	46.2	ND	43.8	ND
7	Plant Inf	1.4	0.03	55.0	ND	48.2	ND
	Coag. Eff	1.2	0.02	45.4	ND	47.0	ND

DWTP	Sample location	DOC	UV_{254}	HBQs FP (ng/L)			
				2,6-DCBQ	DCMBQ	TriCBQ	DBBQ
8	Plant Inf	1.8	0.03	15.5	ND	9.2	ND
	Coag. Eff	1.8	0.03	15.4	ND	8.8	ND
9	Plant Inf	2.0	0.03	21.4	ND	3.6	ND
	Coag. Eff	1.9	0.03	21.3	ND	3.4	ND

ND: not detected, which means the concentration is lower than the detection limit;; Inf: influent; Eff: effluent; Coag.: coagulation.

Table 4.3 Description of the treatment processes at the nine DWTPs.

DWTP	Coagulation	Filtration		Ozonation	UV
	Dose of Alum (mg/L)	Filter Composition	Run length (h)	Dosage (mg/L)	Dosage (mJ/cm^2)
1	26	Anthracite and sand	24	-	15.6
2	9	GAC and sand	48	-	40
3	30	Anthracite and sand	11	-	-
4	10	-	-	2.1	-
5	11	Anthracite and sand	90	1.1	30
6	11	-	-	-	-
7	10	-	-	-	-
8	<5	-	-	-	-
9	<5	-	-	-	-

"-" means that the treatment was not used at this DWTP or was used, but after the chlorination step.

Table 4.4 HBQ yields per unit DOC in the plant influents and in the effluents of each treatment steps.

DWTP	Sample location	HBQs FP per unit DOC (ng/mg)			
		2,6-DCBQ	DCMBQ	TriCBQ	2,6-DBBQ
1	Plant Inf	4.51	0.21	0.44	0.39
	Coag. Eff	6.49	0.34	0.70	0.62
	Filter Eff	6.41	0.33	0.70	0.62
	GAC Eff	5.79	0.26	0.64	0.54
	UV Eff	5.97	0.25	0.63	0.54
2	Plant Inf	6.71	0.11	0.42	0.60
	Coag. Eff	8.56	0.20	0.67	0.69
	GAC Eff	8.48	0.22	0.74	0.72
	UV Eff	8.21	0.21	0.70	0.65
3	Plant Inf	25.08	0.53	1.74	0.41
	Coag. Eff	42.76	1.06	3.29	0.79
	Filter Eff	40.88	1.03	3.29	0.79
4	Plant Inf	31.48	0.40	3.08	0.69
	Coag. Eff	45.49	0.62	3.95	0.95
	Ozone Eff	44.50	0.50	3.21	0.94
	GAC Eff	37.71	0.38	2.50	0.82
5	Plant Inf	6.72	0.32	1.26	0.20
	Coag. Eff	10.02	0.52	2.04	0.28
	Ozone Eff	9.20	0.47	1.92	0.27
	Filter Eff	9.96	0.51	2.07	0.29
	UV Eff	10.71	0.52	2.17	0.29
6	Plant Inf	44.00	ND	34.00	ND
	Coag. Eff	42.00	ND	39.82	ND
7	Plant Inf	39.29	ND	34.43	ND
	Coag. Eff	37.83	ND	39.17	ND
8	Plant Inf	8.61	ND	5.11	ND
	Coag. Eff	8.56	ND	4.89	ND
9	Plant Inf	10.70	ND	1.80	ND
	Coag. Eff	11.21	ND	1.79	ND

ND: not detected; Inf: influent; Eff: effluent; Coag.: coagulation.

Table 4.5 HBQ yields per unit UV_{254} in the plant influents and in the effluents of each treatment steps.

DWTP	Sample location	HBQs FP per unit UV_{254} (ng x cm)			
		2,6-DCBQ	DCMBQ	TriCBQ	2,6-DBBQ
1	Plant Inf	187.1	8.8	18.3	16.3
	Coag. Eff	398.6	20.7	42.9	37.9
	Filter Eff	393.6	20.0	42.9	37.9
	GAC Eff	385.8	17.5	42.5	35.8
	UV Eff	393.3	16.7	41.7	35.8
2	Plant Inf	326.3	5.3	20.7	29.0
	Coag. Eff	599.0	14.0	47.0	48.0
	GAC Eff	615.0	16.3	53.8	52.5
	UV Eff	646.3	16.3	55.0	51.3
3	Plant Inf	698.6	14.6	48.6	11.4
	Coag. Eff	2423.3	60.0	186.7	45.0
	Filter Eff	2316.7	58.3	186.7	45.0
4	Plant Inf	1278.8	16.3	125.0	28.1
	Coag. Eff	1971.1	26.7	171.1	41.1
	Ozone Eff	3026.0	34.0	218.0	64.0
	GAC Eff	2564.0	26.0	170.0	56.0
5	Plant Inf	441.4	20.7	82.9	12.9
	Coag. Eff	626.3	32.5	127.5	17.5
	Ozone Eff	751.7	38.3	156.7	21.7
	Filter Eff	746.7	38.3	155.0	21.7
	UV Eff	750.0	36.7	151.7	20.0
6	Plant Inf	3813.3	-	2946.7	-
	Coag. Eff	5133.3	-	4866.7	-
7	Plant Inf	1833.3	-	1606.7	-
	Coag. Eff	2270.0	-	2350.0	-
8	Plant Inf	516.7	-	306.7	-
	Coag. Eff	513.3	-	293.3	-
9	Plant Inf	713.3	-	120.0	-
	Coag. Eff	710.0	-	113.3	-

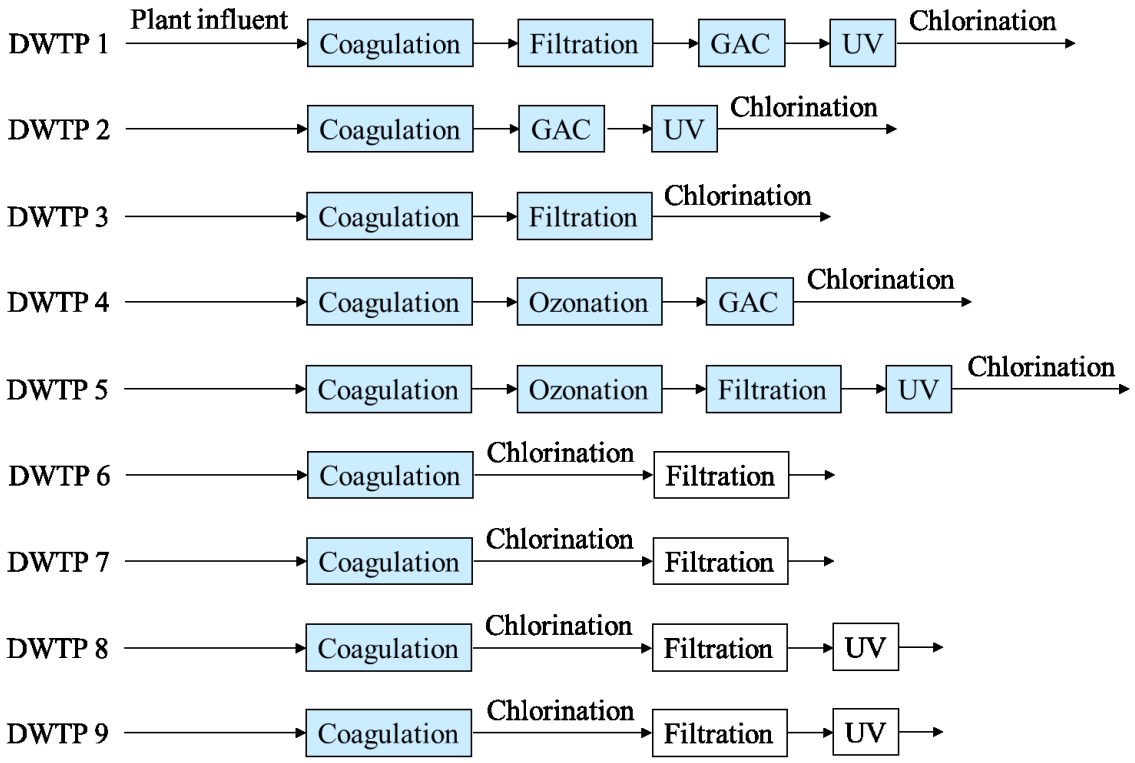


Figure 4.1 Treatment processes and sampling points at the nine DWTPs. Water samples were collected after each treatment process (blue box) before chlorination.

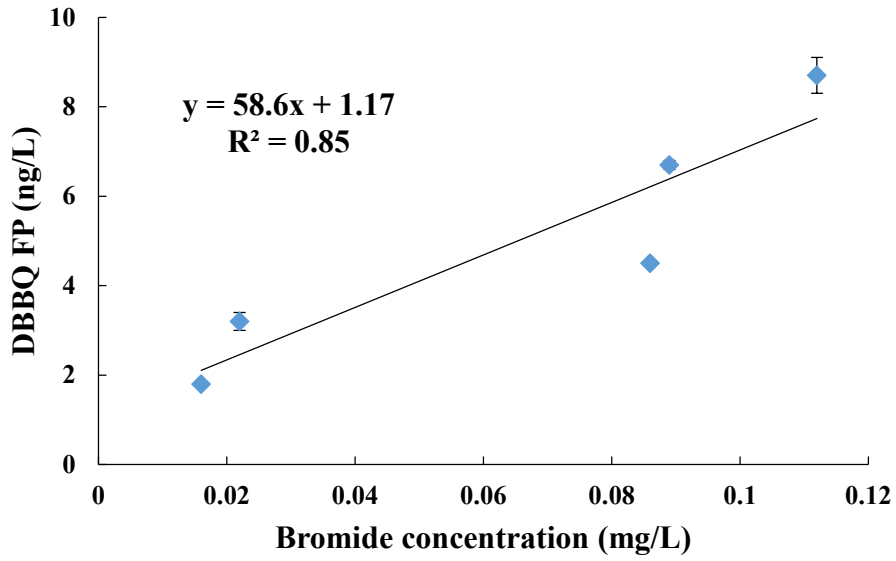


Figure 4.2 Relationship between bromide concentration and 2,6-DBBQ FP in the plant influents from five DWTPs 1-5.

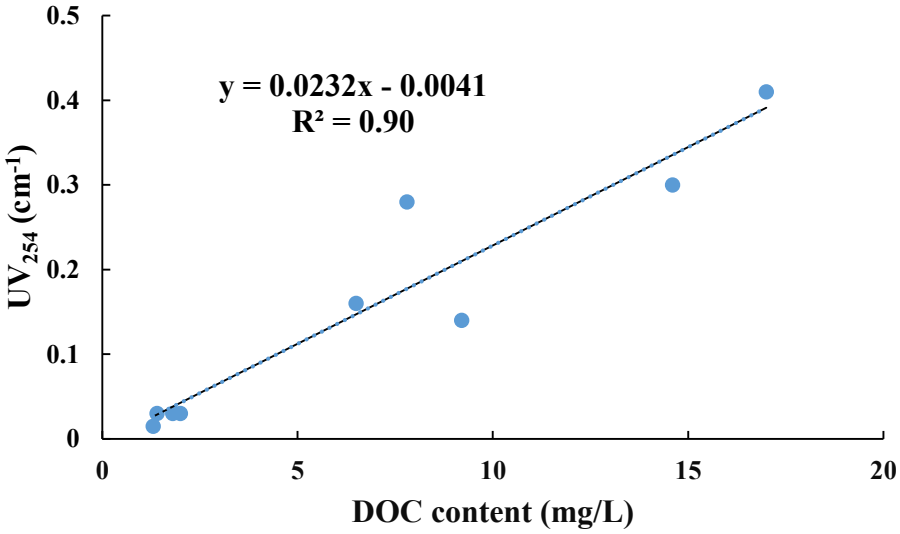


Figure 4.3 Relationship between DOC content and UV_{254} level in the plant influents from DWTPs 1-9.

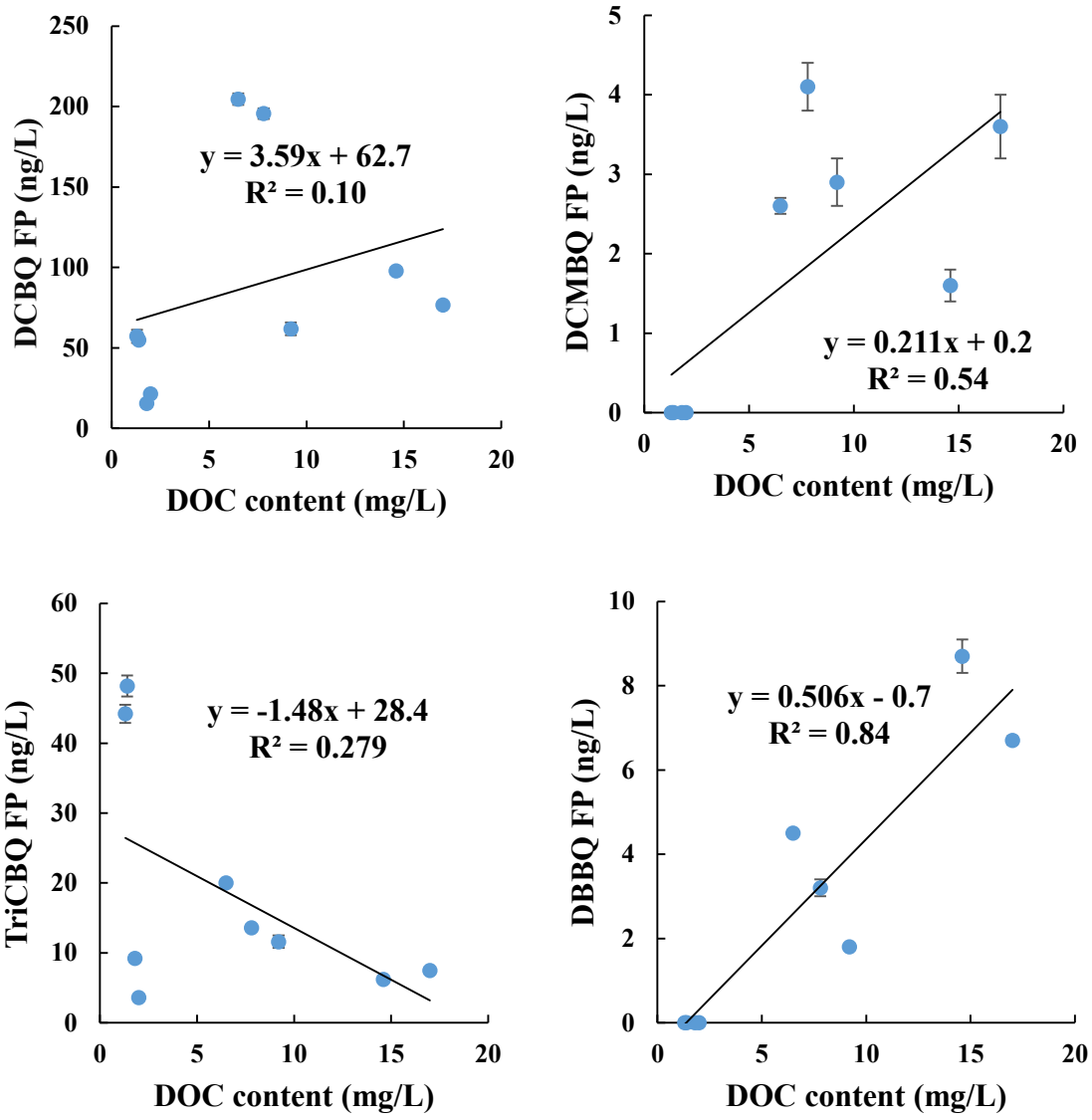


Figure 4.4 Relationship between four HBQs FPs and DOC contents of the plant influents at the nine DWTPs (see Table 4.1).

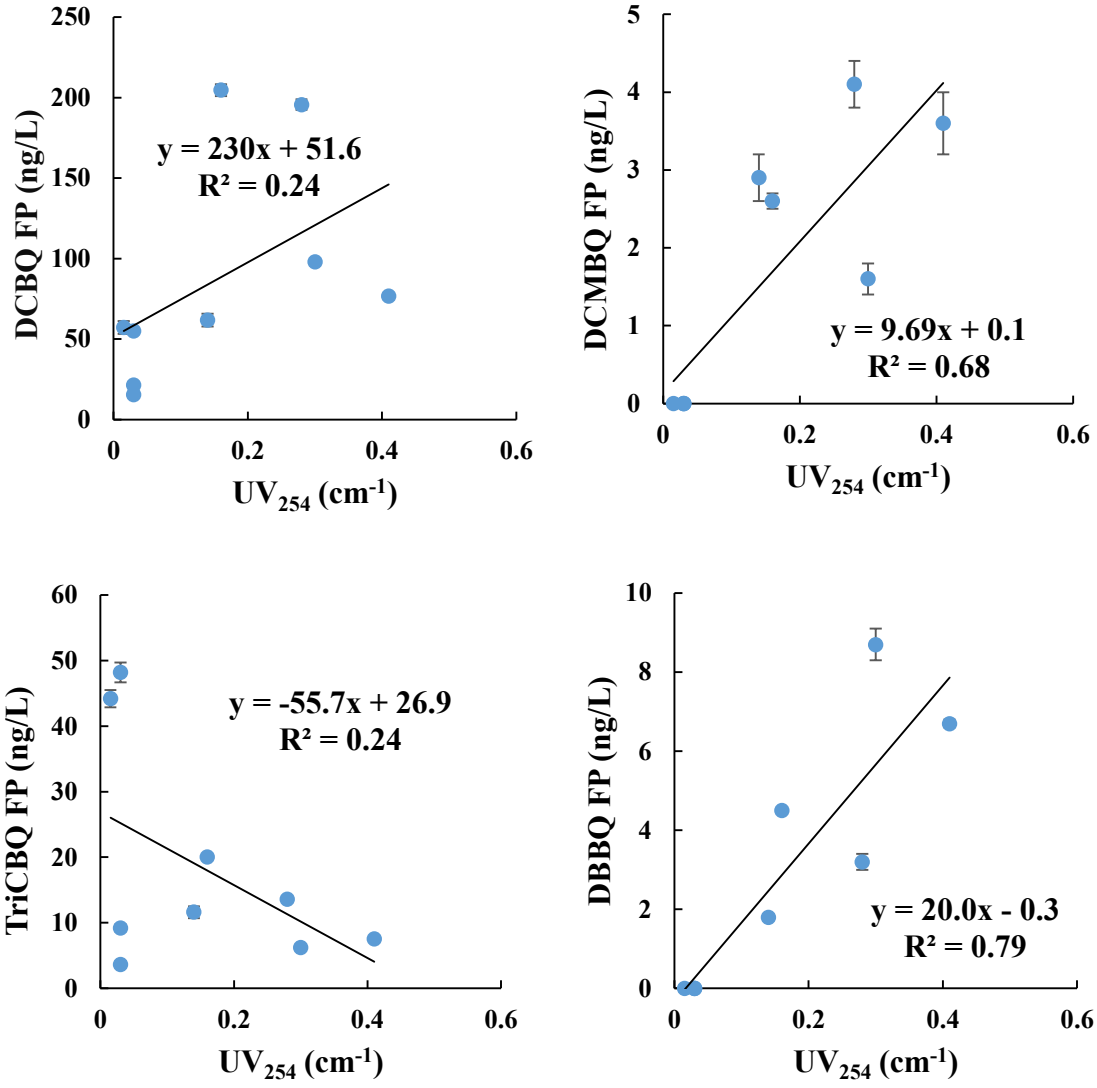


Figure 4.5 Relationship between four HBQs FPs and UV_{254} levels of the plant influents at the nine DWTPs (see Table 4.1).

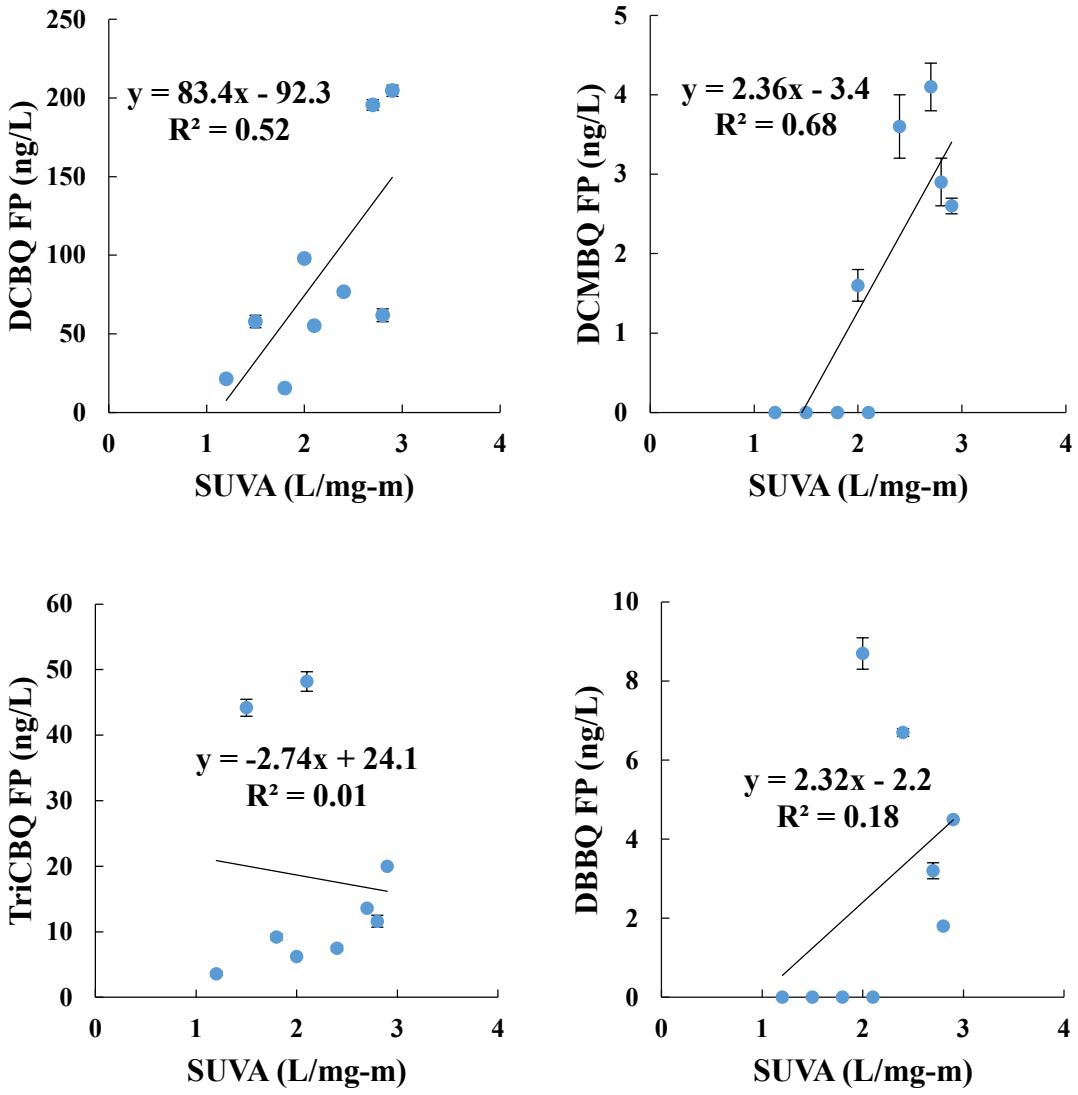


Figure 4.6 Relationship between four HBQs FPs and SUVA values of the plant influents at the nine DWTPs (see Table 1).

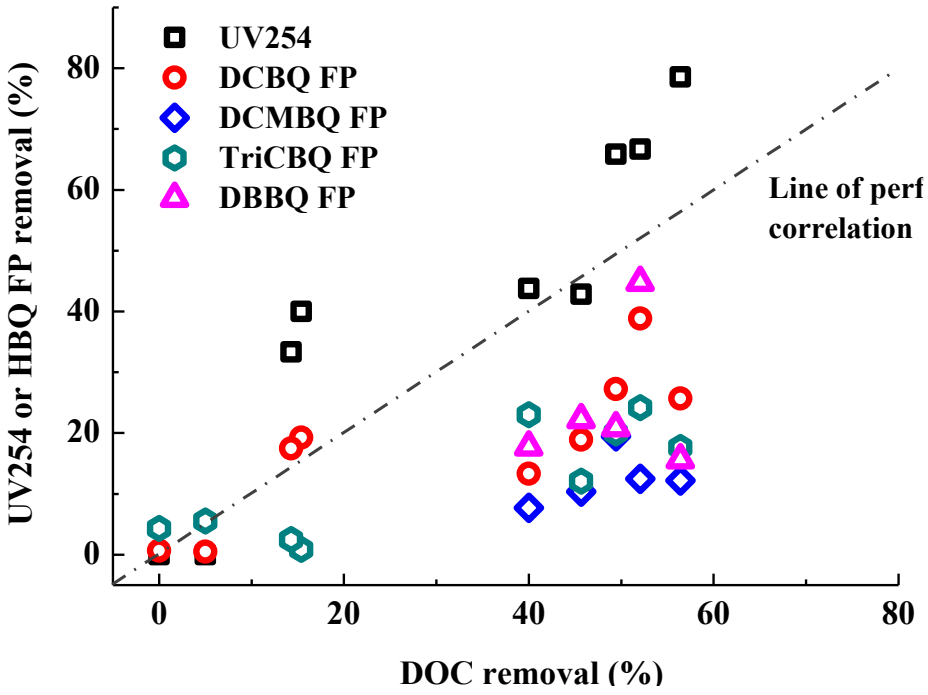


Figure 4.7 Relationships between the removal of HBQ precursors and the removal of DOC or the removal of UV_{254} by coagulation.

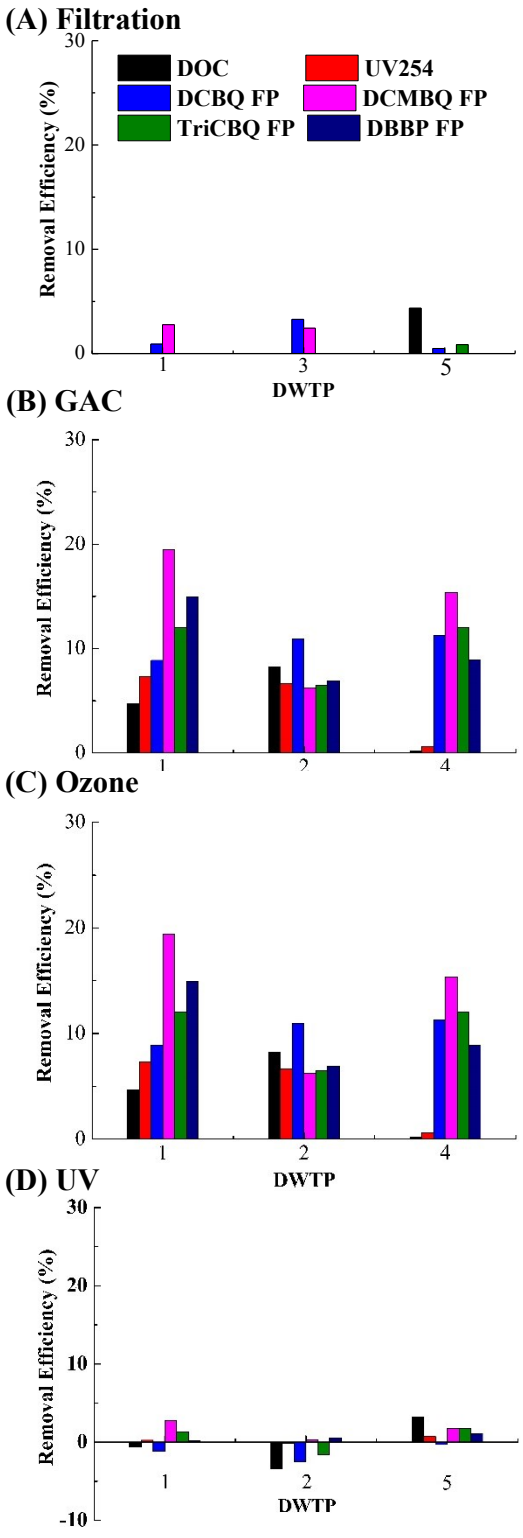


Figure 4.8 Removal of DOC, UV_{254}, and HBQ FPs by water treatment steps. (A) filtration, (B) GAC, (C) ozonation, and (C) UV at selected DWTPs.

5 TRANSFORMATION OF HALOBENZOQUINONES IN DISINFECTED WATER

5.1 INTRODUCTION

When disinfection byproducts (DBPs) are formed, some will undergo spontaneous transformation reactions in the drinking water treatment plant (DWTP) and distribution system (DWDS).[1,2] The DBP species and concentrations in the water arriving at customers' tap may be different from when the water originally left the DWTP.[3,4] The transformation process can largely affect human exposure and health risk of these DBPs.[5,6] Our previous studies have shown that HBQs cannot be eliminated by drinking water treatment steps, thus they will enter the DWDS and finally be transported to water consumers. HBQs are not stable at neutral pH or after exposure to UV irradiation;[7,8] however, the transformation processes of HBQs and their transformation products have not been identified or shown to exist in DWTPs and DWDSs.

Benzoquinone derivatives (BQs) can undergo spontaneous reactions both in the environment and within living organisms.[9-13] BQs in water can undergo redox, photochemical, and nucleophilic reactions to produce products such as semiquinones, benzene-1,2,4-triols, hydroquinones, and hydroxyl-quinones.[14-19] Based on the chemical properties of BQ, we hypothesize that HBQs can undergo oxidation reactions to form hydroxyl-halo-benzoquinones (OH-HBQs) in water and that these OH-HBQs are a stable form of HBQ DBPs in drinking water. To confirm this hypothesis, we conducted studies 1) to identify the transformation products of HBQs to elucidate the transformation pathways; 2) to confirm whether OH-HBQs exist as DBPs in drinking water samples and 3) to demonstrate the toxicological relevance of OH-HBQs.

To identify transformation products, we used high resolution triple quadrupole time-of-flight (QTOF) mass spectrometry to obtain the mass spectra, accurate mass measurements, and tandem mass spectra of the products. To quantitatively examine the transformation kinetics and determine the products in laboratory reactions, we developed a SPE-UHPLC-MS/MS method using triple quadrupole ion-trap (QTRAP) mass spectrometry. We further confirmed these products in the field samples. Finally, we evaluated the *in vitro* toxicity of both the HBQs and the OH-HBQs to elaborate the toxicological relevance of the transformation products.

5.2 EXPERIMENTAL SECTION

5.2.1 Chemicals and solvents

2,6-dibromo-(1,4)-benzoquinone (2,6-DBBQ) was purchased from Indofine Chemical Company (Hillsborough, NJ). 3,5-dichloro-2-methyl-(1,4)-benzoquinone (DCMBQ) and 2,3,6-trichloro-(1,4)-benzoquinone (TriCBQ) were synthesized by Shanghai Acana Pharmtech (Shanghai, China); 2,6-dichloro-(1,4)-benzoquinone (2,6-DCBQ) was purchased from Sigma-Aldrich (St. Louis, MO). 3-hydroxyl-2,6-dichloro-(1,4)-benzoquinones (OH-2,6-DCBQ), 5-hydroxyl-2,3,6-trichloro-(1,4)-benzoquinone (OH-DCMBQ), 5-hydroxyl-2,3,6-trichloro-(1,4)-benzoquinone (OH-TriCBQ), and 3-hydroxyl-2,6-dibromo-(1,4)-benzoquinone (OH-DBBQ) were synthesized in our laboratory by dissolving solid 2,6-DCBQ, DCMBQ, TriCBQ and 2,6-DBBQ, respectively, in Optima water for 12 h at 4 ^{o}C. The purity and identity of the synthesized compounds were assessed using UHPLC-ESI-MS analysis. Only one peak was observed in each chromatogram and isotope patterns confirmed the peak as OH-HBQ. Water (Optima LC/MS grade; the grade means that the solvent goes through 0.03 micron

filtration, and the purity is confirmed by UHPLC-UV and HPLC-MS detection), methanol (Optima LC/MS grade) and hydrochloric acid (ACS grade) were purchased from Fisher Scientific (Nepean, ON). Formic acid (HPLC grade, 50% in water) was purchased from Fluka.

5.2.2 Liquid chromatography–mass spectrometry analysis

A liquid chromatography system (UHPLC, Agilent 1290 Infinity Quaternary LC series) was applied with a Luna C18(2) column (100 × 2.0 mm i.d., 3μm; Phenomenex, Torrance, CA) at room temperature to separate the HBQs and their transformation products. The mobile phase consisted of solvent A (0.1% FA in water) and solvent B (0.1% FA in methanol) with a flow rate of 0.17 mL/min. A gradient program was performed as: linearly increased B from 20% to 90% in 20 min; kept B at 90% for 5 min; changed B to 20% for column equilibration at 25.1–30 min. The sample injection volume was 20 μL.

Accurate mass measurements and isotopic patterns were obtained with a quadrupole time-of-flight mass spectrometer (AB SCIEX TripleTOF® 5600, AB SCIEX, Concord, ON, Canada) to identify transformation products of the four HBQs. The conditions of the TripleTOF mass spectrometry experiments were: negative ionization mode; ion source voltage, -4500 V; gas I, 60 arbitrary units; gas II, 60 arbitrary units; curtain gas, 25 arbitrary units; source temperature, 450 ºC; declustering potential (DP), -90 V; collision energy (CE), -40 V; accumulation time, 0.25 s; scan range, m/z 100–1000. The Information Dependent Acquisition (IDA) was utilized to obtain MS/MS spectra. The MS scan range of IDA was m/z 100–700, and the collision energy spread (CES) was

10 V. The accurate masses of HBQs were set in the inclusion list to track the peaks of HBQs at all times.

Multiple-reaction monitoring (MRM) methods were performed using a triple quadrupole trap tandem mass spectrometer (AB SCIEX QTRAP® 5500, Concord, ON, Canada) for the quantification of the four HBQs and their transformation products. The optimized MS instrumental parameters were: ion-spray voltage, -4500 V; source temperature, 450 ^{o}C; Gas I, 50 arbitrary units; Gas II, 60 arbitrary units; Curtain Gas, 30 arbitrary units; Entrance Potential, -10 V; accumulation time for each ion pair, 100 ms. The MRM ion pairs and the optimized values of DP, CE and collision cell exit potential (CXP) are listed in Table 5.1. Analyst® and PeakView® (AB SCIEX) software were used for data analysis. The method confirmed the identity of the peak by matching the relative ratio of two specific parent-product ion pairs, and quantified it by the peak area of one ion pair of higher intensity.

5.2.3 Sample collection and solid phase extraction

Water samples were collected from defined locations of five DWTPs and DWDSs, including source water, water plant effluent, and tap water in the distribution systems of different water ages (halfway, maximum distance). Some samples were also collected from locations that showed high concentrations of regulated-DBPs. Water samples were stored in amber bottles which were precleaned three times by HBQ-free water and methanol. Formic acid (0.25%, v/v) was added to the samples immediately after collection to quench the chlorine residual and stabilize HBQs.[20,21] The samples were transported back to our laboratory in coolers with icepacks and analyzed immediately on arrival. The time between collection and analysis was within two days.

The water samples were extracted for the HBQs and OH-HBQs using Waters Oasis HLB cartridges (6 cc, 200 mg sorbent per cartridge; Milford, MA). The solid phase extraction (SPE) method was improved upon the previous SPE for the four HBQs.[7] In brief, The HLB cartridges were mounted in a VISIPREP SPE manifold (Supelco, Bellefonte, PA) with flow control liners. The cartridges were conditioned using 12 mL of acidic methanol (0.25% FA, v/v) and equilibrated using 12 mL of acidified water (0.25% FA, v/v). An aliquot of 500 mL of each water sample was pumped through the cartridges at a flow rate of approximately 8 mL/min. After sample loading, the cartridges were washed with the mixture of methanol and water to reduce interference and clean up the samples. The ratio of methanol to water and volume of wash solvent were optimized to achieve the best recovery. Finally, the analytes on the cartridges were eluted using 10 mL of methanol (0.25% FA). The eluent was evaporated down to 100 µL under a gentle nitrogen stream (< 5 psi) and then reconstituted with 400 µL of water (0.25% FA).

5.2.4 Quality control and quality assurance

A travel-blank sample (500 mL of Optima water, 0.25% FA) was included in each sampling trip. Two SPE-blank samples (500 mL of Optima water, 0.25% FA) were extracted along with other water samples in each batch of SPE. Analysis-blank samples (500 µL, 20% methanol, 80% water, 0.25% FA) were analyzed between every five samples. These blank samples were analyzed to examine whether contamination occurred during sampling, pretreatment, or analysis. Triplicate extractions and triplicate runs of each extract were performed for each water sample to determine the average concentration and standard error. Recoveries and matrix effects of individual analytes were determined from the spiked water samples.

5.2.5 Cell culture and cytotoxicity testing

The CHO-K1 (Chinese hamster ovary, CCL-61; ATCC, Manassas, VA) cell line was chosen to evaluate the toxicity of HBQs and OH-HBQs. This cell line is widely used in DBP toxicity studies so comparisons can be readily made. The cells were cultured in (1:1) DMEM:F12 media (Gibco), 10% fetal bovine serum (FBS) (Sigma-Aldrich, Oakville, ON, Canada), and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA) and maintained at 37 °C, 5.5% CO_2, 90% humidity. The cytotoxicity of HBQs and OH-HBQs was examined using the real time cell electronic sensing (RT-CES) system (ACEA Biosciences, San Diego, CA).

5.2.6 Cytotoxicity testing using RT-CES method

The cytotoxicity of HBQs and OH-HBQs on CHO-K1 cells was analyzed using the RT-CES system (ACEA Biosciences). Briefly, a 96-well E-plate is electronically connected with the E-plate station located in the CO_2 incubator, which in turn is connected to the system analyzer. The impedance changes caused by cells interacting with microelectrodes of RT-CES are automatically converted to the cell index (CI). CI is calculated via the following equation:

$$CI = \max_{i=1,\ldots N}\left[\frac{R_{cell}(fi)}{R_b(fi)} - 1\right],$$

Where $R_{cell}(fi)$ is the impedance of the micro-electrode when cells are bound and $R_b(fi)$ is the same parameter with no cells bound, and N is the number of frequency points when impedance is measured (N=3), selected by the instrument to maximize signal. When more living cells attached on the micro-electrodes, the R_{cell} value is higher. Thus the number of living cells in each well is positively correlated with CI.

In our experiment, a population of 6000 cells was initially seeded into each well of the 96-well E-plate; the CI reached 1 after about 20 h. The cells in each plate were treated with HBQs or OH-HBQs (diluted in culture media) at given concentrations. The concentration ranges were selected to induce significant differences of cell growth, at 5–75 μM of 2,6-DCBQ, 5–25 μM of DCMBQ, 20–115 μM of TriCBQ, 1–50 μM of 2,6-DBBQ, 25–100 μM of OH-2,6-DCBQ, 15–40 μM of OH-DCMBQ, 12.5–100 μM of TriCBQ and 25–87.5 μM of OH-DBBQ. Solvent controls (cells were cultured in 1% methanol diluted in culture solution) and negative controls (cells were cultured in culture medium solution, without addition of HBQ solution) were set to evaluate the interference from solvent and the natural growth of cells, respectively.

5.3 RESULTS AND DISCUSSION

Figure 5.1 shows the typical total ion chromatograms (TIC) of four HBQs in freshly prepared solution (red), and solutions stored for 3 h (blue), 12 h (purple) and the blank (black) at 4 ^{o}C. The samples were solid standards dissolved in pure water (Optima™ LC/MS grade). Before analysis, methanol was added to adjust the ratio of methanol in the sample to 20%. A new peak was clearly detected in the 3-h old HBQ solution, suggesting a product of HBQ degradation in water. After 12 h, HBQs were completely undetectable and only one peak (new) corresponding to individual HBQs was detected. We carefully examined the accurate mass (Table 5.2) and isotope ratios of the new peaks obtained from IDA analysis (Figure 5.2). A search using PeakView matched the new peak in 2,6-DCBQ solution with OH-DCBQ. We then used the same procedures to examine DCMBQ, TriCBQ, and 2,6-DBBQ. As shown in Figure 5.2, the accurate mass and isotope patterns of the new peaks in the individual solutions of DCMBQ, TriCBQ, and

2,6-DBBQ in water correspond to OH-DCMBQ, OH-TriCBQ, and OH-DBBQ, respectively. The mass accuracy for the most abundant isotope of the four OH-HBQs was 0.4, 0.8, 0, and 0.2 ppm for the four OH-HBQs, respectively (Figure 5.2). Figure 5.3 shows the extracted ion chromatograms (XIC) of OH-DCBQ, OH-DCMBQ, OH-TriCBQ, and OH-DBBQ produced in the DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ solutions, respectively, over 12 h storage time. The formation of OH-DCBQ, OH-DCMBQ, OH-TriCBQ, and OH-DBBQ (Figure 5.3-A, B, C, and D) increased as the solutions of 2,6-DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ aged, respectively.

Figure 5.2 shows that the OH-HBQ compounds form $[M-H]^-$, $[M]^-$, and $[M+H]^-$ as the major ions with negative electrospray ionization. $[M-H]^-$ ion was the most abundant for OH-DCBQ and OH-DBBQ, while $[M+H]^-$ was the most abundant for OH-DCMBQ and OH-TriCBQ under the optimized conditions. The possible ionization pathways are described in Figure 5.4. The formation of $[M+H]^-$ of OH-HBQs is similar to the ESI pathways of the HBQs that were previously reported.[21] The $[M+H]^-$ ions can be explained by two possible processes: one is direct addition of two electrons and one proton; the other is via two steps: OH-HBQ first undergoes transformation to hydroxyl-halodihydroquinone (OH-HDHQ), which then loses one proton. OH-HBQs also form $[M-H]^-$ ions, which are rarely observed from the ionization of HBQs. This finding could be explained by the ionization of hydroxyl group. There were also minor $[M]^-$ ions observed in the mass spectra, which may be produced by direct ionization via addition of an electron.

Having synthesized and confirmed the OH-HBQs, we aimed to confirm the existence of these compounds in the field samples. To achieve this, we developed a

UHPLC-MS/MS method to determine both the OH-HBQs and the parent HBQs. Two pairs of transition ions were used in the MRM measurements and the MS instrumental parameters were optimized. Table 5.1 describes the optimized MRM conditions. The baseline separation of the eight OH-HBQs and HBQs was achieved using a C18 reverse phase LC.

We also optimized the UHPLC and ionization conditions. FA in the mobile phase can stabilize HBQs, while the addition of weak acid in the mobile phase may suppress the signal of negative electrospray ionization.[22] Based on the signals of HBQs and OH-HBQs (Figure 5.13), we used the FA concentration in the mobile phase at 0.1% in the MRM quantification methods.

Figure 5.6 shows typical UHPLC-MS/MS (MRM) chromatograms obtained from analysis of the four HBQs and four OH-HBQs. The identification of the target compounds was based on the criteria: retention times are identical for the two ion transitions of the specific compound and relative intensity (ratio) of these two ion transitions detected in the samples is consistent with that in the standard solutions. When a HBQ was identified, the ion transition with higher abundance (the first ion pair listed in Table 5.1 for each compound) was used for quantification. The UHPLC-MS/MS method was validated for analysis of the four HBQs and four OH-HBQs in tap water. The detection limit of the UHPLC-MS/MS method is 0.01 to 0.7 ng/mL (Table 5.3, LOD1).

The concentrations of HBQs in water have been previously reported to be around several ng/L to several hundred ng/L.[7,20]. Therefore, it is neccessay to concentrate the compounds from water samples prior to the UPHLC-MS/MS analysis. No SPE method for OH-HBQs was available; thus we developed SPE to concentrate these compounds as

well as HBQs. The SPE procedures were optimized, including conditioning of the cartridge, loading of the sample, washing/cleaning, and elution of the analytes. The retention of analytes is dependent on the washing solvent and elution solvent.[23,24] Based on our LC separation (Figure 5.6), methanol is suitable to elute the HBQs and OH-HBQs. The optimized elution condition was 10 mL of methanol (0.25% FA, v/v). The washing step was optimized to remove the interference matrices and retain the desired analytes. The optimized SPE procedures and recoveries are presented in Figure 5.7.

To validate the SPE-UHPLC-MS/MS conditions, we examined the retention time, LOD, LOQ, recovery and matrix effect. Table 5.3 presents the performance of the method: repeatable retention time (STD<0.05 min), sub ng/L LOD (LOD2, 0.02–0.8 ng/L), LOQ (0.07–2.8 ng/L), and recovery (68–96%). Even after SPE, the matrix effect (79–98%) persisted, therefore, the standard addition method was used for quantification of these compounds in authentic water samples.

Having established a SPE-UHPLC-MS/MS method for both HBQs and OH-HBQs, we were able to quantitatively study the transformation of HBQs to OH-HBQs. Figure 5.8 presents the time course of HBQs converting to OH-HBQs over 24 hour after fresh preparation of a HBQ solution. As the concentrations of HBQs decrease, the concentrations of OH-HBQs increase accordingly. After 12 h, the reaction reached equilibrium. The mass balance (sum) of HBQs and OH-HBQs maintained around 80–120%. OH-HBQs were stable for 60 h at the initial pH 7 and initial concentration 50 μg/mL (Figure 5.9), indicating that OH-HBQs are much more stable than HBQs in water.

We further investigated the presence of OH-HBQs in the field water systems where the HBQs were determined. Using the method for the four HBQs and four OH-

HBQs, we analyzed water samples from five DWTPs and DWDSs. The water treatment processes of the five DWTPs are summarized in Table 5.4. Figure 5.10 demonstrates that OH-HBQs are present in the treated tap water but not in the source water. To further confirm the presence of OH-HBQs, we investigated the occurrence frequency and concentrations of OH-HBQs in these samples as summarized in Table 5.5. OH-DCBQ was the most commonly identified OH-HBQ of the four OH-HBQs tested, which was consistent with 2,6-DCBQ being the most frequently detected HBQ. The concentrations of HBQs and OH-HBQs in each water samples are summarized in Table 5.6. The samples containing HBQs were confirmed to also contain OH-HBQs, suggesting that the transformation of HBQs to OH-HBQs may occur in the DWDSs. To further examine this, we investigated the distribution system of Plant 1. A set of samples were collected from different locations and analyzed for both HBQs and OH-HBQs. The results (Figure 5.11) show that the concentrations of HBQs in water samples decreased while OH-HBQs increased with the increasing distance from the DWTP. Repeated samplings show the same trend in Plant 1 (Figure 5.11). Similar results were found in Plant 2 DWDS where 2,6-DCBQ was detected (Figure 5.12). Both laboratory experiments and the field study supported that the decrease of HBQs were correlated with the increase of OH-HBQs.

To assess the health relevance of OH-HBQs as DBPs, we evaluated the toxicity of OH-HBQs compared with that of HBQs. We used the CHO-K1 cells with the RT-CES method (details in SI) that has been established in our laboratory.[25,26] Figure 5.13 illustrates the temporal response of CHO-K1 cells exposed to 2,6-DCBQ and OH-DCBQ (as a representation of the tested eight compounds). Each well was dosed with the tested compounds when the cell index (CI) was 1 (after about 20-h growth). The cells displayed

concentration-dependent response curves. At any given time point, as the concentration of 2,6-DCBQ or OH-DCBQ increased, the normalized CI decreased, demonstrating a concentration-dependent cytotoxic effect on CHO-K1 cells. Cytotoxic responses of DCMBQ, OH-DCMBQ, TriCBQ, OH-TriCBQ, 2,6-DBBQ, and OH-DBBQ are similar to those in Figure 5.13.

Based on the temporal cytotoxicity profile, we calculated the IC_{50} for each compound on CHO-K1 cells and summarized these results in Table 5.7. Generally, the IC_{50} of HBQs and OH-HBQs are at μM level. The IC_{50} of trihalomethanes (THMs) on CHO cells (72 h) were reported at the range of 3.96 to 11.5 mM[27], and the IC_{50} of haloacetic acids (HAAs) were at the range of 8.90 μM to 17.5 mM[28] (data summarized in Table 5.8). The IC_{50} of HBQs and OH-HBQs (16 to 91 μM) are much lower (more toxic) than most of the regulated DBPs, indicating that chronic cytotoxicities of HBQs and OH-HBQs are significantly higher than those of the regulated DBPs. The IC_{50} of the eight compounds are in the order: DCMBQ < 2,6-DBBQ < OH-DCMBQ < 2,6-DCBQ < OH-DBBQ < TriCBQ < OH-DCBQ < OH-TriCBQ. Comparing HBQ and OH-HBQ in pairs, the IC_{50} are: 2,6-DCBQ < OH-DCBQ, DCMBQ < OH-DCMBQ, TriCBQ < OH-TriCBQ and 2,6-DBBQ < OH-DBBQ, indicating that the addition of hydroxyl group to HBQ decreases the cytotoxicity and the transformation process is partially a detoxifying process. The IC_{50} values of the four HBQs are DCMBQ < 2,6-DBBQ < 2,6-DCBQ < TriBQ, and the IC_{50} of the four OH-HBQs are OH-DCMBQ < OH-DBBQ < OH-DCBQ < OH-TriBQ, i.e., the same order as HBQs. This indicated that the basic HBQ structure likely plays a key role in determining the toxicity of the corresponding OH-HBQ.

5.4 CONCLUSION

Four halobenzoquinones (HBQs) have been detected in treated drinking water and have shown potency in producing reactive oxygen species and inducing damage to cellular DNA and proteins. These HBQs are unstable in drinking water. The fate and behavior of these HBQs in drinking water distribution systems is unclear. Here we report the high resolution mass spectrometry identification of the transformation products of HBQs as halo-hydroxyl-benzoquinones (OH-HBQs) in water under realistic conditions. To further examine the kinetics of transformation, we developed a solid phase extraction with ultra high performance liquid chromatography tandem mass spectrometry (SPE-UHPLC-MS/MS) method to determine both the HBQs and OH-HBQs. The method provides reproducible retention times (SD < 0.05 min), limits of detection (LOD) at sub-ng/L levels, and recoveries of 68%–96%. Using this method, we confirmed that decrease of HBQs correlated with the increase of OH-HBQs in both the laboratory experiments and several distribution systems, supporting that OH-HBQs were more stable forms of HBQ DBPs. To understand the toxicological relevance of the OH-HBQs, we studied *in vitro* toxicity with CHO-K1 cells and determined the IC_{50} of HBQs and OH-HBQs ranging from 15.9 to 72.9 μM. While HBQs are 2 fold more toxic than OH-HBQs, both HBQs and OH-HBQs are substantially more toxic than the regulated DBPs.

5.5 REFERENCES

1. Glezer, V.; Harris, B.; Tai, N.; Lev, L. O. Hydrolysis of haloacetonitriles: linear free energy relationship, kinetics and prducts. *Water Res* **1999**, *33* (8), 1938–1948.
2. Urbansky, E. T. The fate of the haloacetates in drinking water- chemical kinetics in aqueous solution. *Chem Rev* **2001**, *101* (11), 3233–3243.

3. Brisson, I. J; Levallois, P.; Tremblay, H.; Serodes, J.; DeBlois, C.; Charrois, J.; Taguchi, V.; Boyd, J. M.; Li, X.-F.; Rodriguez, M. J. Spatial and temporal occurrence of N-nitrosamines in seven drinking water distribution systems. *Environ Monit Assess* **2013**, *185* (9), 7693–7708.
4. Carter, J. M.; Moran, M. J.; Zogorski, J. S.; Price, C. V. Factors associated with sources, transport and fate of chloroform and three other trihalomethanes in untreated groundwater used for drinking water. *Environ Sci Technol* **2012**, *46* (15), 8189–8197.
5. Farre, M. L.; Perez, S.; Kantiani, L.; Barcelo, D. Fate and toxicity of emerging pollutants, their metabolites and transformation products. *Trends Anal Chem* **2008**, *27* (11), 991–1007.
6. Escher, B. I.; Fenner, K. Recent advances in environmental risk assessment of transformation products. *Environ Sci Technol* **2011**, *45* (9), 3835–3847.
7. Zhao, Y.; Qin, F.; Boyd, J. M.; Anichina, J.; Li, X.-F. Characterization and determination of chloro- and bromo-benzoquinones as new chlorination disinfection byproducts in drinking water. *Anal Chem* **2010**, *82* (11), 4599–4605.
8. Qian, Y.; Wang, W.; Boyd. J. M.; Wu M.; Hrudey, S. E.; Li, X.-F. UV-induced transformation of four halobenzoquinones in drinking water. *Environ Sci Technol* **2013**, *47* (9), 4426–4433.
9. Uchimiya, M.; Stone, A. T. Reversible redox chemistry of quinones: impact on biogeochemical cycles. *Chemosphere* **2009**, *77* (4), 451–458.
10. Hubig, S. M.; Kochi, J. K. Electrom-transfer mechanisms with photoactivated quinones. The encounter complex versus the Rehm-Weller paradigm. *J Am Chem Soc* **1999**, *121* (8), 1688–1694.
11. Arumugam, S.; Popik, V. V. Dual reactivity of hydroxy- and methoxy- substituted o-quinone methides in aqueous solutions: hydration versus tautomerization. *J Org Chem* **2010**, *75* (21), 7338–7346.
12. Wang, W.; Qian Y.; Boyd, J. M.; Wu, M.; Hrudey, S. E.; Li, X.-F. Halobenzoquinones in swimming pool waters and formation from personal care products. *Environ Sci Technol* **2013**, *47* (7), 3275–3282.
13. Monks, T. J.; Hanzlik, R. P.; Cohen, G. M.; Ross, D.; Graham, D. G. Quinone chemistry and toxicity. *Toxicol Appl Pharmacol* **1992**, *112* (1), 2–16.

14. Bolton, J. L.; Trush, M. A.; Penning, T. M.; Dryhurst, G.; Monks, T. J. Role of quinones in toxicity. *Chem Res Toxicol* **2000**, *13* (3), 135–160.
15. Brunmark, A.; Cadenas, E. Redox and addition chemistry of quinoid compounds and its biological implications. *Free Radic Biol Med* **1989**, *7* (4), 435–477.
16. Gorner, H. Photoprocesses of p-benzoquinones in aqueous solution. *J Phys Chem A* **2003**, *107* (51), 11587–11595.
17. Kutyrev, A. A.; Moskv, V. V. Nucleophilic reactions of quinones. *Russ Chem Rev* **1991**, *47* (38), 134–158.
18. Sarr, D. H.; Kazunga, C.; Charles, M. J.; Pavlovich, J. G.; Aitken, M. D. Decomposition of tetrachloro-1,4-benzoquinone (*p*-chloranil) in aqueous solution. *Environ Sci Technol* **1995**, *29* (11), 2735–1740.
19. Zhu, B.-Z.; Kalyanaraman, B.; Jiang, G.-B. Molecular mechanism for metal-independent production of hydroxyl rdicals by hydrogen peroxide and halogenated quinones. *Proc Natl Acad Sci USA* **2007**, *104* (45), 17575–17578.
20. Zhu, B.-Z.; Zhu, J.-G.; Mao, L.; Kalyanraman, B.; Shan, G.-Q. Detoxifying carcinogenic polyhalogented quinones by hydroxamic acids via an unuaual double lossen rearrangement mechanism. *Proc Natl Acad Sci USA* **2010**, *107* (48), 20686–20690.
21. Huang, R.; Wang, W.; Qian, Y.; Boyd, J. M.; Zhao, Y.; Li, X.-F. Ultra pressure liquid chromatography-negative electrospray ionization mass spectrometry determination of twelve halobenzoquinones at ng/L levels in drinking water. *Anal Chem* **2013**, *85* (9), 4520–4529.
22. Wu, Z.; Gao, W.; Phelps, M. A.; Wu, D.; Miller, D. D.; Dalton, J. T. Favorable effects of weak acids on negative-ion electrospray ionization mass spectrometry. *Anal Chem* **2004**, *76* (3), 839–847.
23. Poole, C. F. New trends in solid-phase extraction. *Trends Anal Chem*. **2003**, *22* (6), 362–373.
24. Kruve, A.; Auling, R.; Herodes, K.; Leito, I. Study of liquid chromatography/electrospray ionization mass spectrometry matrix effect on the example glyphpste analysis from cereals. *Rapid Commun Mass Spectrom* **2011**, *25* (21), 3252–3258.

25. Du, H. Y.; Li, J. H.; Moe, B.; McGuigan, C. F.; Shen, S. W.; Li, X.-F. Cytotoxicity and oxidative damage induced by halobenzoquinones to T24 bladder cancer cells. *Environ Sci Technol* **2013**, *47* (6), 2823–2830.
26. Boyd, J. M.; Huang, L.; Xie, L.; Moe, B.; Gabos, S.; Li, X.-F. A cell-microelectronic sensing technique for profiling cytotoxicty of chemicals. *Anal Chim Acta* **2008**, *789* (1), 83–90.
27. Plewa, M. J.; Wagner, E. D. *Mammalian Cell Cytotoxicity and Genotoxicity of Disinfection By-products*. Water Research Foundation: Denver, 2009; pp: 107–108.
28. Plewa, M. J.; Simmons, J. E.; Richardson, S. D.; Wagner, E. D. Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drikning water disinfection by-products. *Environ Mol Mutagen* **2010**, *51* (8-9), 871–878.

Table 5.1 Optimized MS conditions for analysis of HBQs and OH-HBQs.

Compound	Parent ion (m/z)	Product ion (m/z)	DP	CE	CXP
2,6-DCBQ	177	113	100	24	13
	177	141	100	20	13
OH-DCBQ	191	163	50	23	15
	191	83	50	33	10
DCMBQ	191	127	85	24	11
	191	155	85	20	11
OH-DCMBQ	205	177	50	22	10
	205	169	60	23	10
TriCBQ	211	35	80	18	11
	211	175	80	20	11
OH-TriCBQ	225	173	40	35	6
	225	154	40	42	13
2,6-DBBQ	267	79	100	50	10
	267	80	100	50	10
OH-DBBQ	281	79	36	65	10
	283	81	51	53	12

DP: declustering potential; CE: collision energy; CXP: collision cell exit potential.

Table 5.2 Theoretical and tested isotope pattern of transformation products of the four HBQs.

Parent HBQ	Transformation Product	Theoretical Mass[M-H]- m/z	ratio %	Theoretical Mass[M]- m/z	ratio %	Theoretical Mass[M+H]- m/z ratio %		Measured Mass m/z ratio %	
2,6-DCBQ	OH-DCBQ	190.93082	100.000	191.93865	100.000	192.94647	100.000	190.9309	100.0
$C_6H_2O_2Cl_2$	$C_6H_2O_3Cl_2$	191.93420	6.804	192.94203	6.814	193.94986	6.824	191.9359	14.5
		192.92797	64.752	193.93579	64.753	194.94362	64.754	192.9284	63.6
		193.93132	4.395	194.93915	4.402	195.94698	4.408	193.9328	9.2
		194.92530	10.737	195.93312	10.738	196.94095	10.738	194.9261	9.1
		195.92858	0.724	196.93641	0.725	197.94424	0.726		
2,6-DCMBQ	OH-DCMBQ	204.94647	100.000	205.95430	100.000	206.96212	100.000	204.9465	31.5
$C_7H_4Cl_2O_2$	$C_7H_4Cl_2O_3$	205.94985	7.936	206.95768	7.946	207.96551	7.956	205.9531	8.2
		206.94363	64.830	207.95146	64.831	208.95928	64.831	206.9455	13.1
		207.94698	5.128	208.95481	5.135	209.96264	5.141	206.9622	100.0
		208.94099	10.787	209.94882	10.788	210.95664	10.788	207.9497	5.1
		209.94425	0.845	210.95208	0.846	211.95991	0.848	208.9593	65.5
		210.94576	0.091	211.95359	0.091	212.96142	0.091	209.9623	5.4
		211.94856	0.005	212.95639	0.005	213.96422	0.005	210.9561	11.0

Parent HBQ	Transformation Product	Theoretical Mass[M-H]- m/z	ratio %	Theoretical Mass[M]- m/z	ratio %	Theoretical Mass[M+H]- m/z ratio %		Measured Mass m/z ratio %	
TriCBQ	OH-TriCBQ	224.89185	100.000	225.89968	100.000	226.90750	100.000	224.8924	28.6
$C_6HCl_3O_2$	$C_6HCl_3O_3$	225.89522	6.794	226.90305	6.804	227.91088	6.814	225.8995	13.6
		226.88896	96.730	227.89679	96.731	228.90461	96.732	226.9075	100.0
		227.89232	6.561	228.90015	6.571	229.90798	6.580	227.8971	19.0
		228.88614	31.444	229.89397	31.444	230.90179	31.445	228.9049	88.3
		229.88947	2.126	230.89730	2.129	231.90512	2.132	229.9074	5.8
		230.88355	3.516	231.89138	3.517	232.89920	3.517	230.9020	28.1
2,6-DBBQ	OH-DBBQ	278.82979	51.190	279.83761	51.190	280.84544	51.190	278.8295	39.0
$C_6H_2Br_2O_2$	$C_6H_2Br_2O_3$	279.83316	3.483	280.84099	3.488	281.84882	3.493	279.8354	7.2
		280.82777	100.000	281.83560	100.000	282.84342	100.000	280.8289	100.0
		281.83114	6.798	282.83897	6.808	283.84680	6.818	281.8337	15.5
		282.82581	49.234	283.83363	49.235	284.84146	49.235	282.8377	91.1
		283.82916	3.339	284.83699	3.344	285.84481	3.349	283.8349	10.5
		284.83060	0.388	285.83843	0.388	286.84626	0.389	284.8409	27.5

M: the initial analyte.

Table 5.3 Retention times, LODs, LOQs, recovery and matrix effect of the SPE-UHPLC-ESI-MS/MS method for analysis of HBQs and OH-HBQs.

Analytes	Retention time (min)	LOD1 (ng/mL)	LOD2 (ng/L)	LOQ (ng/L)	Recovery1 (%)	Recovery2 (%)	Matrix effects (%)
2,6-DCBQ	9.61 ± 0.01	0.08	0.07	0.2	92 ± 1	96 ± 2	97 ± 3
OH-DCBQ	7.76 ± 0.01	0.02	0.03	0.07	82 ± 3	92 ± 8	88 ± 3
DCMBQ	17.31 ± 0.01	0.01	0.7	2.4	69 ± 1	96 ± 2	98 ± 5
OH-DCMBQ	16.72 ± 0.01	0.06	0.4	1.2	62 ± 4	76 ± 2	93 ± 2
TriCBQ	14.67 ± 0.02	0.7	0.8	2.8	70 ± 3	78 ± 5	83 ± 8
OH-TriCBQ	12.61 ± 0.01	0.7	0.7	2.5	68 ± 3	79 ± 2	79 ± 2
2,6-DBBQ	11.96 ± 0.01	0.04	0.05	0.2	88 ± 5	95 ± 5	95 ± 5
OH-DBBQ	8.34 ± 0.01	0.02	0.04	0.1	83 ± 3	85 ± 4	95 ± 1

1) LOD1 is the LOD of the UHPLC-MS/MS method (no SPE) from analysis of 5 ng/mL standards and blanks, which is estimated as the concentration when the signal-to-noise ratio equals 3. The noise is determined by 3 times of the average peak height of 7 blank samples, and the signal is determined by the average peak height of triplicate 5 ng/mL standard samples.

2) LOD2 is the LOD of the SPE-UHPLC-MS/MS method for analysis of HBQs in the treated water samples, which is also estimated as the concentration when the signal-to-noise ratio equals 3. The noise is determined by 3 times of the average peak height of triplicate blank samples that were concentrated by SPE and then analyzed by UHPLC-

MS/MS. The signal is determined by the average peak height of triplicate 5 ng/mL standard samples directly analyzed using UPLC-MS/MS analysis.

3) LOQ is the LOQ of the SPE-UHPLC-MS/MS method for analysis of HBQs in the treated water samples, which is estimated as the concentration when the signal-to-noise ratio equals 10. The determination of noise and signal is the same as LOD2.

4) Recovery 1 and recovery 2 were determined by analysis of tap water samples spiked with twelve HBQs at 20 and 10 ng/L each, respectively.

5) Recovery (%) = (Concentration of pre-spiked sample – Concentration of no-spiked sample) / Concentration of standard sample, where the pre-spiked sample represents the sample spiked before SPE, the no-spike sample represents the sample without spike. The concentrations of these two sets of samples were determined by standard addition method. The standard sample is the standard solution prepared in pure solvent.

6) Matrix effect (%) = (Peak area of post-spiked sample – Peak area of no-spike sample) / Peak area of standard sample

Table 5.4 Summary of water treatment processes used in the five DWTPs.

DWTP #	1	2	3	4	5
Dissolved air flotation					×
Coagulation	×	×	×	×	
Sedimentation	×	×	×	×	
Filtration	×	×	×	×	×
UV		×	×	×	×
Chlorine	×	×	×	×	×
Ozone					×
Chloramines	×	×	×	×	

Table 5.5 The occurrence frequency and concentrations of the four HBQs and OH-HBQs in treated water collected from five water treatment plants.

Compound	Frequency	Con. (ng/L)	Compound	Frequency	Con. (ng/L)
2,6-DCBQ	34/37	nd─20	OH-DCBQ	34/37	nd─20
DCMBQ	11/37	nd─4	OH-DCMBQ	12/37	nd─7
TriCBQ	10/37	nd─20	OH-TriCBQ	6/37	nd─20
2,6-DBBQ	6/37	nd─10	OH-DBBQ	6/37	nd─10

Note: nd: not detected. The concentration is lower than the detection limit.

Table 5.6 HBQ and OH-HBQ concentrations in water samples from five water treatment plants (DWTP 1-5).

Water Plant	Sampling date	Location	2,6-DCBQ	DCMBQ	TriCBQ	2,6-DBBQ	OH-DCBQ	OH-DCMBQ	OH-TriCBQ	OH-DBBQ
Plant 1	2012.12.05	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	nd	nd	0.7±0.1	nd	nd	nd	nd	nd
		3	nd	nd	0.4±0.1	nd	nd	nd	nd	nd
		4	nd	nd	nd	nd	nd	nd	nd	nd
	2013.2.24	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	7.3±1.1	1.6±0.2	nd	nd	0.3±0.3	0.5±0.2	nd	nd
		3	4.2±0.5	0.7±0.3	1.1±0.2	nd	0.7±0.2	1.4±0.4	nd	nd
		4	2.5±0.5	nd	0.9±0.2	nd	1.1±0.4	1.9±0.4	nd	nd
	2013.6.24	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	8.0±0.8	3.1±0.1	10.3±1.1	4.7±0.5	6.2±0.4	1.4±0.2	3.2±0.2	5.7±0.5
		3	6.1±0.2	0.8±0.2	6.4±0.6	6.6±0.8	12.4±0.3	3.9±0.2	7.6±1.1	8.2±0.9
		4	4.1±0.3	0.7±0.2	2.3±0.4	9.2±0.6	19.9±0.2	5.0±0.2	14.3±0.8	9.6±0.5
	2013.9.25	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	19.7±1.3	3.6±0.2	20.3±1.8	4.7±0.5	1.0±0.1	0.5±0.2	5.2±0.5	2.7±0.5
		3	14.1±0.9	1.6±0.5	17.4±0.8	7.6±0.5	9.6±0.2	1.9±0.2	9.6±1.7	9.6±0.9
		4	1.3±0.3	1.0±0.2	4.3±0.4	10.2±0.3	18.3±0.3	6.0±0.2	20.3±1.8	10.2±0.3
Plant 2	2013.8.13	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	7.3±2.8	1.9 ±0.2	nd	nd	2.5±0.1	7.0±0.2	nd	nd

Water Plant	Sampling date	Location	2,6-DCBQ	DCMBQ	TriCBQ	2,6-DBBQ	OH-DCBQ	OH-DCMBQ	OH-TriCBQ	OH-DBBQ
Plant 3	2013.3.4	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	8.8±1.1	nd	nd	nd	1.1±0.1	nd	nd	nd
		3	5.6±0.3	nd	nd	nd	2.5±0.2	nd	nd	nd
		4	2.8±0.2	nd	nd	nd	5.6±0.3	nd	nd	nd
		5	4.4±0.1	nd	nd	nd	3.1±0.5	nd	nd	nd
		6	5.2±0.2	nd	nd	nd	1.6±0.5	nd	nd	nd
		7	3.3±0.2	nd	nd	nd	3.2±0.3	nd	nd	nd
		8	3.4±0.2	nd	nd	nd	4.1±0.9	nd	nd	nd
		9	4.1±0.1	nd	nd	nd	1.1±0.2	nd	nd	nd
		10	5.1±0.2	nd	nd	nd	0.5±0.4	nd	nd	nd
	2013.4.15	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	7.6±0.9	nd	nd	nd	1.7±0.7	nd	nd	nd
		3	3.7±0.9	nd	nd	nd	1.4±0.5	nd	nd	nd
		4	3.6±1.0	nd	nd	nd	0.9±0.7	nd	nd	nd
	2013.6.3	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	9.2±0.4	nd	nd	nd	0.7±0.7	nd	nd	nd
		3	5.9±1.0	nd	nd	nd	1.4±0.3	nd	nd	nd
		4	5.4±1.0	nd	nd	nd	2.6±1.0	nd	nd	nd
	2013.9.3	1	nd	nd	nd	nd	nd	nd	nd	nd

Water Plant	Sampling date	Location	2,6-DCBQ	DCMBQ	TriCBQ	2,6-DBBQ	OH-DCBQ	OH-DCMBQ	OH-TriCBQ	OH-DBBQ
		2	9.9±0.9	nd	nd	nd	1.7±0.4	nd	nd	nd
		3	8.7±0.8	nd	nd	nd	2.4±0.8	nd	nd	nd
		4	8.2±0.8	nd	nd	nd	4.5±1.8	nd	nd	nd
Plant 4	2013.3.4	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	7.2±1.0	nd	nd	nd	4.5±0.2	nd	nd	nd
	2013.4.15	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	8.9±0.8	nd	nd	nd	3.5±3.4	nd	nd	nd
	2013.6.3	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	9.9±0.9	nd	nd	nd	nd	nd	nd	nd
	2013.9.3	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	11.1±0.8	nd	nd	nd	5.4±0.8	nd	nd	nd
Plant 5	2013.8.13	1	nd	nd	nd	nd	nd	nd	nd	nd
		2	7.5±0.2	2.1±0.2	nd	nd	4.1±0.5	1.1±0.4	nd	nd
		4	4.2±0.4	0.9±0.1	nd	nd	7.2±0.9	6.2±0.6	nd	nd

1) Location 1 is raw water, 2 is water plant effluent, 3 and 4 are treated water in the distribution water system that is half-distance or maximum distance to the water plant, respectively.

2) Plant 2 and 3 share the same water distribution system.

3) nd: not detected. The concentration is below the detection limit.

Table 5.7 IC_{50} values of the four HBQs and four OH-HBQs on CHO-K1 cells.

	IC50 (μM)		
Compound	24h	48h	72h
2,6-DCBQ	27.3 ± 1.0	35.5 ± 1.0	41.5 ± 1.3
OH-DCBQ	61.0 ± 3.0	69.5 ± 5.6	90.6 ± 33.6
DCMBQ	11.4 ± 0.5	13.7 ± 0.5	15.9 ± 0.9
OH-DCMBQ	20.4 ± 0.6	21.5 ± 0.8	24.0 ± 1.1
TriCBQ	45.5 ± 2.5	63.7 ± 2.1	72.9 ± 3.6
OH-TriCBQ	64.4 ± 3.7	67.1 ± 4.7	69.8 ± 6.8
2,6-DBBQ	19.8 ± 1.5	29.2 ± 1.8	35.5 ± 0.7
OH-DBBQ	42.8 ± 6.2	51.8 ± 3.4	50.4 ± 7.6

Note: IC_{50} is the concentration of the compound determined from a regression analysis of the data (Figure 5), at which cell density is reduced to 50% of the negative control

Table 5.8 The CHO cell chronic cytotoxicity of US EPA regulated DBPs.

DBPs	Chemical Class	IC_{50} (mM)
Chloroform	Halomethane	9.92[27]
Bromodichloromethane	Halomethane	11.5[27]
Dibromochloromethane	Halomethane	No data
Bromoform	Halomethane	3.96[27]
Monochloroacetic acid	Haloacetic acid	0.944[28]
Dichloroacetic acid	Haloacetic acid	11.470[28]
Trichlorocetic acid	Haloacetic acid	17.520[28]
Monobromoacetic acid	Haloacetic acid	0.0089[28]
Dibromoacetic acid	Haloacetic acid	0.500[28]
Bromate		0.963[28]
Chlorate		No data

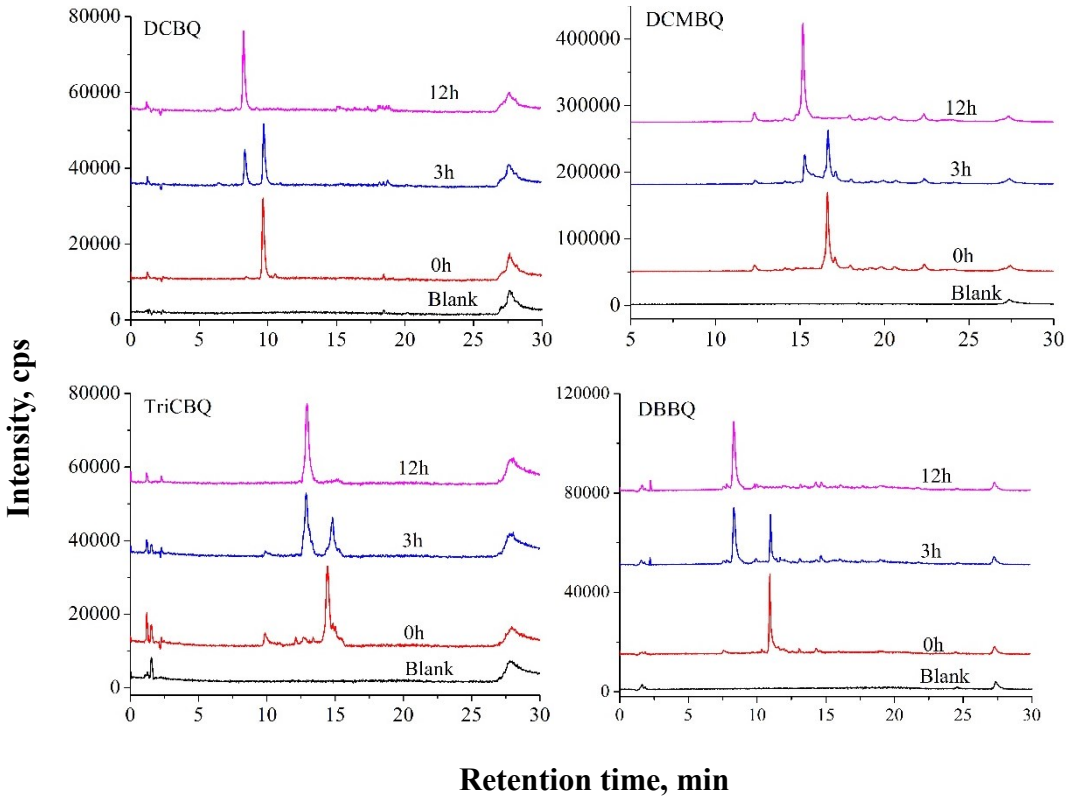


Figure 5.1 Total ion scan chromatograms of HBQ solutions.

Blank solution (only solvent, black) and (A) 2,6-DCBQ, (B) DCMBQ, (C) TriCBQ and (D) 2,6-DBBQ in freshly-prepared (red), 3-h old (blue), 12-h old (purple) solution.

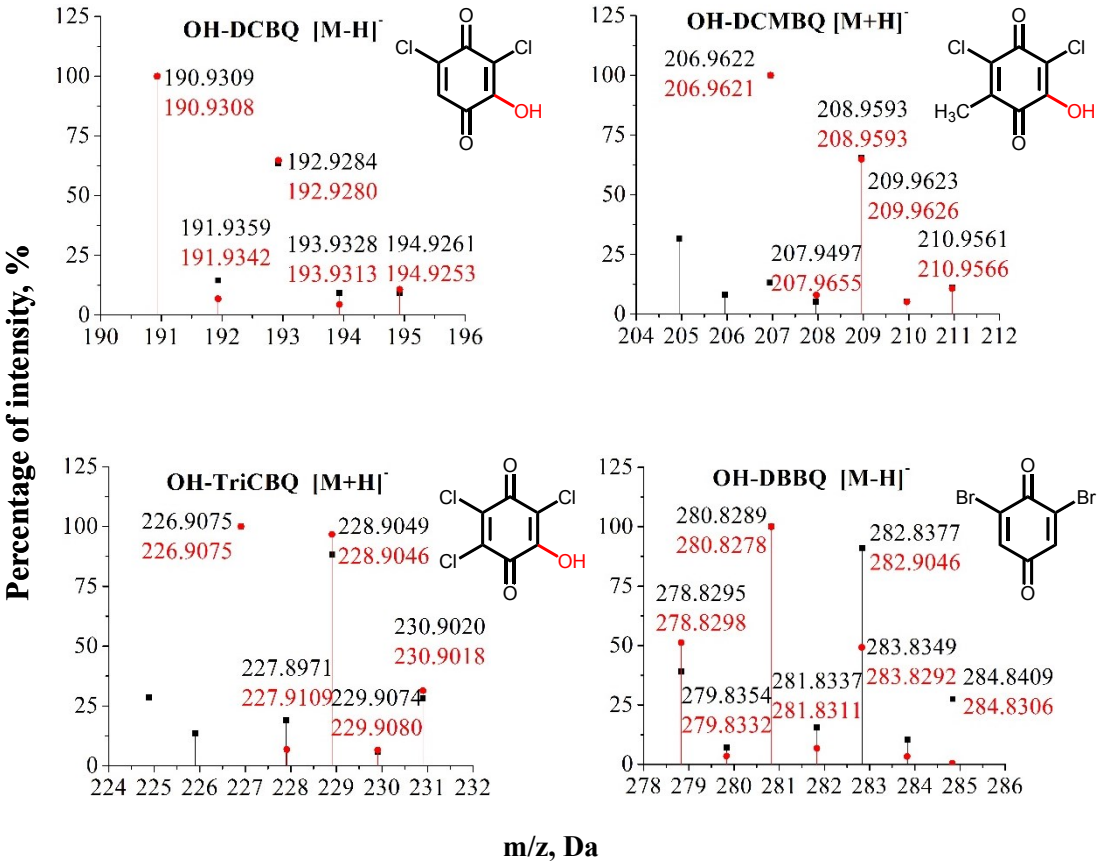


Figure 5.2 Isotope patterns of four OH-HBQs produced in the solutions of HBQs. The red trace is the theoretical value and the black trace is the measured value.

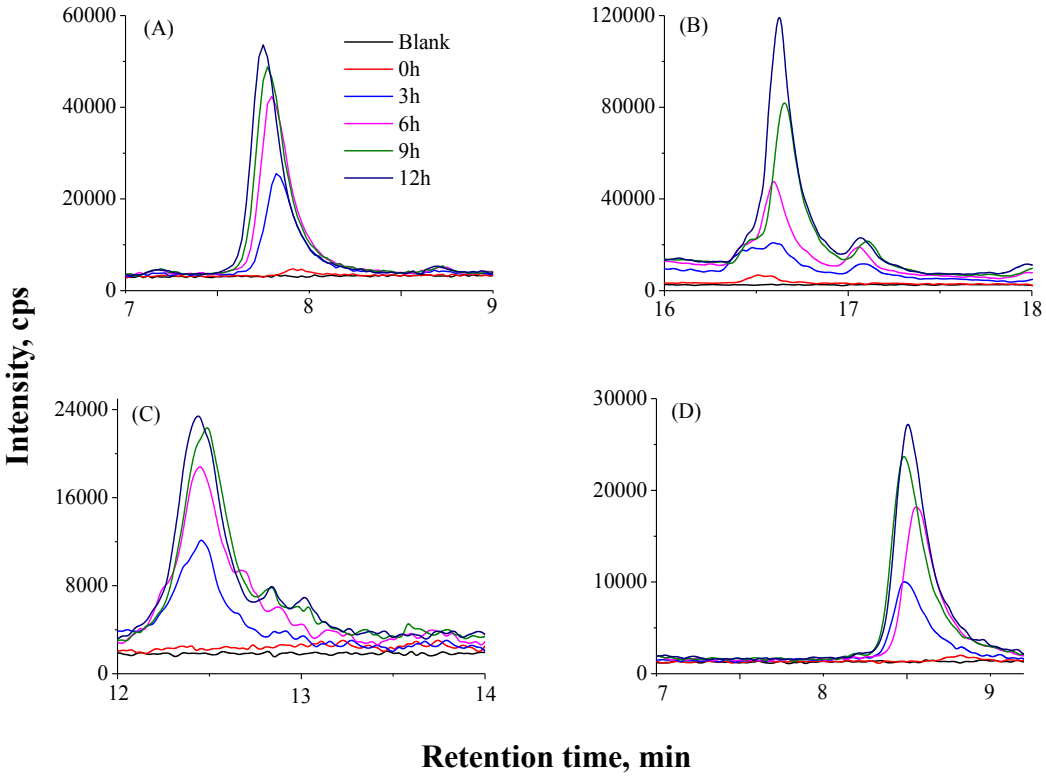


Figure 5.3 Specific extracted ion chromatograms (XIC) of HBQs in solutions over 12 h storage time.

(A) m/z range from 190 to 195 of 2,6-DCBQ solution; (B) m/z range from 205 to 210 of DCMBQ solution; (C) m/z range from 225 to 230 of TriCBQ solution; (D) m/z range from 279 to 285 of 2,6-DBBQ solution.

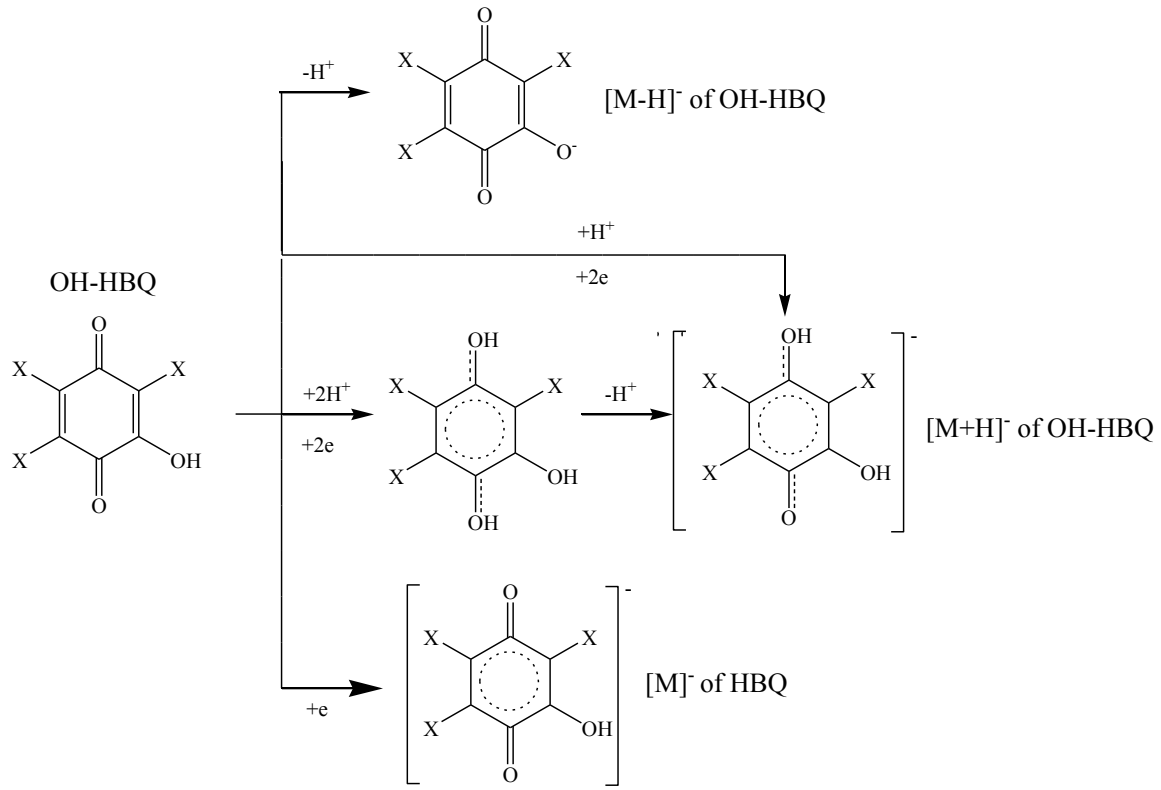


Figure 5.4 Proposed ionization processes of OH-HBQs.

X represents Cl, Br or CH_3. M represents the parent compound.

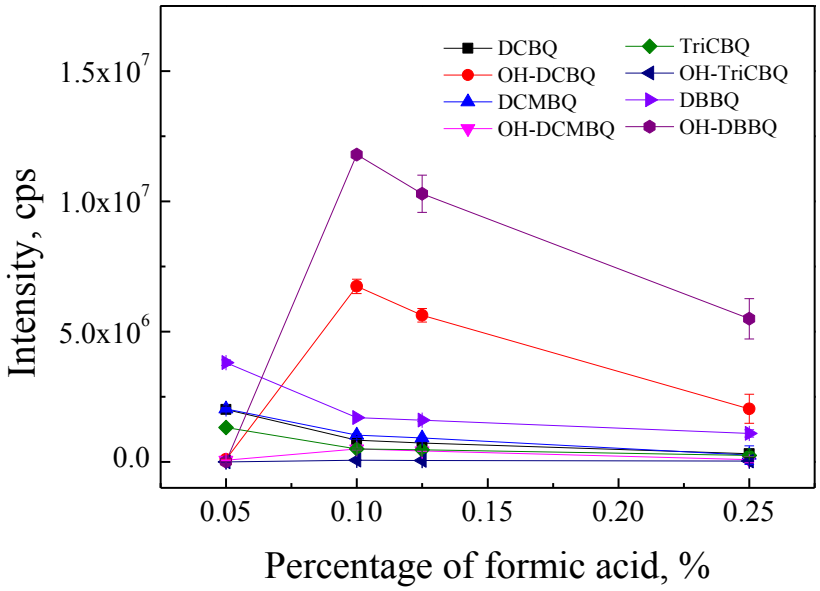


Figure 5.5 The intensity of HBQs and OH-HBQs as a function of the percentage of formic acid in the mobile phase of the LC-MS/MS analysis.

The concentrations of analytes were 100 ng/mL each.

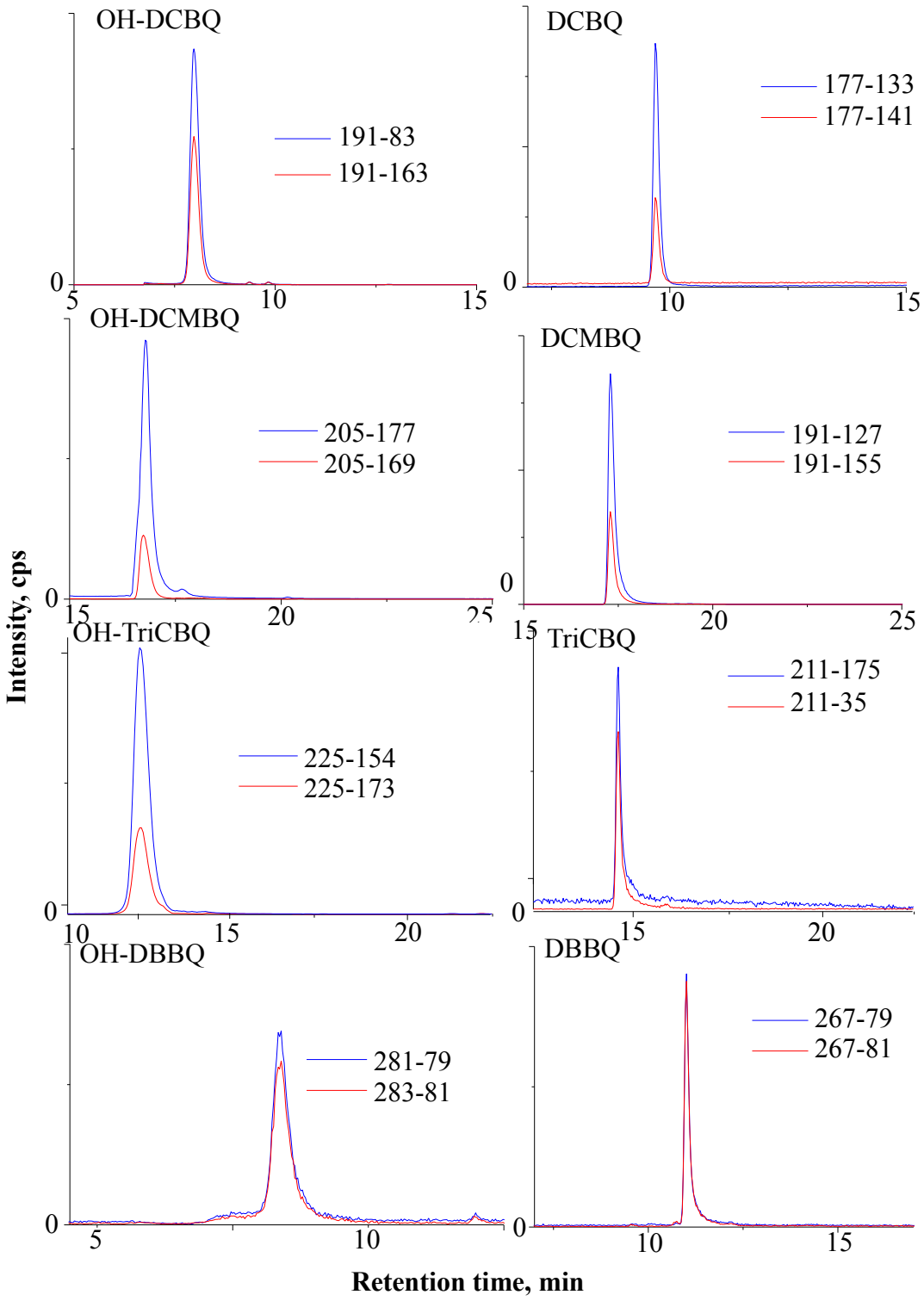


Figure 5.6 MRM chromatograms of four OH-HBQs and corresponding HBQs.

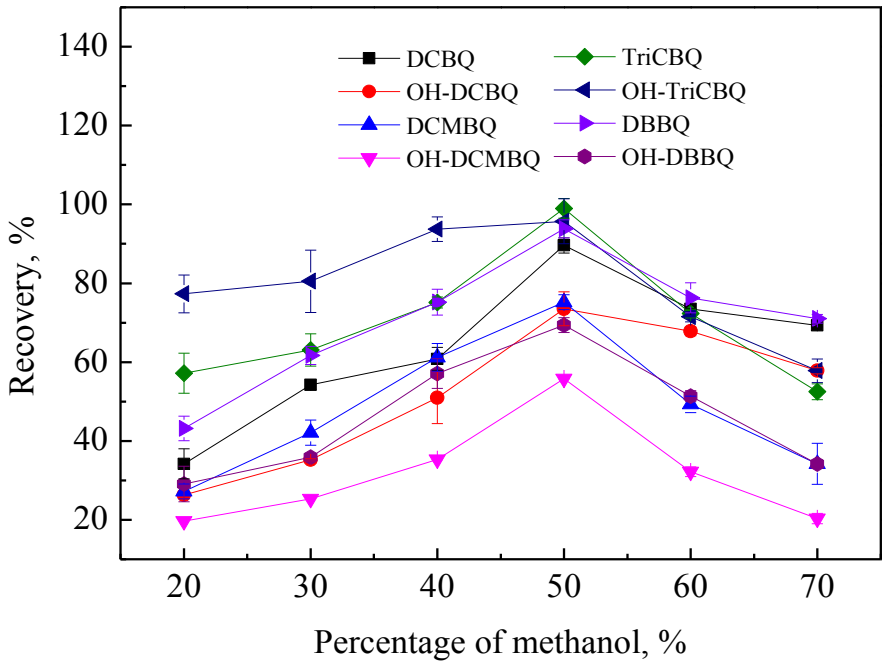


Figure 5.7 Recoveries of HBQs and OH-HBQs as a function of the content (%) of the methanol in the washing solution for SPE.

The initial concentration of each HBQs and OH-HBQs was 5 ng/L.

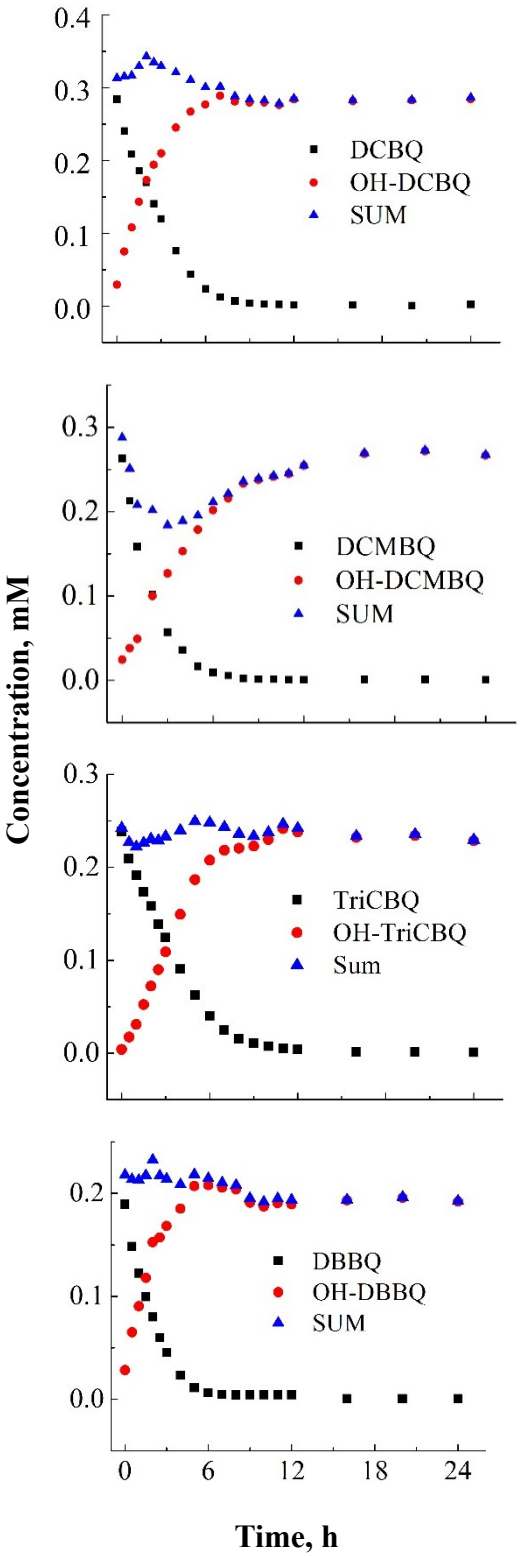


Figure 5.8 Time course of HBQs to OH-HBQs.

Initial HBQ solution was 50 µg/mL at pH 4.5 and was maintained at 4 ºC.

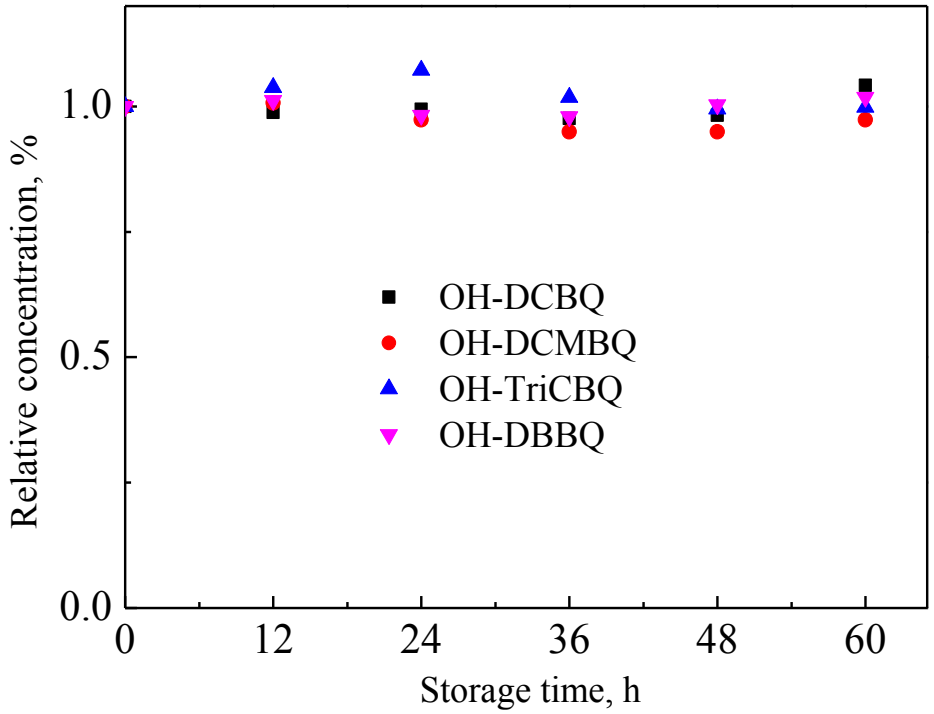


Figure 5.9 Stability of OH-HBQs in water at pH 7.0 over 60 h. Initial concentration was 50 µg/mL.

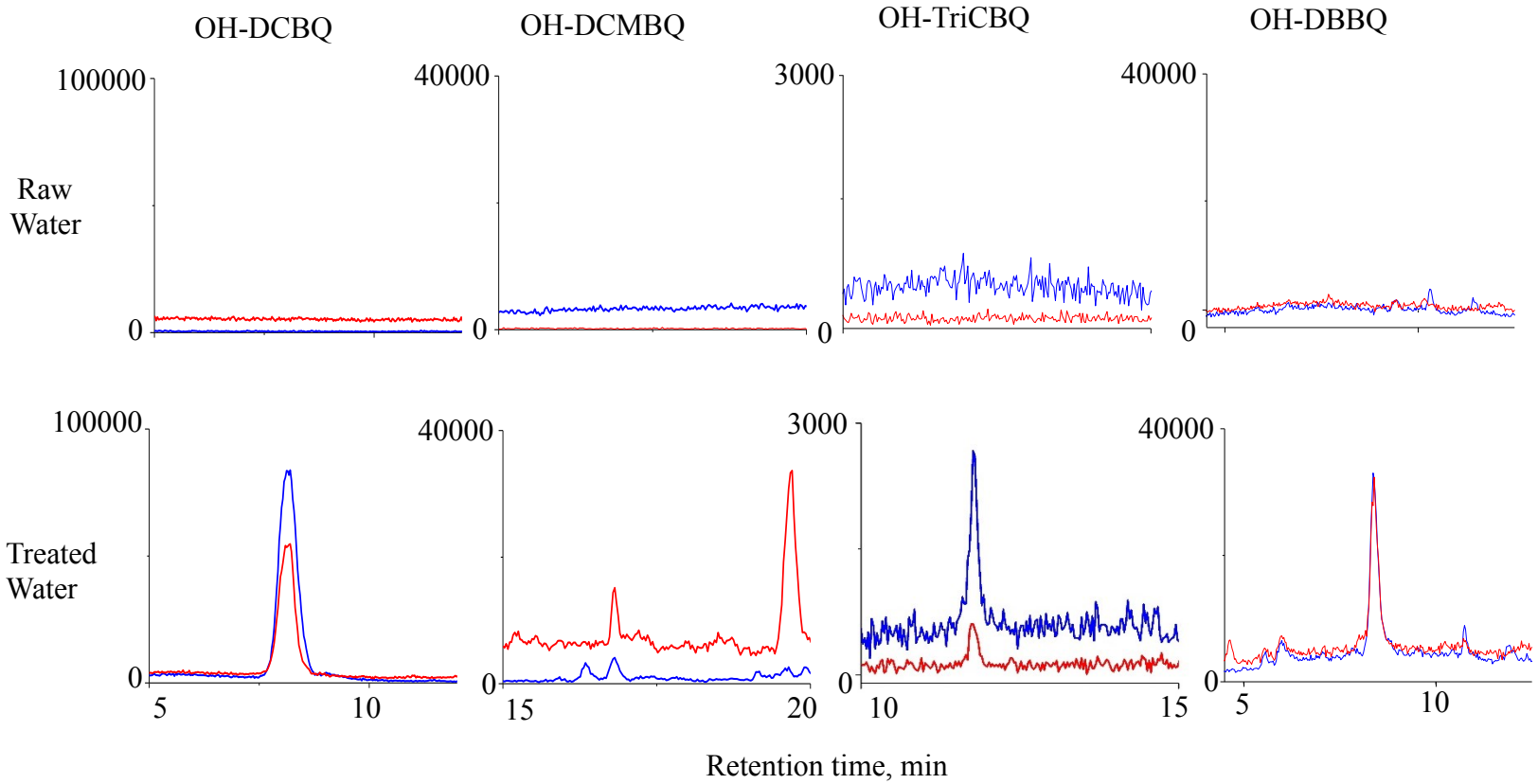


Figure 5.10 MRM chromatograms of four OH-HBQs obtained from the extracts of source water and treated water samples.

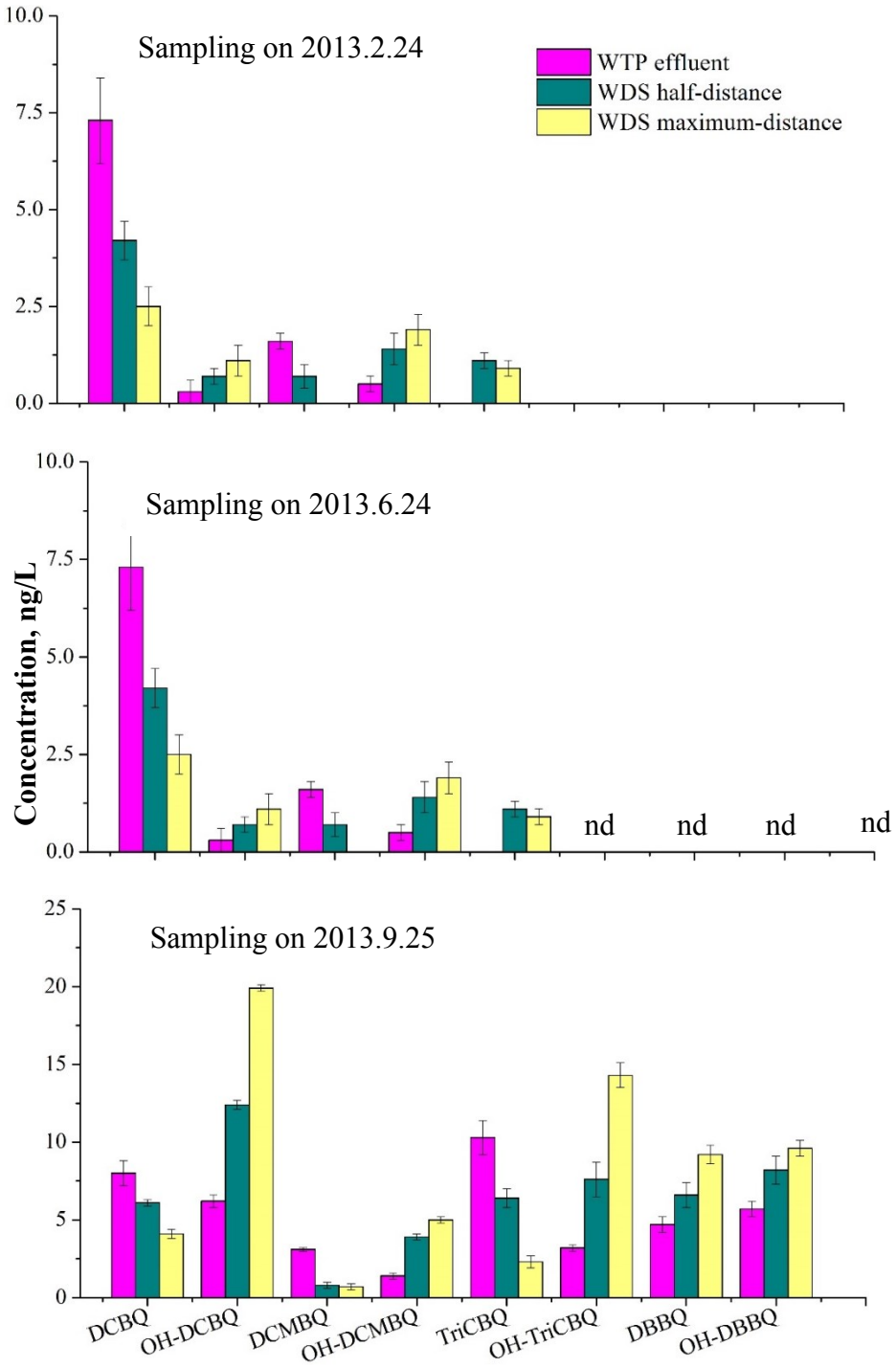


Figure 5.11 The concentrations of HBQs and OH-HBQs in Plant 1.

Samples were collected on 2013.2.24, 6.24 and 9.25.

nd: not detected, which concentration is lower than the detection limit.

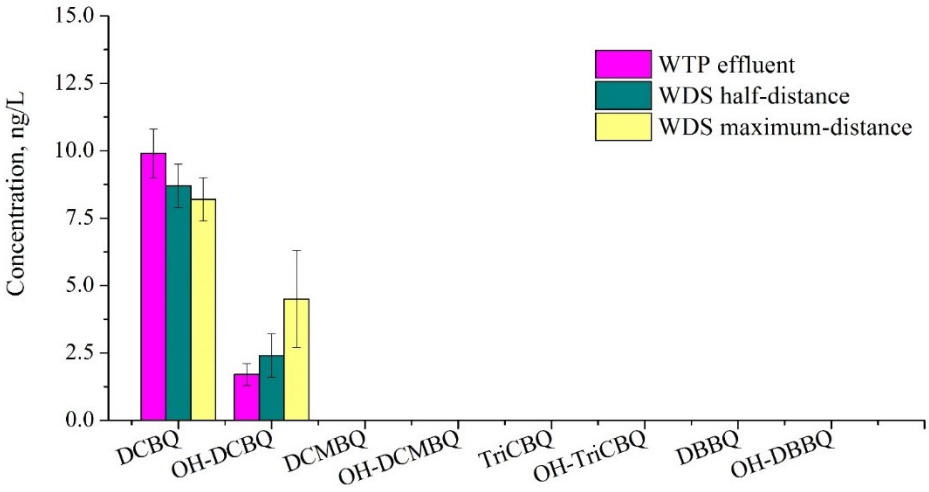


Figure 5.12 The concentrations of HBQs and OH-HBQs in Plant 2.

Samples were collected on 2013.9.4.

nd: not detected, which concentration is lower than the detection limit.

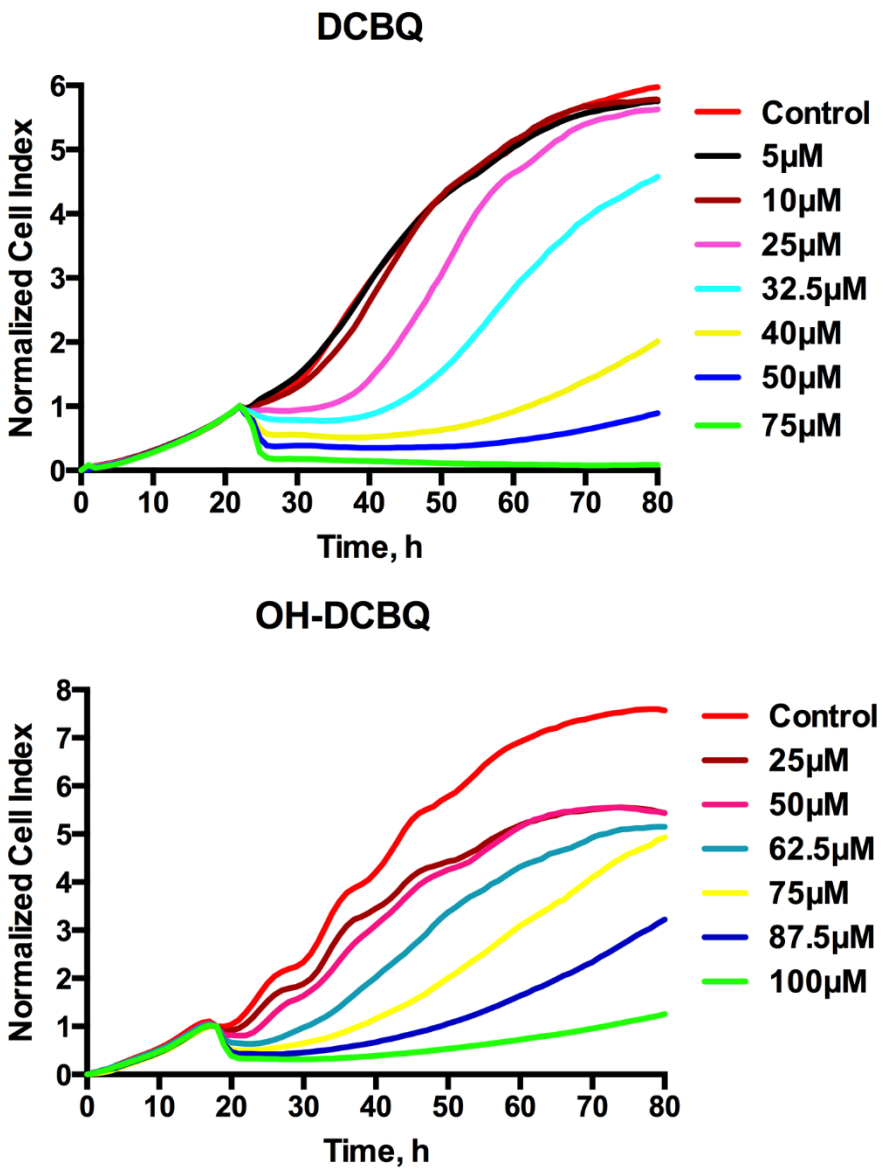


Figure 5.13 The RT-CES profiles of (A) 2,6-DCBQ and (B) OH-DCBQ on CHO-K1 cells.

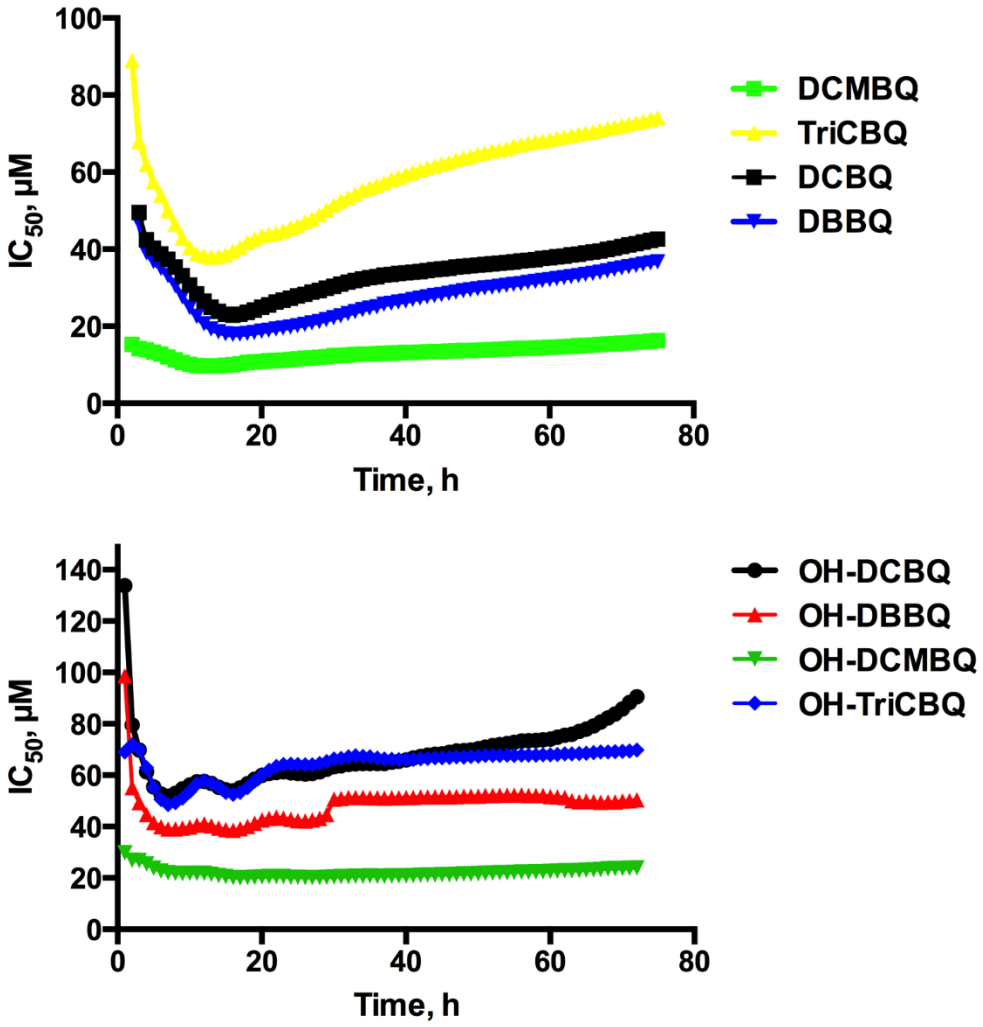


Figure 5.14 IC_{50} histogram of (A) HBQs and (B) OH-HBQs on CHO-K1 cells.

6 CONJUGATION OF HALOBENZOQUINONES WITH GLUTATHIONE IN AQUEOUS SOLUTION AND HEPG2 CELLS

6.1 INTRODUCTION

In the past five years, we have consistently identified four halobenzoquinones (HBQs) as disinfection byproducts (DBPs) in drinking water and recreational water in North America: 2,6-dichloro-(1,4)-benzoquinone (2,6-DCBQ), 3,5-dichloro-2-methyl-(1,4)-benzoquinone (DCMBQ), 2,3,6-trichloro-(1,4)-benzoquinone (TriCBQ), and 2,6-dibromo-(1,4)-benzoquinone (2,6-DBBQ).[1-4] *In vitro* cytotoxicity experiments confirmed that HBQs are highly cytotoxic and potentially genotoxic. HBQs are capable of producing reactive oxygen species (ROS), dysfunctioning cellular antioxidant systems, oxidizing protein and DNA, breaking DNA strands, and forming DNA adducts.[5-7]

Glutathione (GSH), the most abundant non-protein thiol in cells, serves as the major endogenous antioxidant protecting cells from HBQ toxicity.[6,8] A concentration-dependent depletion of the cellular GSH level was correlated with increased HBQ cytotoxicity in T24 bladder cancer cells.[6] Here, we confirm that the cellular GSH depletion by HBQs is due to both conjugation between GSH and HBQs and oxidation of GSH to glutathione disulfide (GSSG). Complex reaction mechanisms involving Michael addition, nucleophilic substitution, free radicals, desulfurization and disulfide are elucidated.

6.2 EXPERIMENTAL SECTION

6.2.1 Chemicals and solvents

3,5-Dichloro-2-methyl-(1,4)-benzoquinone (DCMBQ, ≥98%) and 2,3,6-trichloro-(1,4)-benzoquinone (TriCBQ, ≥98%) were synthesized by Shanghai Acana Pharmtech (Shanghai, China). 2,6-Dibromo-(1,4)-benzoquinone (2,6-DBBQ, ≥98%) was purchased from Indofine Chemical Company (Hillsborough, NJ). 2,6-Dichloro-(1,4)-benzoquinone (2,6-DCBQ, ≥98%), L-glutathione reduced (HPLC grade, ≥98%), and L-glutathione oxidized (HPLC grade, ≥98%) were purchased from Sigma-Aldrich (St. Louis, MO). Optima LC-MS grade water (0.03 micron filtration minimizes particles) and methanol (0.1 micron filtration minimizes particles) were purchased from Fisher Scientific (Nepean, ON). The purity was confirmed by UHPLC-UV and HPLC-MS detection. Formic acid (HPLC grade, 50% in water) was purchased from Fluka. Superoxide dismutase (SOD) was purchased from MP Biomedicals. Ethyl alcohol (EtOH), 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO).

6.2.2 Liquid chromatography–mass spectrometry analysis

The separation of conjugates was achieved by an ultra-high performance liquid chromatography system (UHPLC, Agilent 1290 Infinity Quaternary LC series) coupled with a Luna C18(2) column (100 × 2.0 mm i.d., 3μm; Phenomenex, Torrance, CA) at room temperature (25 ^{o}C). The mobile phase consisted of solvent A (0.1% FA in water) and solvent B (0.1% FA in methanol) with a flow rate of 0.17 mL/min. We optimized the gradient program and set the final method as: linearly increased B from 2% to 50% in 30 min; rapidly increased B from 50% to 90% in 0.01 second and kept until 35 min; finally

changed B to 2% in 0.01 second and kept until 40 min for column equilibration. The sample injection volume was 20 μL.

A quadrupole time-of-flight mass spectrometer (AB SCIEX TripleTOF® 5600 MS, AB SCIEX, Concord, ON, Canada) was connected with UHPLC to obtain the isotope pattern and fragment information of the conjugation products. Signal intensity and mass resolution were tuned every three hours using a specific external tuning solution (AB SCIEX calibration solution for the AB SCIEX TripleTOF 5600 system, Concord, ON, Canada). To obtain the information of all possible unknown conjugation products and reduce the interference from background, we developed an UHPLC-Triple TOF MS (Information Dependent Acquisition, IDA) method. In the IDA method, we set two parallel experiments: (1) negative ToF MS survey scan and (2) negative product ion scan. For the ToF survey scan, the specific conditions were: ion source voltage, -4500 V; gas I, 60 arbitrary units; gas II, 60 arbitrary units; curtain gas, 25 arbitrary units; source temperature, 450 ºC; declustering potential (DP), -90 V; accumulation time, 0.25 s; and scan range, m/z 100–3000. For the negative product ion scan, a maximum of four parent ions in each survey scan will be selected for MS/MS analysis. The criteria to initiate the MS/MS scan is set as follows: (a). the m/z of the parent ion is greater than 100, and smaller than 1250 (the maximum m/z that the instrument can measure); (b). the intensity of the parent ion is higher than 50 cps; (c). the charge state of the parent ion is between 1 and 4; (d). the isotope within 4 Da is excluded in the same cycle. The background is subtracted dynamically. The related parameters were set as follows: collision energy (CE), -40 V; collision energy spread (CES), 10 V; accumulation time, 0.2 s; and scan range, m/z 30–3000. The accurate masses of HBQs were set in the inclusion list to track

the peaks of HBQs at all times. PeakView™ (AB SCIEX) software was used for data analysis.

The stock solution of GSH (100 mmol/L in water) was prepared daily prior to the experiments. Reaction mixtures were prepared by mixing 10 μmol solid standard of HBQ with 10 mL aqueous GSH solution using vortex. The reaction mixtures contained 1 mmol/L HBQ with varying concentrations of GSH at 0.1, 0.3, 0.5, 1, 3, 5, 10 and 100 mmol/L in 10 mL of aqueous solutions. All reactions were conducted in amber bottles with Teflon cap to avoid light irradiation. The reaction mixtures were diluted for 10 times prior to UHPLC-QToF MS analysis.

6.2.3 Electron paramagnetic resonance analysis

Electron paramagnetic resonance (EPR) spectroscopy analysis was performed at room temperature using a Bruker Elexys E-500 spectrometer. 200 μL of the reaction solution was transferred to a flat cell for immediate scan. In all analysis, the Q value was at 1900 ± 100, and the frequency was kept at 9.8143 ± 0.0001 GHz. The scan range was from 3445 G to 3540 G, modulation amplitude was 1.0 G, and the sweep time was 60 s.

We dissolved solid standard of HBQ (purity ≥98%) in methanol (Optima LC-MS grade) to obtain 100 mM HBQ stock solution. GSH was dissolved in water (Optima LC-MS grade) to obtain 100 mM GSH solution in prior of the experiment. The stock solution was mixed and diluted in water for EPR analysis. DMPO was added to water and then mixed with the stock solution of 2,6-DBBQ/2,6-DCBQ and GSH for spin trapping experiments. To account for solvent effects, we analyzed a series of controls, including 1) pure water (Optima LC-MS grade); 2) 1% methanol (Optima LC-MS grade) in water; 3)

100 mM DMPO in water; and 4) 50 mM GSH with 100 mM DMPO in water. No free radical was detected in any of the negative control samples.

6.2.4 Collection of Treated Cell and Culture Medium

Hepatocellular carcinoma cell line HepG2 was obtained from the American Type Culture Collection (ATCC). The cells were incubated in 60 mm dishes and stored in a humidified 37 ºC, 5% CO_2 incubator. The culture medium was Eagle's Minimum Essential Medium (ATCC; #30-2003) supplemented with 10% fetal bovine serum (Sigma; #F1051) and 1% of 1000 U penicillin/1000 µg streptomycin solution (Gibco; #15140-122). Until the concentration of cells reached about 1×10^{6}/mL, HBQs were dosed at concentrations of ½ IC_{50} at 24 h: 36 µM for 2,6-DCBQ, 85 µM for DCMBQ, 97.5 µM for TriCBQ, and 85 µM for 2,6-DBBQ. Untreated cells (no HBQs were added) were included as negative control. Cells were collected by trypsinization after certain time of exposure and washed by Dulbecco's phosphate-buffered saline (PBS) three times. The cell pellets were resuspended in 100 µL of ice-cold formic acid (5%), homogenized for 1 min, and centrifuged at 10000 g at 4 ºC for 10 min.

6.3 RESULTS AND DISCUSSION

6.3.1 Identification of GSH with 2,6-DCBQ by UHPLC-MS/MS

Figure 6.1 shows typical chromatograms of the UHPLC-QToF MS analysis of aqueous reaction solution containing 1 mM 2,6-DCBQ with varying concentrations of GSH at A) 0.1 mM, B) 1 mM, and C) 5 mM, respectively. The reaction mixtures were diluted with water ten times prior to LC-MS/MS analysis. Comparing the chromatograms of the reaction mixtures with those of the blank, pure solutions of GSSG, GSH, and 2,6-DCBQ

(Figure 6.2), several new peaks are clearly separated from GSSG, GSH and 2,6-DCBQ in the reaction mixture of 2,6-DCBQ with GSH. GSH and 2,6-DCBQ can readily form conjugates and the reaction can complete within 5 min.

To identify the structures of these conjugation products, I developed and used a UHPLC-QToF (IDA) method to acquire the accurate mass measurements by ToF scan, and to obtain the MS/MS spectra of candidate precursors by product ion scan in the same run. Figure 6.3 shows the scan spectra of the parent ions of four conjugates (1-1, 2-1, 3-1, and 4-1) and their MS/MS spectra (1-2, 2-2, 3-2, and 4-2). For example, Peak 7 at retention time 16.2 min in Figure 6.1 has molecular ion (m/z 753.1287) and the isotopic pattern shown in Figure 6.3(2-1). The measured accurate masses correspond to chemical formula $[C_{26}H_{34}N_6O_{14}S_2Cl]^-$ with a mass accuracy of 4.3 ppm. Its MS/MS spectrum was obtained by the IDA product ion scan, as shown in Figure 6.3(2-2). Several characteristic fragments of GSH-conjugates were identified in the MS/MS spectrum: the fragment m/z 306.0772 corresponding to GSH; m/z 272.0893 resulting from the elimination of H_2S from GSH; m/z 254.0786 from the elimination of both H_2S and H_2O; m/z 143.0463 and m/z 128.0375 attributing to the cleavage of the γGlu-Cys amide bond of 272.0893. These substructure fragments confirmed this peak corresponds to a GSH conjugate. The fragments m/z 172.9472 and 206.9346 correspond to the S-MCHQ and S-SH-MCHQ radicals, supporting that the formation of the sulfur-quinone bond corresponds to the conjugation of GSH with 2,6-DCBQ. Fragments m/z 444.0549 and m/z 480.0332 correspond to S-SG-HQ and S-SG-MCBQ radicals, respectively. Fragments m/z 624.0875 and m/z 717.1554 are formed from the elimination of Glu or H_2O from 2-MC-

3,6-DiSG-HQ. All fragment peaks in the dependent product ion scan correspond to substructures of 2-MC-3,6-DiSG-HQ, with mass accuracy better than 3.2 ppm.

Figure 6.3 presents the MS and MS/MS spectra of mono-, di-, tri- and tetra-SG conjugates. The ToF scan spectra (1-1, 2-1, 3-1, and 4-1) of the parent ions (blue line) match with their theoretical isotope patterns of $[M-H]^-$ (for compounds 1-3) and $[M-2H]^{2-}$ (for compound 4) (red line). Their MS/MS spectra (Figures 6.3.1-2, 2-2, 3-2, and 4-2) also match with the fragments of the proposed chemical structures. Similarly, we used the accurate masses of parent ions and their MS/MS spectra to identify other products.

In total, we identified eleven conjugates of 2,6-DCBQ with GSH, including mono-SG-BQ, di-SG-BQ, tri-SG-BQ, and tetra-SG-BQ conjugates as well as their isobaric isomers. Table 6.1 summarizes the chemical formula, putative structures, and formation pathways (presented with simplified reaction components) of the 11 conjugates identified in the reaction mixture of 2,6-DCBQ and GSH. It is noted that the same accurate mass (formula) may represent several isobaric positional isomers of a conjugate. The isomers always share similar MS and MS/MS spectra and thus the position of the substitution of SG is not distinguished. The chemical structures of isomers are further proposed based on the dipole moment (calculated using Chem3D Ultra^{TM}). The isomer with high dipole moment is of high polarity, thus its retention time on C_{18} column is shorter. For example, Peak 5 (retention time of 10.7 min) and Peak 7 (retention time of 14.5 min) have the same MS and MS/MS spectra corresponding to MC-DiSG-BQ. We propose that Peak 5 is 2-MC-5,6-DiSG-BQ with a higher dipole moment of 7.204 Debye, and Peak 7 is 2-MC-3,6-SG-BQ with a lower dipole moment of 2.570 Debye. In addition, HBQ always coexisted with halo-semiquinone (HSQ) and halohydroquinone (HHQ)

through reversible redox reaction.[9] Neither LC separation nor MS spectrum can distinguish the co-existing $[M-H]^-$ ion of HHQ, $[M]^-$ ion of HSQ, and $[M+H]^-$ ion of HBQ. Therefore, peak 4-14 may represent the mixture of three chemical forms.

6.3.2 The reaction pathways between GSH with 2,6-DCBQ

The identification of various GSH conjugates led to further investigation into the binding stoichiometry of 2,6-DCBQ with GSH. When the ratio of GSH/2,6-DCBQ was 0.1, mono-SG and di-SG substituted 2,6-DCBQ formed, including 2,6-DC-3,5-DiSG-BQ (Peak 9) and 2,6-DC-3-SG-HQ (Peak 10). Michael addition of GSH on 2,6-DCBQ forms 2,6-DC-3-SG-HQ that can be oxidized by oxygen or 2,6-DCBQ to form 2,6-DC-3-SG-BQ. Subsequently, a second GSH attacks 2,6-DC-3-SG-BQ to form 2,6-DC-3,5-DiSG-HQ and 2,6-DC-3,5-DiSG-BQ. The proposed reaction pathways are as follows.

2,6-DCBQ + GSH ↔ 2,6-DC-3-SG-HQ (Figure 6.1, Peak 10)

2,6-DC-3-SG-HQ (Peak 10) + O_2 ↔ 2,6-DC-3-SG-BQ (Figure 6.1, Peak 10) + H_2O_2

2,6-DC-3-SG-HQ (Peak 10)+ 2,6-DCBQ ↔ 2,6-DC-3-SG-BQ (Figure 6.1, Peak 10) + DCHQ

2,6-DC-3-SG-BQ + GSH ↔ 2,6-DC-3,5-DiSG-HQ (Figure 6.1, Peak 9)

2,6-DC-3,5-DiSG-HQ + O_2 ↔ 2,6-DC-3,5-DiSG-BQ (Figure 6.1, Peak 9) + H_2O_2

2,6-DC-3,5-DiSG-HQ + 2,6-DCBQ ↔ 2,6-DC-3,5-DiSG-BQ (Figure 6.1, Peak 9) + DCHQ

When the GSH/2,6-DCBQ was increased to 1, additional dechlorinated SG conjugates were formed, including 2-MC-5,6-DiSG-HQ (Peak 5), 2-MC-3,6-DiSG-HQ (Peak 7), 2,6-DiSG-HQ (Peak 12), 2-MC-3,5-DiSG-HQ (Peak 13), and 2-MC-6-SG-HQ

(Peak 14). The loss of chlorine from 2,6-DCBQ to form these conjugates indicated that GSH substitutes the chlorine on the BQ ring through nucleophilic reaction. HHQs are believed to not react with GSH. Because of the higher electron density of HHQs, chlorine would be unfavorable as a leaving group.[10]

2,6-DCBQ + GSH ↔ 2-MC-6-SG-BQ (Figure 6.1, Peak 14) + HCl

2-MC-6-SG-BQ + GSH ↔ 2,6-DiSG-BQ (Figure 6.1, Peak 12) + HCl

These dechlorinated conjugation products can further undergo Michael addition to form more glutathionyl conjugation products. A rearrangement reaction may occur for the formation of 2-MC-3,5-DiSG-HQ.

2-MC-6-SG-BQ + GSH ↔ 2-MC-5,6 (or 3,6; or 3,5)-DiSG-HQ (Figure 6.1, Peak 5, 7 or 14)

When GSH/2,6-DCBQ was increased to 5, triSG conjugates (Peak 4 and 6) and TetraSG-BQ (Peak 3) emerged, and some mono- and di-SG conjugates still existed (Figure 6.1C). These conjugates all coexisted and achieved chemical equilibrium. Even when the ratio of GSH/2,6-DCBQ is as high as 100, mono-SG substituted conjugates were still detectable and did not completely transform to tri- and tetra-SG substituted conjugates.

2-MC-5,6 (or 3,6; or 3,5)-DiSG-BQ + GSH ↔ 2-MC-3, 5, 6-TriSG-HQ (Figure 6.1, Peak 6)

2-MC-5,6 (or 3,6; or 3,5)-DiSG-BQ + GSH ↔ TriSG-BQ (Figure 6.1, Peak 4)+ HCl

2,6-DiSG-BQ + GSH ↔ TriSG-HQ (Figure 6.1, Peak 4)

TriSG-BQ + GSH ↔ TetraSG-HQ

2-MC-3, 5, 6-TriSG-BQ + GSH ↔ TetraSG-BQ (Figure 6.1, Peak 3) + HCl

The redox reaction between hydroquinone (HQ) to benzoquinone (BQ) derivatives can be a two electron reduction or two sequential one-electron reduction steps through the formation of semiquinone radical.[11] To examine the possible production of semiquinone radicals (SQ) in the reaction process, we analyzed the reaction mixture of 2,6-DCBQ and GSH at varying molar ratios using EPR. Figures 6.4 show that 2,6-dichloro-semiquinone radical ($DCSQ^{\bullet -}$) was identified (g=2.00538), and the intensity of the radical was decreased as a function of increased GSH level. When GSH was increased to 100 μM, $DCSQ^{\bullet -}$ was undetectable.

2,6-DCBQ can undergo one-electron transfer reaction, forming 2,6-dichloro-(1,4)benzosemiquinone radical ($DCSQ^{\bullet -}$):

2,6-DCBQ + e^- ↔ $DCSQ^{\bullet -}$ (Figure 6.4A and 6.5A)

Sequential one-electron transfer reaction forms 2,6-dichloro-(1,4)hydroquinone (DCHQ)

$DCSQ^{\bullet -}$ + e^- + $2H^+$ ↔ DCHQ

In attempt to trigger more transient free radicals, we analyzed the mixture using EPR spin trapping with 100 mM DMPO. Figure 6.5 shows the signals detected when DMPO was added to the reaction mixture of 2,6-DCBQ with GSH. In addition to $DCSQ^{\bullet -}$, we triggered the apparent signal of DMPO/$^{\bullet}$OH spin adduct. However, the signal of DMPO/$^{\bullet}$OH spin adduct was not depleted when DMSO or SOD was added (Figure 6.6). Thus the DMPO/$^{\bullet}$OH signal is not an indication of the formation of free superoxide or hydroxyl radicals in the reaction system, but from the direct oxidation of DMPO by 2,6-DCBQ.[12,13] The mechanism is described as follows:

2,6-DCBQ + DMPO $\xrightarrow{\text{Light}}$ $2,6\text{-}DCBQ^{\bullet -}$ + $DMPO^{\bullet +}$

$DMPO^{+\bullet} + H_2O \rightarrow DMPO/^{\bullet}OH + H^{+}$

With the increase of GSH, both the $DCSQ^{\bullet-}$ and the $DMPO/^{\bullet}OH$ were decreased. When the GSH was increased to 5 mM, all free radical species were undetectable. The conjugation of GSH to 2,6-DCBQ will increase the electron intensity, thus the conjugation products are more easily oxidized than 2,6-DCBQ. Conjugation products tend to undergo two-election oxidation from the HQ form to the BQ form, instead a step-by-step one-election oxidation. Thus we did not identify any SQ radical of conjugation products.

$DCHQ + O_2 \leftrightarrow 2,6\text{-}DCBQ + H_2O_2$

$H_2O_2 + DCSQ^{\bullet-} \leftrightarrow HO^{\bullet}$ (Figure 3 A) $+ OH^{-} + 2,6\text{-}DCBQ$ [14]

$DCSQ^{\bullet-}$ radical or hydroxyl radical oxidized GSH to form GSSG[15] [16]

$DCSQ^{\bullet-} + e^{-} + 2GSH \leftrightarrow DCHQ + GSSG$ (Figure 1, Peak 2)

$2HO^{\bullet} + 2GSH \leftrightarrow GSSG$ (Figure 1, Peak 2) $+ H_2O$

The amount of hydroxyl radical is very limited, thus the major oxidant is SQ radical.

In summary, three typical reactions between GSH and 2,6-DCBQ were involved: nucleophilic substitution of chlorine on the BQ ring to form glutathionyl BQs; Michael addition of GSH to the BQ ring to form glutathionyl HQs; and reversible redox reaction between HBQ and HSQ or HHQ along with oxidizing GSH to GSSG (Figure 6.7). With the increase of the GSH/2,6-DCBQ ratio, the conjugation level of GSH to 2,6-DCBQ is increased, that is, mono and di-SG substituted BQs are further glutathionylated to tri- and tetra-SG BQs. Figure 6.8 illustrates the proposed pathways of GSH conjugation to 2,6-DCBQ. The reaction mechanism between TriCBQ and GSH is similar.

6.3.3 The reaction between GSH with DCMBQ

When DCMBQ was incubated with GSH, five conjugation products and GSSG were identified in the mixture using UHPLC-MS/MS. The name, formula, and simplified formation mechanisms of these conjugates are summarized in Table 6.2. In addition to the three typical reactions, we observed substitution of a methyl group by a glutathionyl group. The proposed reaction processes are shown in Figure 6.9. GSH attacks the methyl-connected carbon on the BQ ring of TriSG-MBQ (Compound 9-1) to form Compound 9-2, with a subsequent elimination of a hydrogen on methyl and a SG group to form compound 9-3. Addition of GSH or H_2O (H_2O can serve to provide hydrogen) to the double bond can form a HQ compound 9-4 and BQ compound 9-5. A similar reaction process forms TetraSG-HQ (Compound 9-7) and TetraSG-BQ (Compound 9-8).

6.3.4 The reaction between GSH with 2,6-DBBQ

We analyzed 2,6-DBBQ solution using EPR, and found 2,6-dibromo-(1,4)benzosemiquinone ($DBSQ^{\bullet-}$) radical in aqueous solution in natural aerobic/light environment. When 1 mM 2,6-DBBQ was incubated with GSH at a series of concentrations, only the $DBSQ^{\bullet-}$ radical was found and the intensity of the radical was decreased with the increase of the GSH/2,6-DBBQ ratio (Figure 6.10). The signal intensity of the $DBSQ^{\bullet-}$ radical was completely undetectable when the GSH concentration was as high as 300 μM ([GSH]/[2,6-DBBQ]=0.3). In addition to the $DBSQ^{\bullet-}$ radical, we also detected the formation of the DMPO/$^{\bullet}$OH adduct using DMPO spin trapping (Figure 6.11). Similar with 2,6-DCBQ. the intensity of $DBSQ^{\bullet-}$ and DMPO/$^{\bullet}$OH was decreased with the increase of GSH level.

Ten glutathionyl conjugates were identified from the reaction of 2,6-DBBQ with GSH using UHPLC-MS/MS: TetraSG-HQ (Peak 3), TriSG-HQ (Peak 4), 2-MB-TriSG-HQ (Peak 5), 2-MB-3,5-DiSG-HQ (Peak 6), 2-MB-TriSG-HQ (Peak 7), 2-MB-5,6-DiSG-HQ (Peak 9), 2-MB-3-SG-HQ (Peak 10), 2,6-DB-3,5-DiSG-HQ (Peak 12), 2-MB-6-SG-HQ (Peak 14), and 2,6-DiSG-HQ (Peak 16). (Figure 6.12 and Table 6.3). These products are formed from the reactions of free radical, Michael addition, and nucleophilic substitution, similar to the 2,6-DCBQ-GSH reaction. A difference is that the substitution of bromine by GSH is favored over Michael addition. Several debrominated compounds were identified at low GSH/2,6-DBBQ level, including 2-MB-3-SG-HQ (Peak 10), 2,6-DB-3,5-DiSG-HQ (Peak 12), and 2-MB-6-SG-HQ (Peak 14), and 2,6-DiSG-HQ (Peak 16) (Figure 6.12A). Thus the reaction is following the order: substitution of bromine by GSH > Michael addition of GSH on BQ ring > substitution of chlorine by GSH.

In addition, some minor products were identified in the mixture of 2,6-DBBQ with GSH, including MB-SG-G-HQ (Peak 5, 8 and 11), and MB-SSG-HQ (Peak 13). G is desulfurized glutathione, and SSG is disulfide glutathione. We propose the formation pathway of these G or SSG conjugates as follows (Figure 6.13): It has been reported that the β-elimination of cysteine or GSH forms dehydropeptide 13-2 and releases hydrogen sulfide (H_2S).[17-19] DBSQ radical reacts with compound 13-2 to form a carbon-centered radical 13-3. GSH donates a hydrogen to the oxidizing carbon-centered radical, acting as a free radical scavenger. This reaction happens very rapidly, thus we could not capture the intermediate radical.[20] The conjugation product 13-4 deforms to more stable HQ-form compound 13-5: 2,6-DB-3-G-HQ anion. 2,6-DB-3-G-HQ anion is further oxidized to 2,6-DB-3-G-BQ (compound 13-6). GS substitutes bromine on the 2,6-DB-3-G-BQ to form 2-

MB-5-G-6-SG-HQ (compound 13-7) or 2-MB-5-SG-6-G-HQ (compounds 13-8), corresponding to Figure 6.12, Peaks 5 and 8. On the other hand, GSH reacts with H_2S forming GSSH[21], and finally forming GSS conjugates.

$GSH+H_2S \rightarrow GSSH+H_2$

2,6-DBBQ+GSSH → MB-GSS-BQ (Figure 6.12, Peak 13) + HBr

6.3.5 Identification of conjugation products in HBQ-treated HepG2 cells

After confirming that 2,6-DCBQ can react with GSH in aqueous solution, we aimed to further study how HBQs react with GSH inside cells. When HepG2 cells were exposed to 2,6-DCBQ, GSSG and eight conjugation products of 2,6-DCBQ and GSH were identified in the cell extracts. Based on their retention times, accurate masses, and MS/MS spectra, the eight GSH conjugates were the same as those identified in the reaction mixture of GSH and 2,6-DCBQ at molar ratio of 5:1. The three conjugates identified at the molar ratio of GSH/2,6-DCBQ of 0.1:1 were not identified in cells. This is reasonable because the cellular level of GSH is between 1 to 11 mM,[8] more than 100 times higher than the dose of 2,6-DCBQ (36 μm).

We have collected cells after 10 min, 20 min, 30 min, 2 h, and 4 h exposure to 2,6-DCBQ, and the intensities of each conjugate as a function of exposure time are shown in Figure 6.14. Only mono and di-SG conjugates were identified after 10-min exposure. Eight conjugates were all identified in the cell extracts after 20-min treatment. The intensity of less glutathionylated conjugates was reduced with the increase of the intensity of more glutathionylated conjugates in 4 h. The result further supported the sequential conjugation of GSH on 2,6-DCBQ. Similarly, the conjugation products identified in DCMBQ or 2,6-DBBQ treated HepG2 cells were also found to be the same

as those detected in the mixtures of GSH/DCMBQ or GSH/2,6-DBBQ at 5:1 ratio, respectively. Only TetraSG-BQ was not identified in DCMBQ/2,6-DBBQ treated cells. The sequential conjugation trends were also observed in HepG2 cells treated by DCMBQ and TriCBQ.

6.4 CONCLUSION

In this chapter, we have examined the reaction products of GSH with HBQs both in aqueous solution and HepG2 cells. Mono, di, tri and tetraSG-HBQ conjugates were identified. The species and intensities of conjugates were changed from mono and diSG-HBQ conjugates to tri and tetraSG–HBQ conjugates when the ratio of GSH to HBQs was increased from 0.1 to 5. Three typical reactions were involved: the redox cycle reactions between HBQs and HHQs to form HSQ free radicals and GSSG, Michael addition of GSH on BQ, and nucleophilic substitution of halo group by GSH. The reaction follows the order: formation of HSQ radical > substitution of bromine by GSH > Michael addition of GSH on BQ ring > substitution of chlorine by GSH. In addition, we observed the unique conjugation products of GSH on 2,6-DBBQ, which were desulfurized or disulfide GSH conjugates. The conjugates identified in HepG2 cells were all identified in aqueous solution of GSH/HBQs at 5.

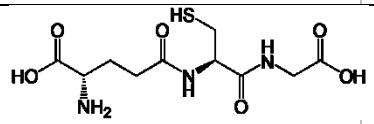
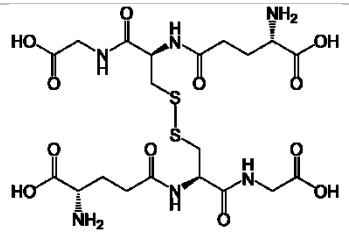
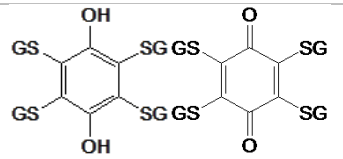
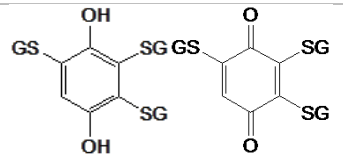
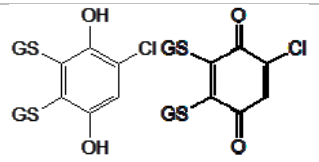
6.5 REFERENCES

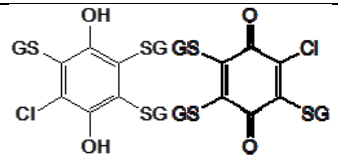
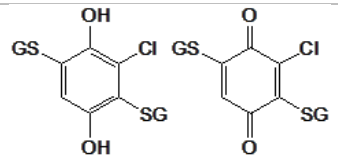
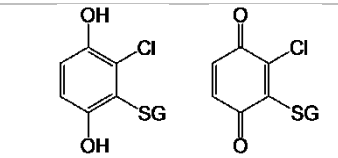
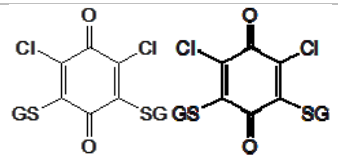
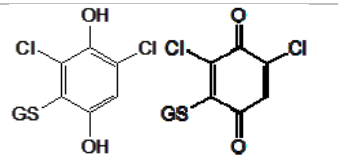
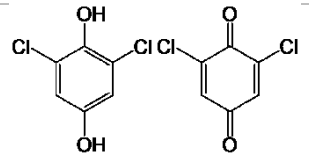
1. Qin, F.; Zhao, Y.-Y.; Zhao, Y.; Boyd, J. M.; Zhou, W.; Li, X.-F. A toxic disinfection by-product, 2,6-dichloro-1,4-benzoquinone, identified in drinking water. *Angew Chem Int Ed* **2010**, *49* (4), 790–792.
2. Zhao, Y.; Qin, F.; Boyd, J. M.; Anichina, J.; Li, X.-F. Characterization and determination of chloro- and bromo-benzoquinones as new chlorination disinfection byproducts in drinking water. *Anal Chem* **2010**, *82* (11), 4599–4605.

3. Zhao, Y.; Anichina, J.; Lu, X.; Bull, R. J.; Krasner, S. W.; Hrudey, S. E.; Li, X.-F. Occurrence and formation of chloro- and bromo-benzoquinones during drinking water disinfection. *Water Res* **2012**, *46* (14), 4351–4360.
4. Wang, W.; Qian, Y.; Boyd, J. M.; Wu, M.; Hrudey, S. E.; Li, X.-F. Halobenzoquinones in swimming pool waters and their formation from personal care products. *Environ Sci Technol* **2013**, *47* (7), 3275–3282.
5. Du, H. Y.; Li, J. H.; Moe, B.; McGuigan, C. F.; Shen, S. W.; Li, X. F. Cytotoxicity and oxidative damage induced by halobenzoquinones to T24 bladder cancer cells. *Environ Sci Technol* **2013**, *47* (6), 2823–2830.
6. Li, J. H.; Wang, W.; Zhang, H. Q.; Le, X. C.; Li, X. F. Glutathione-mediated detoxification of halobenzoquinone drinking water disinfection byproducts in T24 Cells. *Toxicol Sci* **2014**, *141* (2), 335–343.
7. Li, J. H.; Wang, W.; Moe, B.; Wang, H. L.; Li, X. F. Chemical and toxicological characterization of halobenzoquinones, an emerging class of disinfection byproducts. *Chem Res Toxicol* **2015**, *28* (3), 306–318.
8. Rhieu, S. Y.; Urbas, A. A.; Bearden, D. W.; Marino, J. P.; Lippa, K. A.; Reipa, V. Probing the intracellular glutathione redox potential by in-cell NMR spectroscopy. *Angew Chem Int Edit* **2014**, *53* (2), 447–450.
9. Gunner, M. R.; Madeo, J.; Zhu, Z. Modification of quinone electrochemistry by the proteins in the biological electron transfer chains: examples from photosynthetic reaction centers. *J Bioenerg Biomembr* **2008**, *40* (5), 509–519.
10. Song, Y.; Wagner, B. A.; Witmer, J. R.; Lehmler, H. J.; Buettner, G. R. Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones. *Proc Natl Acad Sci USA* **2009**, *106* (24), 9725–9730.
11. El-Najjar, N.; Gali-Muhtasib, H.; Ketola, R. A.; Vuorela, P.; Urtti, A.; Vuorela, H. The chemical and biological activities of quinones: overview and implications in analytical detection. *Phytochem Rev* **2011**, *10* (3), 353–370.
12. Monroe, S.; Eaton, S. S. Photo-enhanced production of the spin adduct 5,5-dimethyl-1-pyrroline-N-oxide/center dot OH in aqueous menadione solutions. *Arch Biochem Biophys* **1996**, *329* (2), 221–227.

13. Alegria, A. E.; Ferrer, A.; Santiago, G.; Sepulveda, E.; Flores, W. Photochemistry of water-soluble quinones. Production of the hydroxyl radical, singlet oxygen and the superoxide ion. *J Photochem Photobio A* **1999**, *127* (1-3), 57–65.
14. Zhu, B. Z.; Mao, L.; Huang, C. H.; Qin, H.; Fan, R. M.; Kalyanaraman, B.; Zhu, J. G. Unprecedented hydroxyl radical-dependent two-step chemiluminescence production by polyhalogenated quinoid carcinogens and H_2O_2. *Proc Natl Acad Sci USA* **2012**, *109* (40), 16046–16051.
15. Madrasi, K.; Joshi, M. S.; Gadkari, T.; Kavallieratos, K.; Tsoukias, N. M. Glutathiyl radical as an intermediate in glutathione nitrosation. *Free Radic Biol Med* **2012**, *53* (10), 1968–1976.
16. Wardman, P. Evaluation of the "radical sink" hypothesis from a chemical-kinetic viewpoint. *J Radioanal Nucl Chem* **1998**, *232* (1-2), 23–27.
17. Zhao, Y.; Wang, H.; Xian, M. Cysteine-activated hydrogen sulfide (H_2S) donors. *J Am Chem Soc* **2011**, *133* (1), 15–17.
18. Zhao, Y.; Biggs, T. D.; Xian, M. Hydrogen sulfide (H_2S) releasing agents: chemistry and biological applications. *Chem Commun* **2014**, *50* (80), 11788–11805.
19. Fonvielle, M.; Mellal, D.; Patin, D.; Lecerf, M.; Blanot, D.; Bouhss, A.; Santarem, M.; Mengin-Lecreulx, D.; Sollogoub, M.; Arthur, M.; Etheve-Quelquejeu, M. Efficient access to peptidyl-RNA conjugates for picomolar inhibition of non-ribosomal FemXWv aminoacyl transferase. *Chem Eur J* **2013**, *19* (4), 1357–1363.
20. Willson, R. L. Free radical repair mechanisms and the interactions of glutathione and vitamins C and E. In *Radioprotectors and Anticarcinogens*, Academic Press: London, 1982; pp 1–23.
21. Chung, H. S.; Wang, S. B.; Venkatraman, V.; Murray, C. I.; Van Eyk, J. E. Cysteine oxidative posttranslational modifications emerging regulation in the cardiovascular system. *Circ Res* **2013**, *112* (2), 382–392.

Table 6.1 The formula, possible structures and formation mechanism of each new peak identified in the mixture of 2,6-DCBQ with GSH.

No.	Retention time (min)	Name	Formula	Structure	Formation
1	3.2	Glutathione	$C_{10}H_{17}N_3O_6S$ (GSH)		Reactant
2	4.9	Glutathione disulfide	$C_{20}H_{32}N_6O_{12}S_2$ (GSSG)		2GS
3	7.6	2,3,5,6-Tetraglutathionyl-(1,4)hydroquinone/ benzoquinone	$C_{46}H_{66}N_{12}O_{26}S_4$ (TetraSG-HQ) (TetraSG-BQ)		4GS+2,6-DCBQ-2Cl-2H
4	8.8	2,3,5-Triglutathionyl-(1,4)hydroquinone/ benzoquinone	$C_{36}H_{51}N_9O_{20}S_3$ (TriSG-HQ) (TriSG-BQ)		3GS+2,6-DCBQ-2Cl-H
5	10.7	2-Monochloro-5,6-diglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{26}H_{35}N_6O_{14}S_2Cl$ (2-MC-5,6-DiSG-HQ) (2-MC-5,6-DiSG-BQ)		2GS+2,6-DCBQ-Cl-H

No.	Retention time (min)	Name	Formula	Structure	Formation
6	11.5	2-Monochloro-3,5,6-triglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{36}H_{50}N_9O_{20}S_3Cl$ (2-MC-3,5,6-TriSG-HQ) (2-MC-3,5,6-TriSG-BQ)		3GS+2,6-DCBQ-Cl-2H
7	14.5	2-Monochloro-3,6-diglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{26}H_{35}N_6O_{14}S_2Cl$ (2-MC-3,6-DiSG-HQ) (2-MC-3,6-DiSG-BQ)		2GS+2,6-DCBQ-Cl-H
8	15.0	2-Monochloro-6-glutathionyl-(1,4) hydroquinone/benzoquinone	$C_{16}H_{20}N_3O_8SCl$ (2-MC-6-SG-HQ) (2-MC-6-SG-BQ)		GS+2,6-DCBQ-Cl-H
9	17.9	2,6-Dichloro-2,5-diglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{26}H_{34}N_6O_{14}S_2Cl_2$ (2,6-DC-2,5-DiSG-HQ) (2,6-DC-2,5-DiSG-BQ)		2GS+2,6-DCBQ-2H
10	19.3	2,6-Dichloro-3-glutathionyl-(1,4) hydroquinone/benzoquinone	$C_{16}H_{19}O_8N_3SCl_2$ (2,6-DC-3-SG-HQ) (2,6-DC-3-SG-BQ)		GS+2,6-DCBQ-H
11	22.3	2,6-Dichloro-(1,4) hydroquinone/benzoquinone	$C_6H_2Cl_2O_2$ (2,6-DCBQ) (2,6-DCHQ)		Reactant

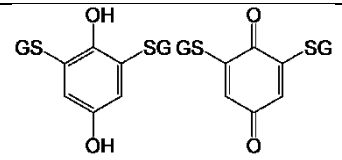
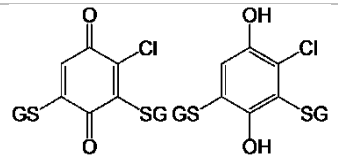
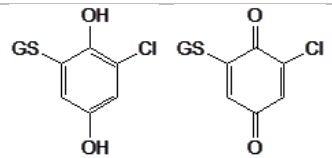
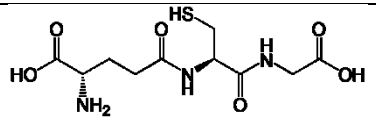
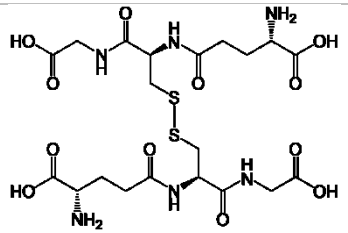
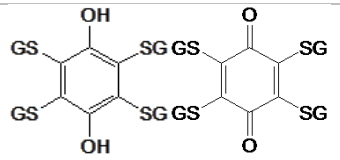
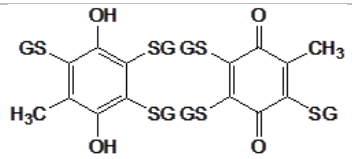
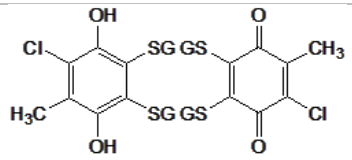
No.	Retention time (min)	Name	Formula	Structure	Formation
12	13.2	2,6-Diglutathionyl-(1,4)hydroquinone/ benzoquinone	$C_{26}H_{34}N_6O_{14}S_2$ (2,6-DiSG-HQ) (2,6-DiSG-BQ)		2GS+2,6-DCBQ-2Cl
13	16.2	2-Monochloro-3,5-diglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{26}H_{33}ClN_6O_{14}S_2$ (2-MC-3,5-DiSG-HQ) (2-MC-3,5-DiSG-BQ)		2GS+2,6-DCBQ-Cl-2H
14	17.3	2-Monochloro-6-glutathionyl-(1,4) hydroquinone/benzoquinone	$C_{16}H_{18}ClN_3O_8S$ (2-MC-6-SG-HQ)		GS+2,6-DCBQ-Cl-H

Table 6.2 The formula, possible structure and formation mechanism of each new peak identified in the mixture of DCMBQ with GSH.

No.	Retention time (min)	Name	Formula	Structure	Formation
1	3.2	Glutathione	$C_{10}H_{17}N_3O_6S$ (GSH)		Reactant
2	4.9	Glutathione disulfide	$C_{20}H_{32}N_6O_{12}S_2$ (GSSG)		2GS
3	7.6	2,3,5,6-Tetraglutathionyl-(1,4)hydroquinone/ benzoquinone	$C_{46}H_{66}N_{12}O_{26}S_4$ (TetraSG-HQ) (TetraSG-BQ)		4GS+2,6-DCBQ-2Cl-CH_3-H
4	12.0	2,3,5-Triglutathionyl-(1,4)hydroquinone/ benzoquinone	$C_{37}H_{53}N_9O_{20}S_3$ (TriSG-MHQ) (TriSG-MBQ)		3GS+DCMBQ-2Cl-H
5	16.1	2-Monochloro-5,6-diglutathionyl-3-methyl-(1,4)hydroquinone/ benzoquinone	$C_{27}H_{37}N_6O_{14}S_2Cl$ (2-MC-5,6-DiSG-MHQ) (2-MC-5,6-DiSG-MBQ)		2GS+DCMBQ-HCl

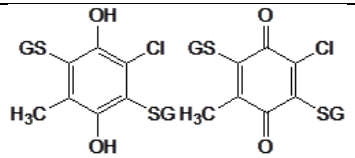
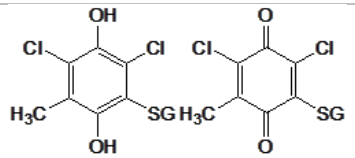
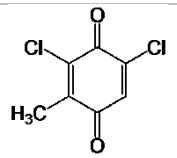
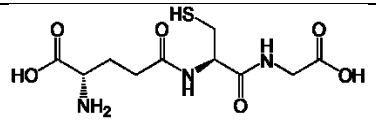
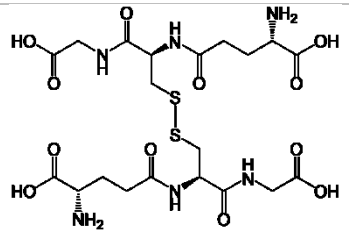
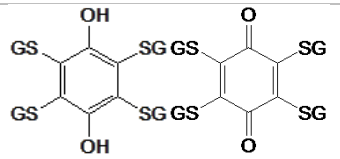
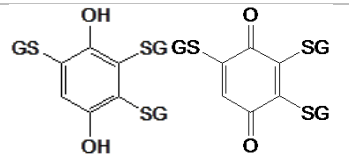
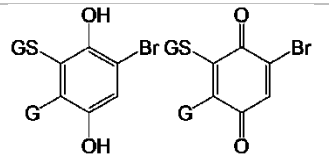
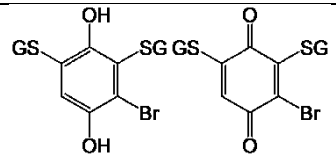
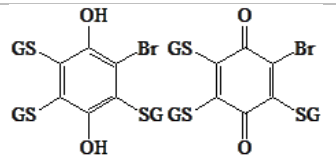
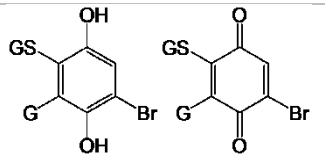
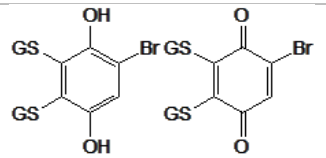
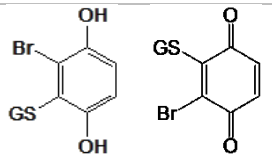
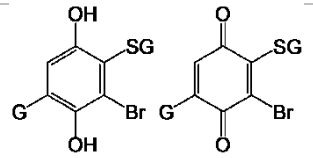
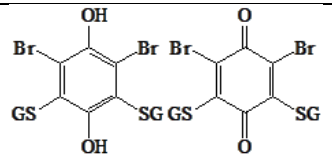
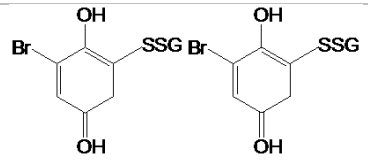
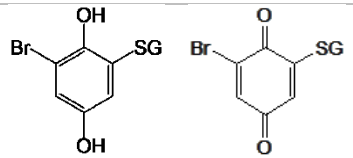
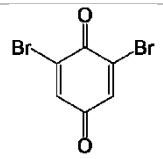
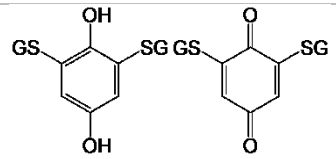
No.	Retention time (min)	Name	Formula	Structure	Formation
6	19.1	2-Monochloro-3,6-diglutathionyl-5-methyl-(1,4)hydroquinone/ benzoquinone	$C_{27}H_{37}N_6O_{14}S_2Cl$ (2-MC-3,6-DiSG-MHQ) (2-MC-3,6-DiSG-MBQ)		2GS+DCMBQ-HCl
7	25.9	2,6-Dichloro-3-glutathionyl-5-methyl-(1,4)hydroquinone/ benzoquinone	$C_{17}H_{21}N_3O_8SCl_2$ (2,6-DC-3-SG-MHQ) (2,6-DC-3-SG-MBQ)		GS+DCMBQ-H
9	30.0	2,6-Dichloro-3-methyl-(1,4)hydroquinone/ benzoquinone	DCMBQ DCMHQ		Reactant

Table 6.3 The formula, possible structure and formation mechanism of each new peak identified in the mixture of 2,6-DBBQ with GSH.

No.	Retention time (min)	Name	Formula	Structure	Formation
1	3.2	Glutathione	$C_{10}H_{17}N_3O_6S$ (GSH)		Reactant
2	4.9	Glutathione disulfide	$C_{20}H_{32}N_6O_{12}S_2$ (GSSG)		2GSH-2H
3	7.6	2,3,5,6-Tetraglutathionyl-(1,4)hydroquinone/ benzoquinone	$C_{46}H_{66}N_{12}O_{26}S_4$ (TetraSG-HQ) (TetraSG-BQ)		4GS+2,6-DBBQ-2Br-2H
4	8.8	2,3,5-Triglutathionyl-(1,4)hydroquinone/ benzoquinone	$C_{36}H_{51}N_9O_{20}S_3$ (TriSG-HQ) (TriSG-BQ)		3GS+2,6-DBBQ-2Br-H
5	10.7	2-Monobromo-5-desulfurized glutathionyl-6-glutathionyl-(1,4)hydroquinone/benzoquinone	$C_{26}H_{36}N_6O_{14}SBr$ (2-MB-5-G-6-SG-HQ) (2-MB-5-G-6-SG-BQ)		2GS+2,6-DBBQ-S-HBr

No.	Retention time (min)	Name	Formula	Structure	Formation
6	11.1	2-Monobromo-3,5-diglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{26}H_{36}N_6O_{14}S_2Br$ (2-MB-3,5-DiSG-HQ) (2-MB-3,5-DiSG-BQ)		2GS+GBBQ-HBr
7	11.7	2-Monobromo-3,5,6-triglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{36}H_{50}N_9O_{20}S_3Br$ (2-MB-TriSG-HQ) (2-MB-TriSG-BQ)		3GS+2,6-DBBQ-HBr
8	14.4	2-Monobromo-5-glutathionyl-6-desulfurized glutathionyl-(1,4)hydroquinone/benzoquinone	$C_{26}H_{36}N_6O_{14}SBr_2$ (2-MB-5-SG-6-G-HQ) (2-MB-5-SG-6-G-BQ)		2GS+2,6-DBBQ-S-HBr
9	15.2	2-Monobromo-5,6-diglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{26}H_{35}N_6O_{14}S_2Br$ (2-MB-5,6-DiSG-HQ) (2-MB-5,6-DiSG-BQ)		2GS+2,6-DBBQ-HBr
10	16.5	2-Monobromo-3,5,6-triglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{16}H_{20}N_3O_8SBr$ (2-MB-3-SG-HQ) (2-MB-3-SG-BQ)		GS+2,6-DBBQ-Br
11	18.4	2-Monobromo-3-glutathionyl-6-desulfurized glutathionyl-(1,4)hydroquinone/benzoquinone	$C_{26}H_{36}N_6O_{14}SBr_2$ (2-MB-3-SG-6-G-HQ) (2-MB-3-SG-6-G-BQ)		2GS+2,6-DBBQ-HBr-S

No.	Retention time (min)	Name	Formula	Structure	Formation
12	19.1	2,6-Dibromo-3,5-diglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{26}H_{34}N_6O_{14}S_2Br_2$ (2,6-DB-3,5-DiSG-HQ) (2,6-DB-3,5-DiSG-BQ)		2GS+2,6-DBBQ-2H
13	19.9	2-Monobromo-6-disulfide glutathionyl-(1,4) hydroquinone/benzoquinone	$C_{16}H_{22}N_3O_8S_2Br$ (MB-GSS-HQ) (MB-GSS-BQ)		SG+S+2,6-DBBQ-Br
14	21.2	2-Monobromo-6-glutathionyl-(1,4) hydroquinone/benzoquinone	$C_{16}H_{18}N_3O_{13}SBr$ (2-MB-6-SG-HQ) (2-MB-6-SG-BQ)		2,6-DBBQ+GS-Br
15	25.6	2,6-Dibromo-(1,4) hydroquinone/benzoquinone	$C_6H_2O_2Br_2$ (DBHQ) (2,6-DBBQ)		
16	13.1	2,6-Diglutathionyl-(1,4) hydroquinone/benzoquinone	$C_{26}H_{34}N_6O_{14}S_2$ (2,6-DiSG-HQ) (2,6-DiSG-BQ)		2GSH+2,6-DCBQ-2Cl-2H

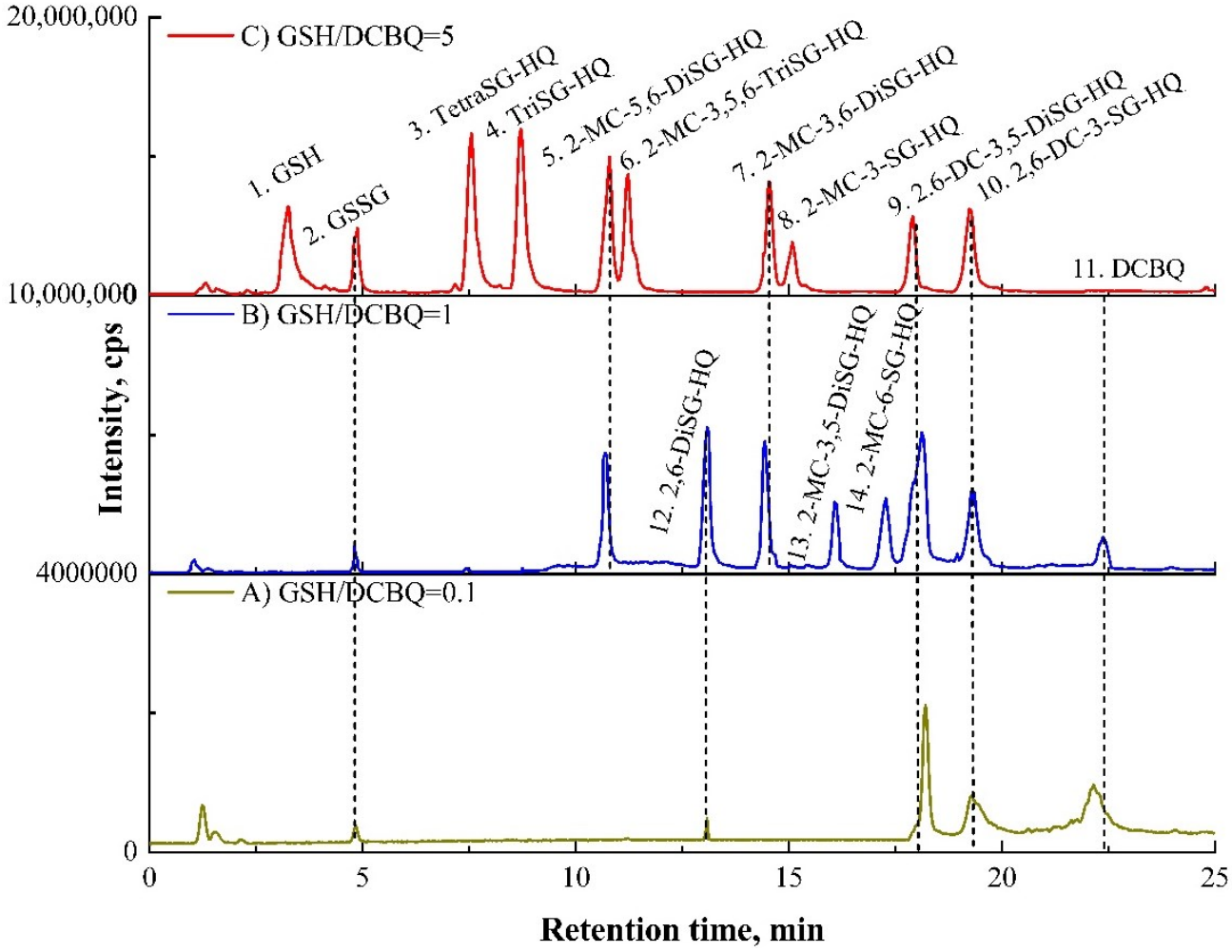


Figure 6.1 The UHPLC-ToF chromatograms of the mixture solution of GSH and 2,6-DCBQ at different ratios. A) GSH/2,6-DCBQ=0.1, B) GSH/2,6-DCBQ=1, and B) GSH/2,6-DCBQ=0.5

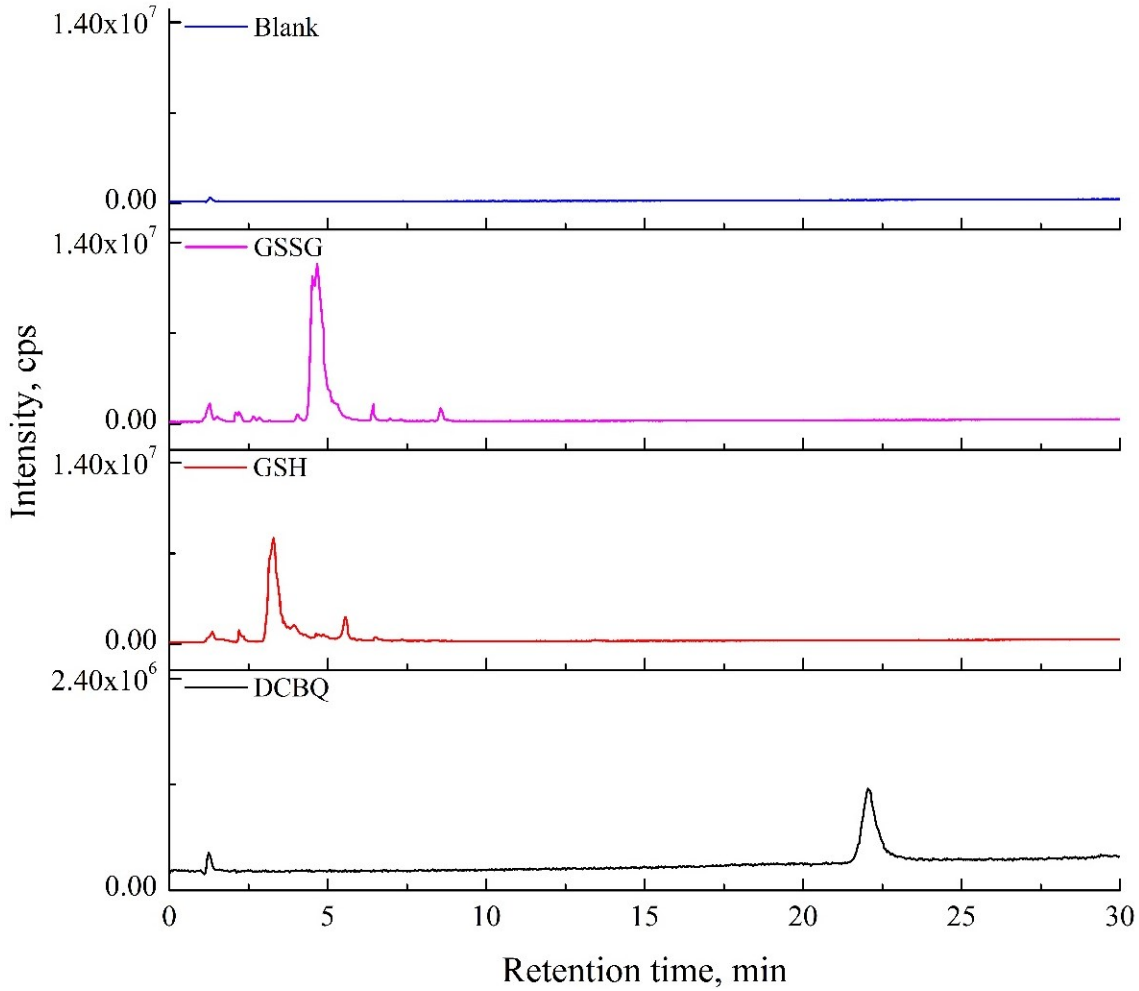
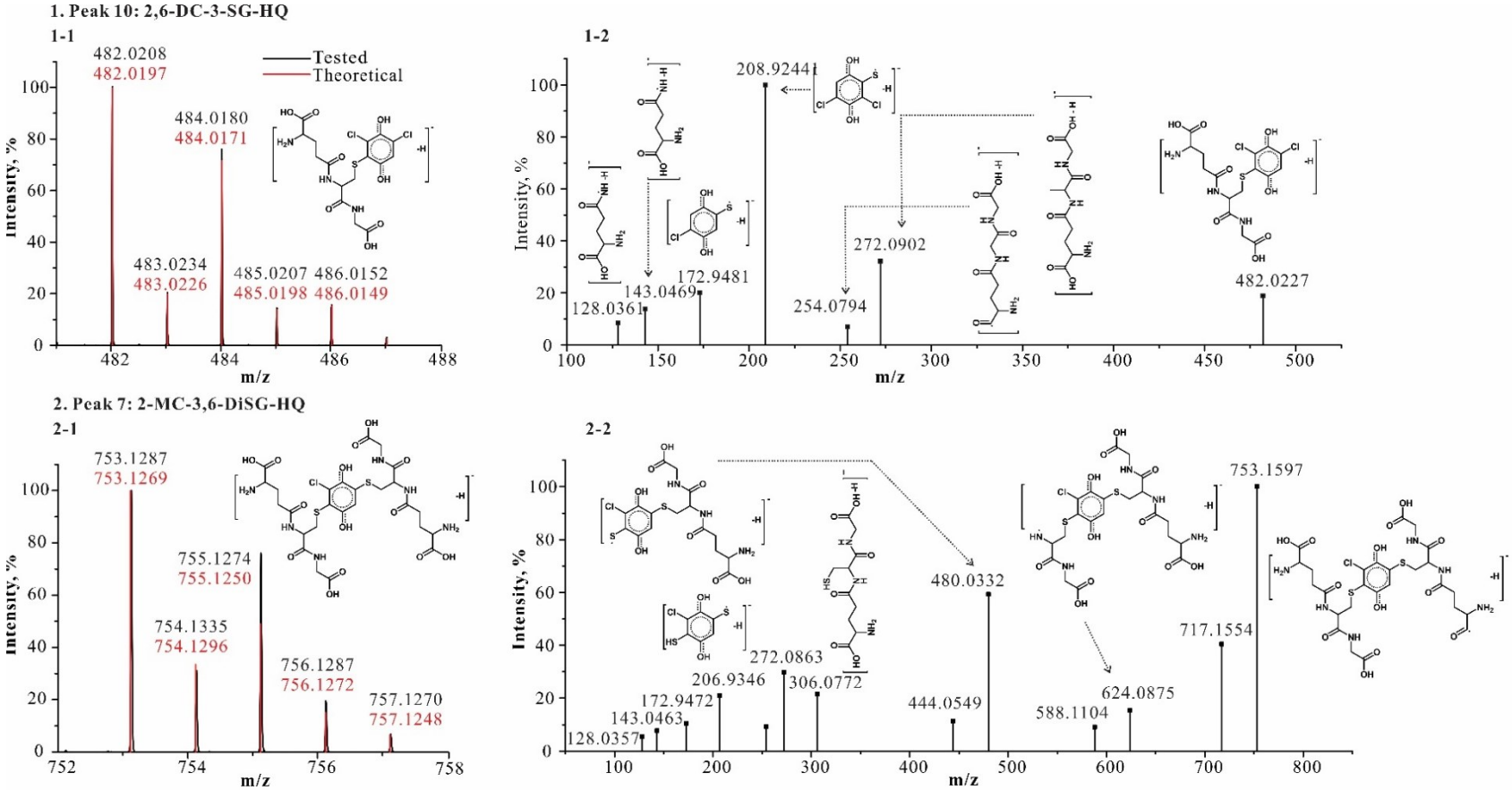


Figure 6.2 The ToF chromatograms of blank, GSSG, GSH and 2,6-DCBQ.


1. Peak 10: 2,6-DC-3-SG-HQ
1-1
Tested
Theoretical
482.0208
482.0197
484.0180
484.0171
483.0234
483.0226
485.0207
485.0198
486.0152
486.0149
Intensity, %
m/z
1-2
208.92441
272.0902
172.9481
143.0469
128.0361
254.0794
482.0227
Intensity, %
m/z
2. Peak 7: 2-MC-3,6-DiSG-HQ
2-1
753.1287
753.1269
755.1274
755.1250
754.1335
754.1296
756.1287
756.1272
757.1270
757.1248
Intensity, %
m/z
2-2
480.0332
753.1597
717.1554
272.0863
206.9346
306.0772
172.9472
143.0463
128.0357
444.0549
588.1104
624.0875
Intensity, %
m/z

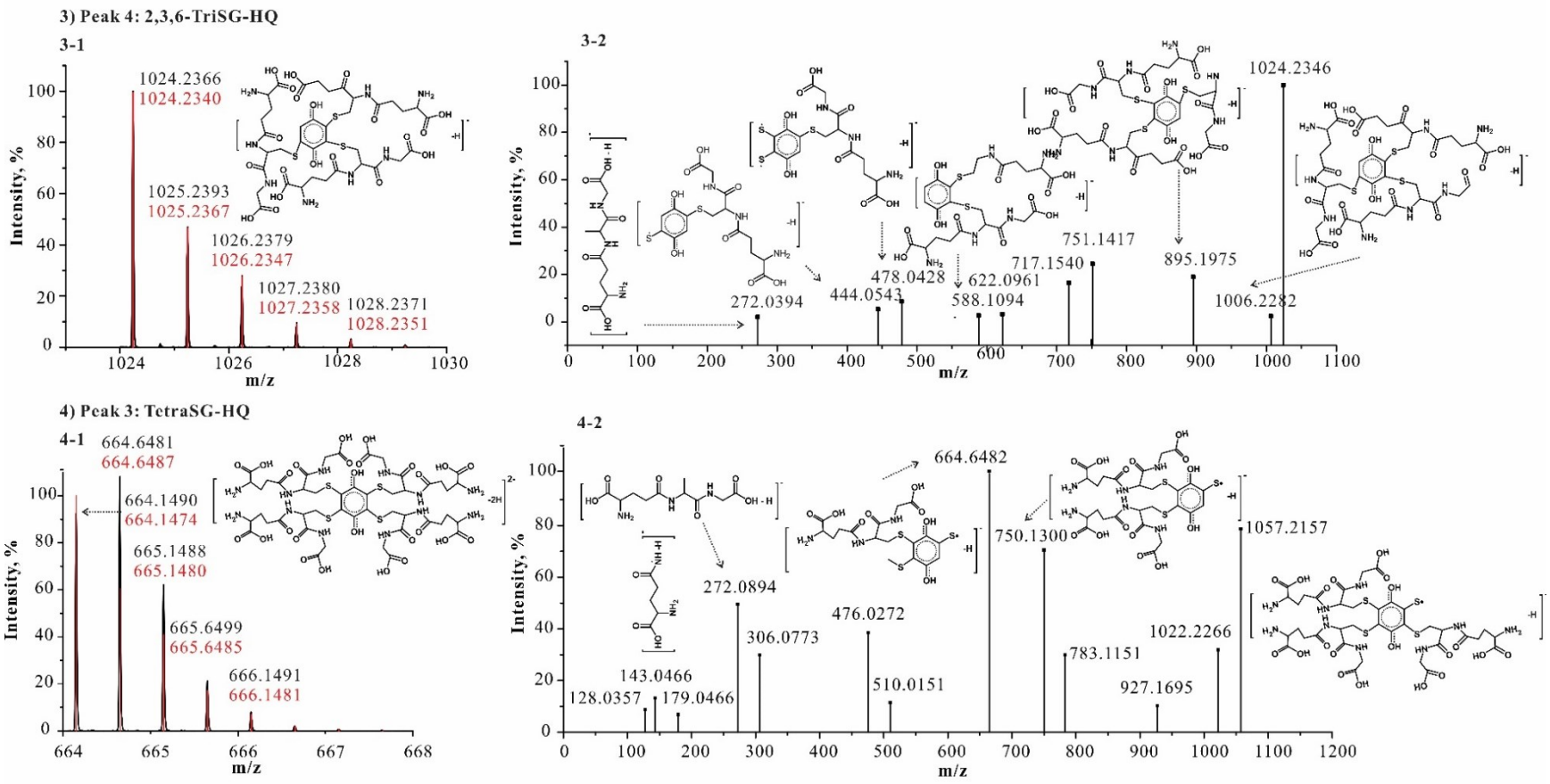


Figure 6.3 The MS and MS/MS spectra of mono, di, tri and tetra-glutathionyl-benzoquinones.

1), 2), 3) and 4) are 2,6-DC-SG-HQ, 2-MC-3,6-DiSG-HQ, TriSG-HQ, and TetraSG-HQ, respectively. 1-1, 2-1, 3-1 and 4-1 are the ToF MS spectra of the parent ions (blue line), in accordance with the theoretical isotope pattern of proposed $[M-H]^-$ or $[M-2H]^{2-}$ anion (red line); 1-2, 2-2, 3-2 and 4-2 are the dependent MS/MS spectra of the parent isotope with highest intensity.

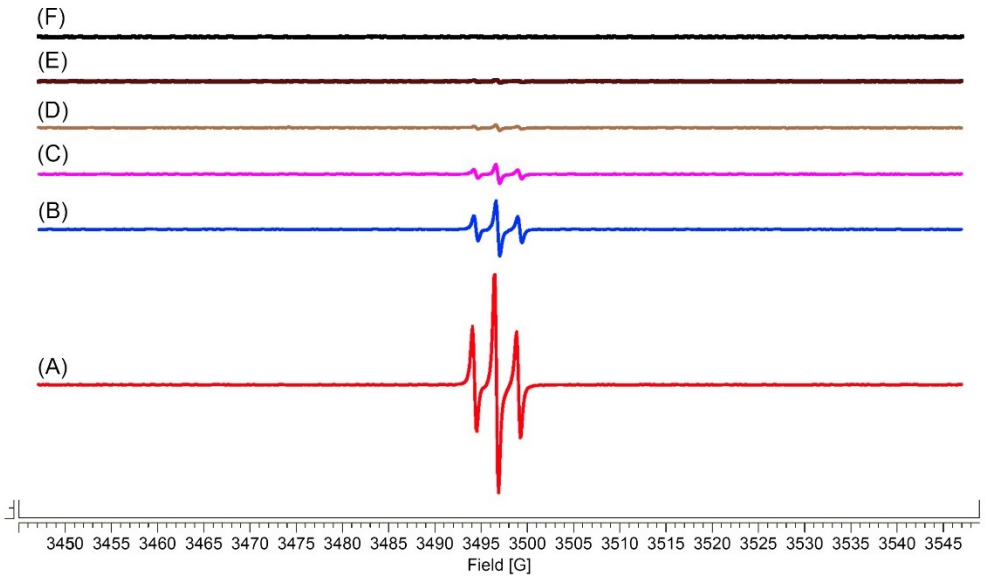


Figure 6.4 Semiquinone radical upon the reaction of GSH and 2,6-DCBQ. [2,6-DCBQ]=1 mM in (A)–(E). (A) [GSH]=0, [GSH]/[2,6-DCBQ]=0, pH=6.8; (B) [GSH]=10 μM, [GSH]/[2,6-DCBQ]=0.01, pH=6.6; (C) [GSH]=30 μM, [GSH]/[2,6-DCBQ]=0.03, pH=6.5; (D) [GSH]=50 μM, [GSH]/[2,6-DCBQ]=0.05, pH=6.4; (E) [GSH]=100 μM, [GSH]/[2,6-DCBQ]=0.1, pH=6.2; (F) [GSH]=300 μM, [GSH]/[2,6-DCBQ]=0.3, pH=6.2

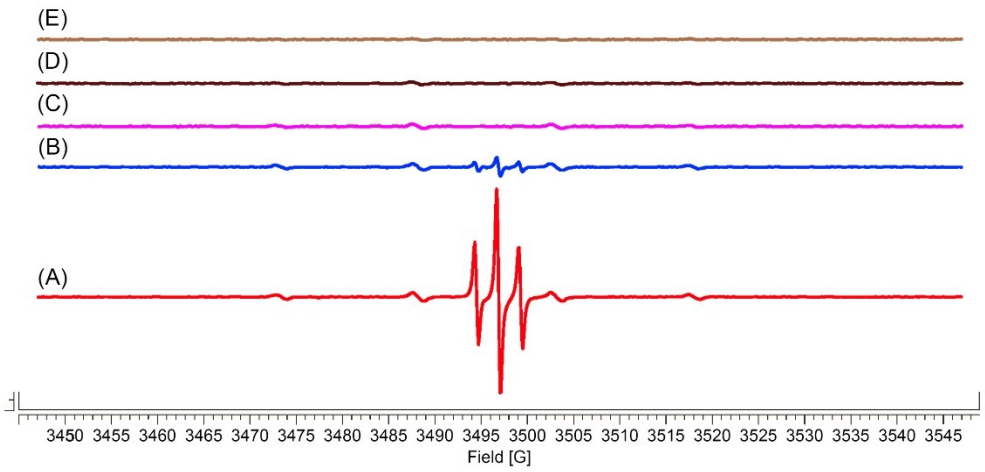


Figure 6.5 ESR spin trapping of the reaction of GSH and 2,6-DCBQ with DMPO. [2,6-DCBQ]=1 mM, and [DMPO]=100 μM in (A)–(E). (A) [GSH]=0, [GSH]/[2,6-DCBQ]=0; (B) [GSH]=100 μM, [GSH]/[2,6-DCBQ]=0.1; (C) [GSH]=500 μM, [GSH]/[2,6-DCBQ]=0.5; (D) [GSH]=1 mM, [GSH]/[2,6-DCBQ]=1; (E) [GSH]=5 mM, [GSH]/[2,6-DCBQ]=5.

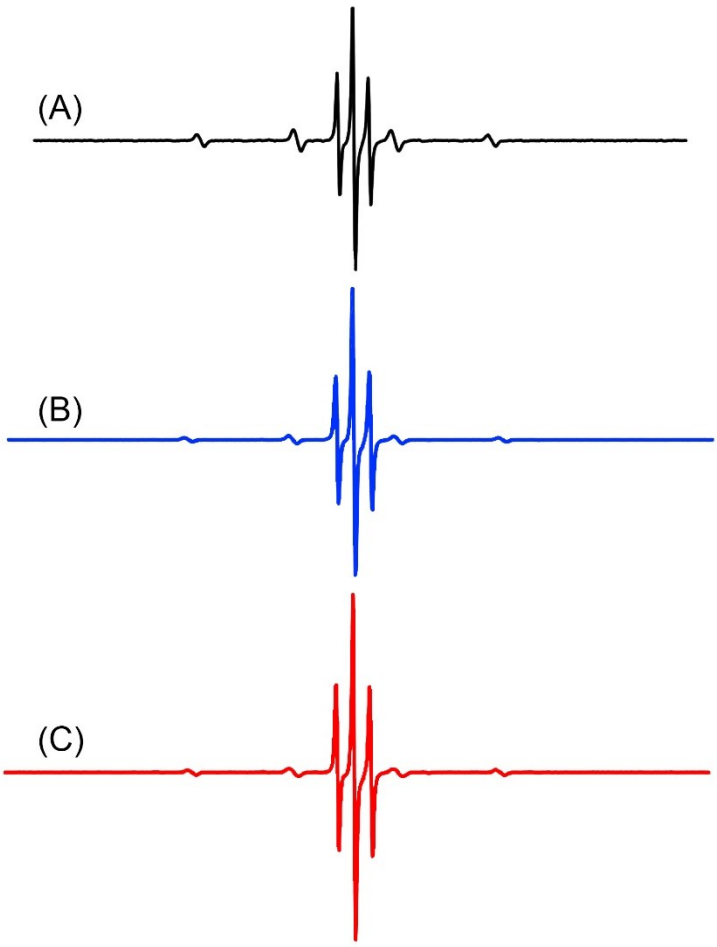


Figure 6.6 ESR spin trapping of the radical in 2,6-DCBQ solution using DMSO. [2,6-DCBQ]=1 mM, and [DMPO]=100 μM in (A)-(C). (A) is without any addition, (B) is with addition of 6 μM SOD, and (C) is with addition of 10% DMSO.

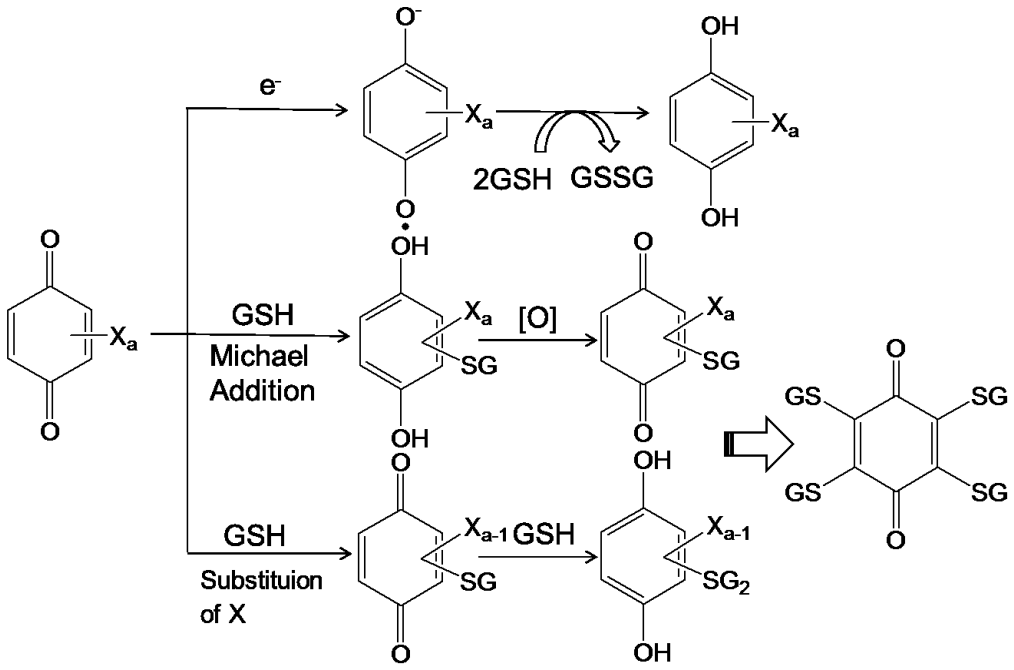


Figure 6.7 Reactions involved in the conjugation of GSH on chlorinated HBQs.
X is substitution group, Cl, Br or CH_3; a is the number of substituted group, equal to 1, 2 or 3

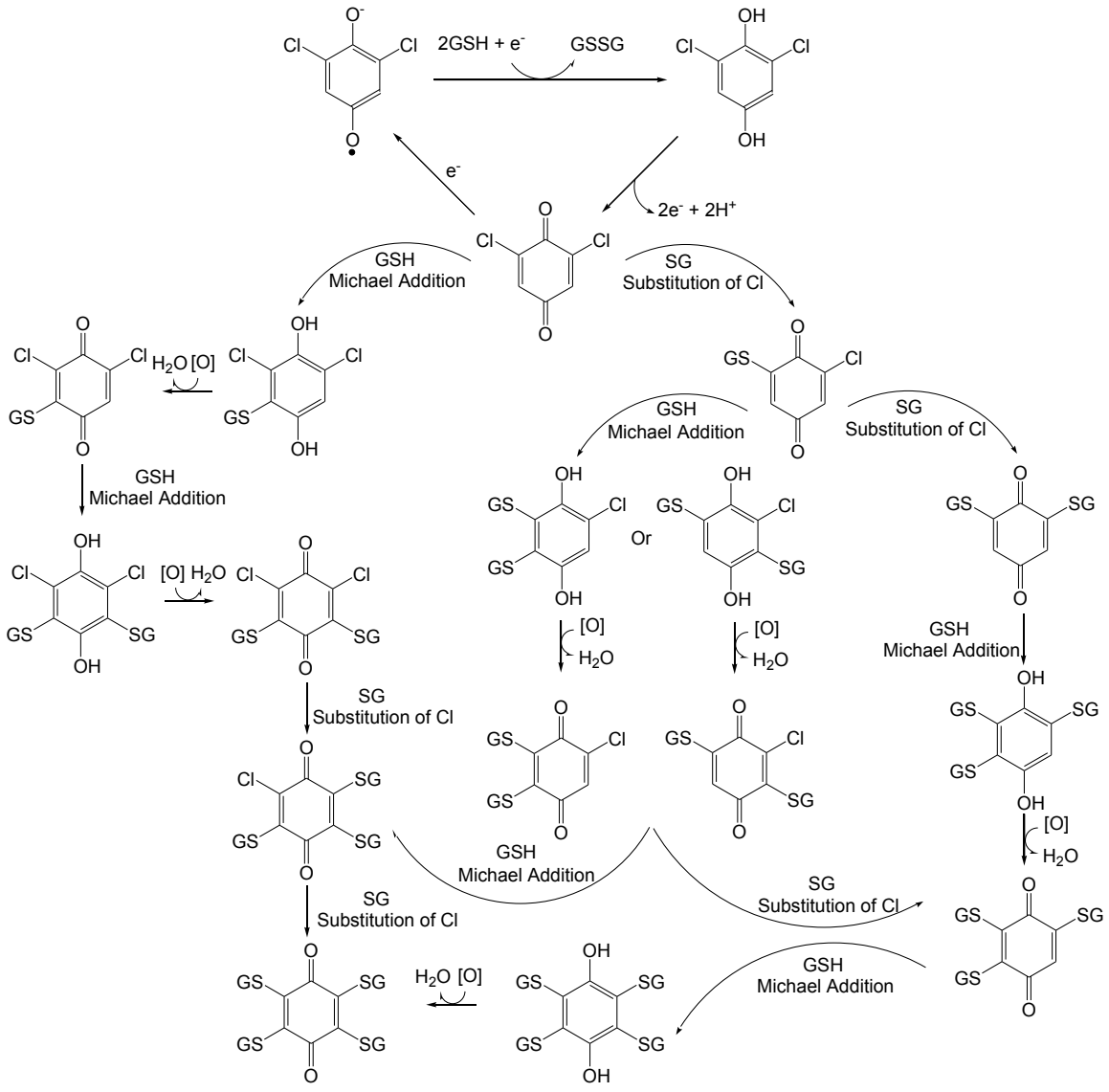


Figure 6.8 Proposed reaction pathways of 2,6-DCBQ with GSH.

[O] is the oxidant, which could be oxygen gas or less glutathionylated quinone.

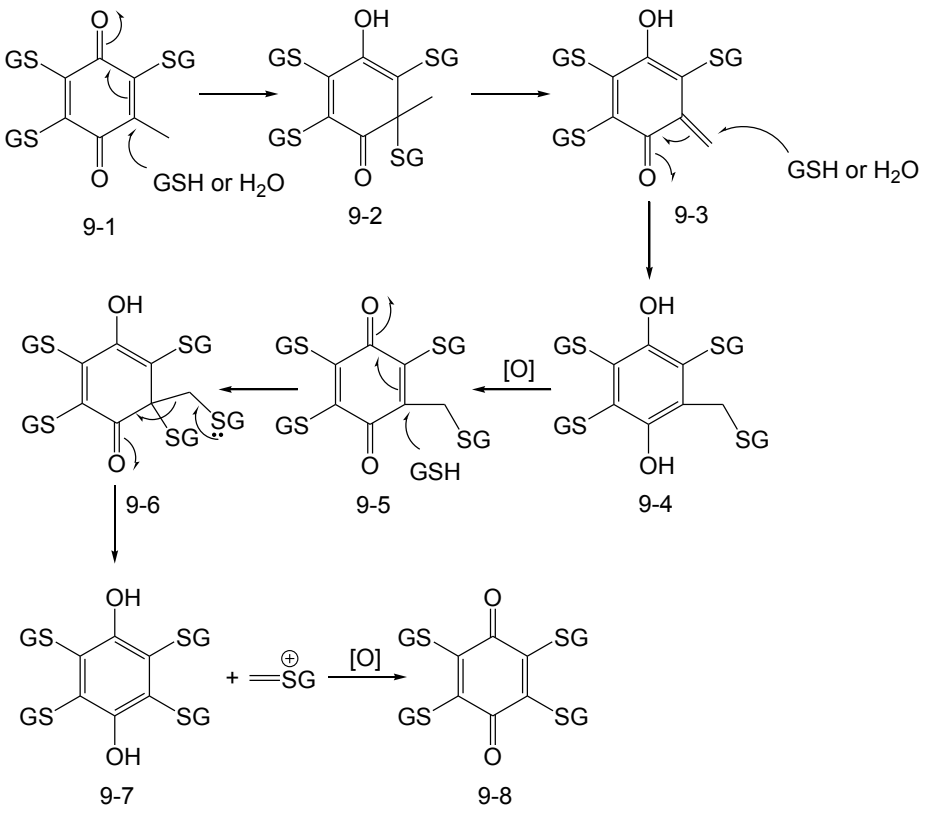


Figure 6.9 Proposed reaction pathway of DCMBQ with GSH for the formation of tetra-glutathionyl-benzoquinone.

[O] is the oxidant, which could be oxygen or less glutathionylated quinone.

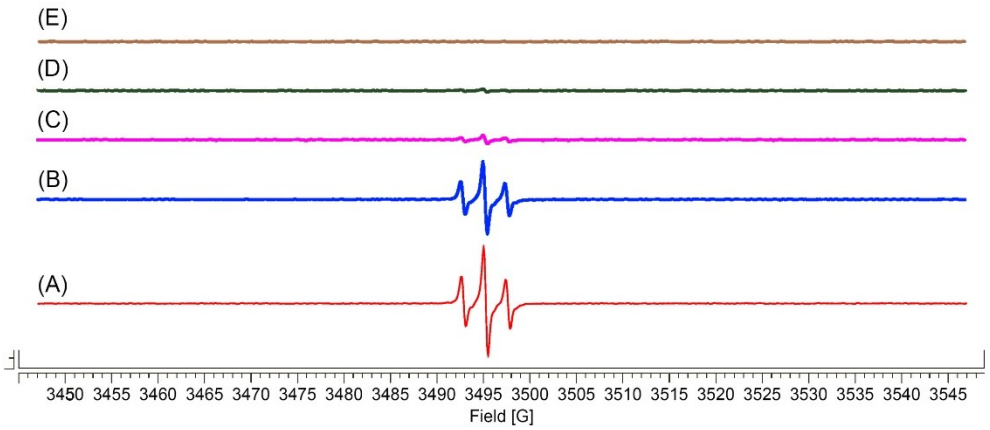


Figure 6.10 Semiquinone radical upon the reaction of GSH and 2,6-DBBQ. [2,6-DBBQ]=1 mM in (A)–(E). (A) [GSH]=0, [GSH]/[2,6-DBBQ]=0, pH=6.8; (B) [GSH]=10 μM, [GSH]/[2,6-DBBQ]=0.01, pH=6.6; (C) [GSH]=50 μM, [GSH]/[2,6-DBBQ]=0.05, pH=6.5; (D) [GSH]=100 μM, [GSH]/[2,6-DBBQ]=0.1, pH=6.4; (E) [GSH]=300 μM, [GSH]/[2,6-DBBQ]=0.3, pH=6.2.

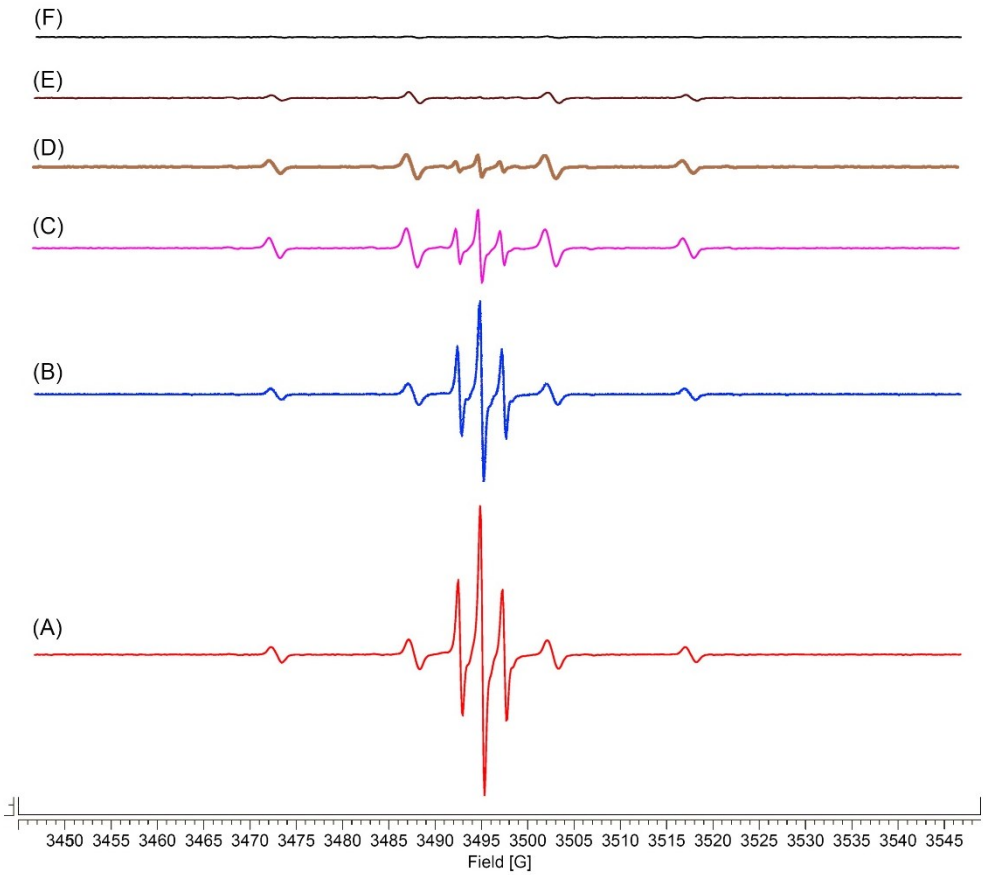


Figure 6.11 Spin trapping of semiquinone and hydroxyl radicals in the reaction of GSH and 2,6-DBBQ using DMPO.

[2,6-DBBQ]=1 mM, and [DMPO]=100 mM were used in (A)–(F). (A) [GSH]=0, [GSH]/[2,6-DBBQ]=0; (B) [GSH]=10 μM, [GSH]/[2,6-DBBQ]=0.01; (C) [GSH]=50 μM, [GSH]/[2,6-DBBQ]=0.05; (D) [GSH]=100 μM, [GSH]/[2,6-DBBQ]=0.1; (E) [GSH]=300 μM, [GSH]/[2,6-DBBQ]=0.3; (F) [GSH]=500 μM, [GSH]/[2,6-DBBQ]=0.5.

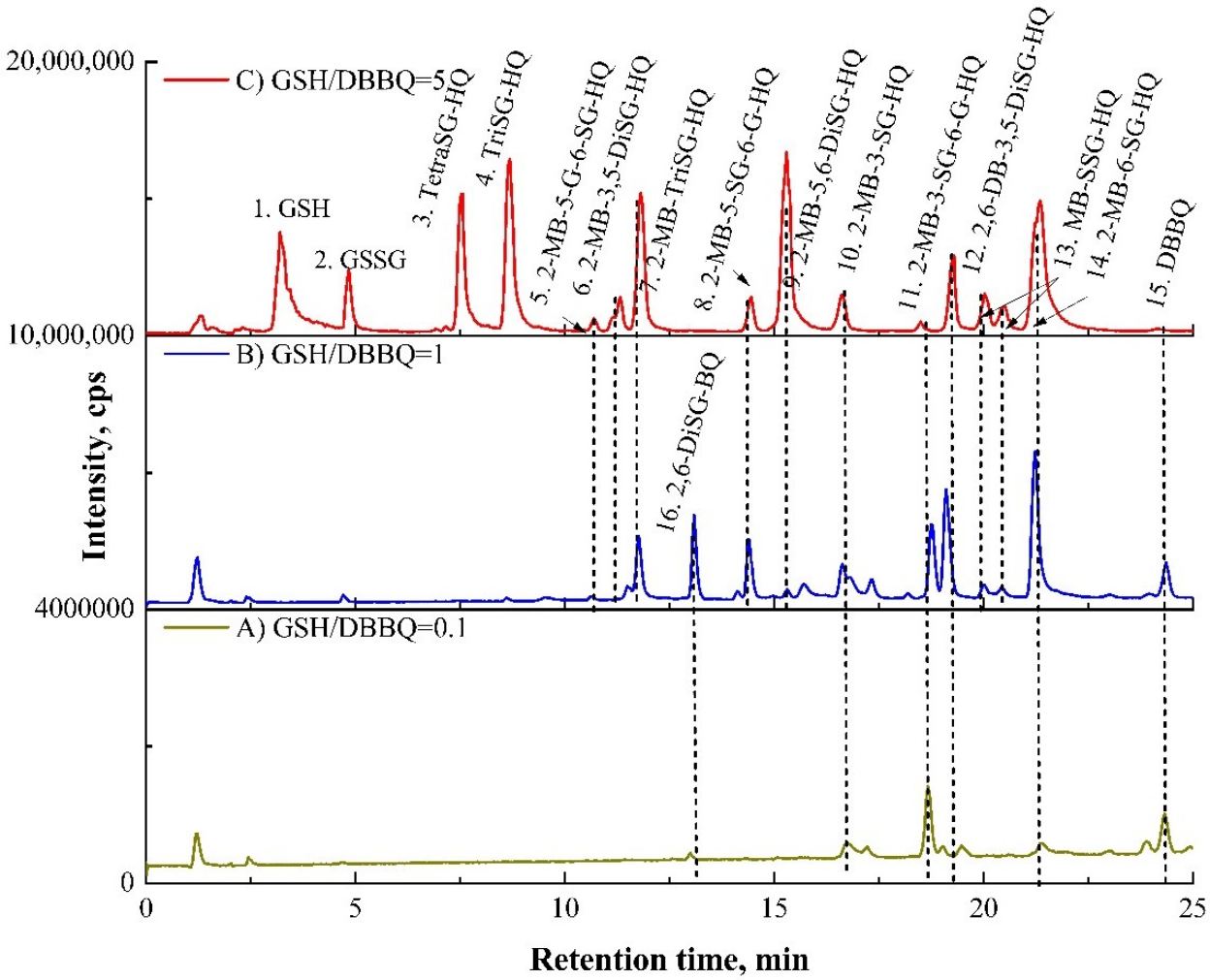


Figure 6.12 The UHPLC-ToF chromatograms of the mixture solution of GSH and 2,6-DBBQ at different ratios. A) GSH/2,6-DBBQ=0.1, B) GSH/2,6-DBBQ=1, and C) GSH/2,6-DBBQ=5

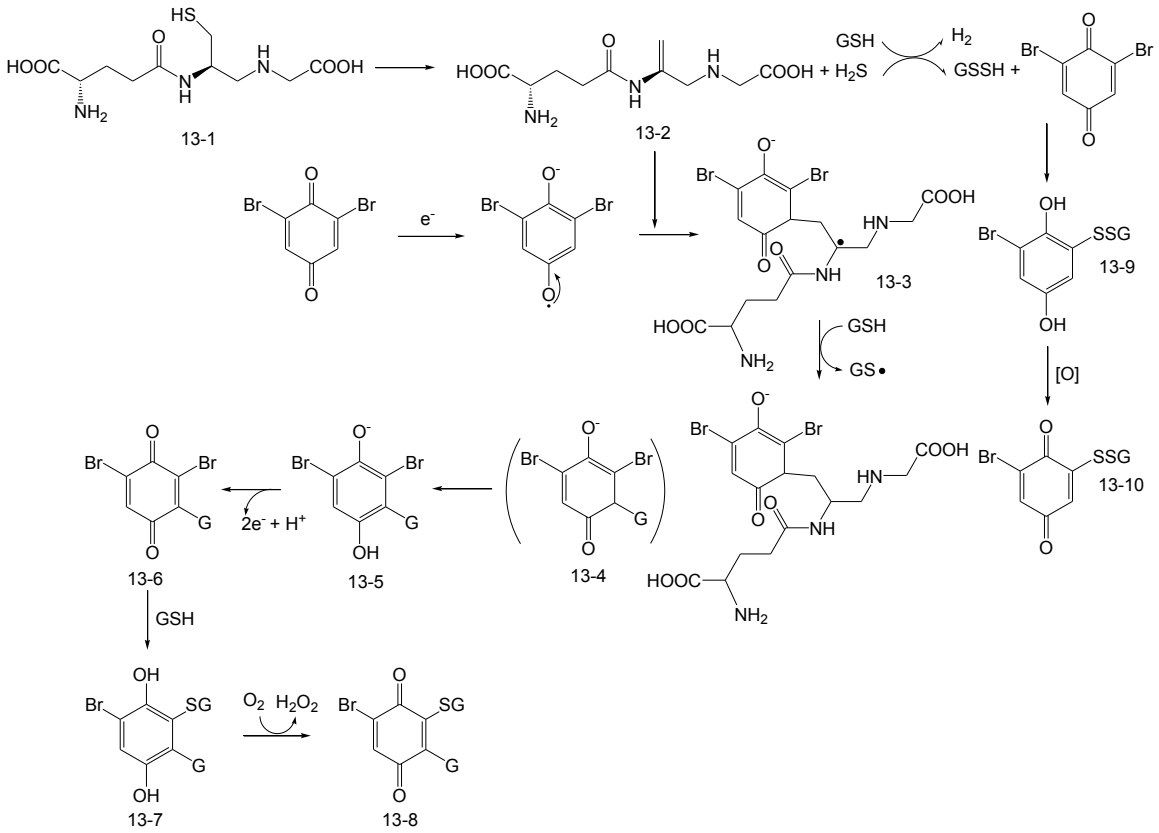


Figure 6.13 Proposed reaction pathway of 2,6-DBBQ with GSH for the formation of G or SSG conjugates.

[O] is the oxidant, which could be oxygen or other glutathionylated quinone.

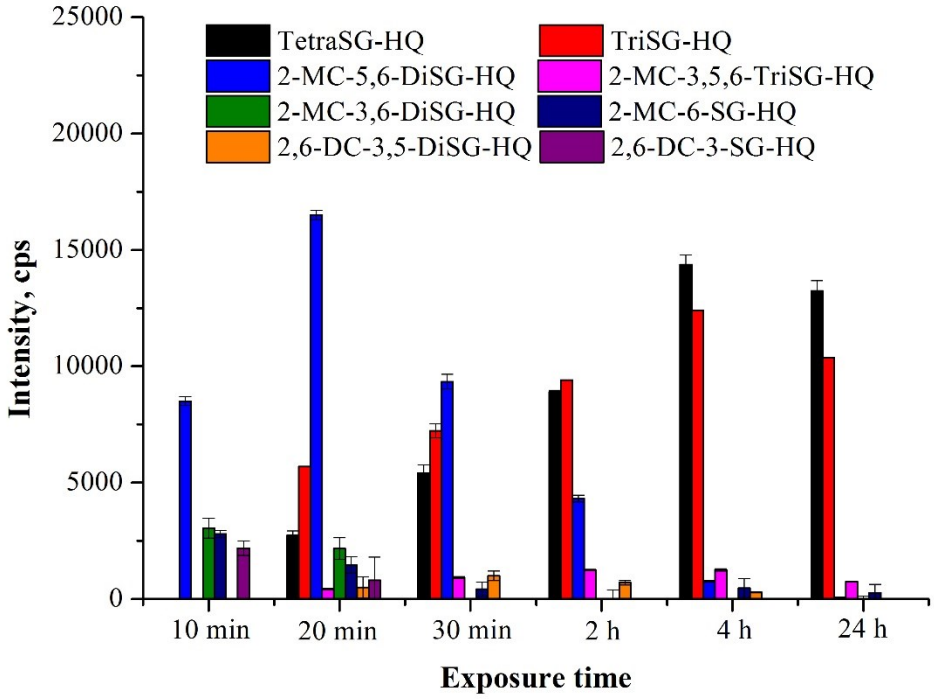


Figure 6.14 The ToF intensity of the molecular ion of 2,6-DCBQ-GSH conjugates in 2,6-DCBQ treated cells as a function of exposure time.

7 CONCLUSIONS AND SYNTHESIS

7.1 INTRODUCTION

Disinfection by-products (DBPs) are a group of compounds unintentionally created from organic matter reacting with chemicals used in the water disinfection process.[1] In epidemiological studies, DBP exposure through drinking water has been associated with various chronic diseases. DBP exposure has shown a consistent correlation with an increased incidence in bladder cancer[2–4] and inconsistently with adverse reproductive outcomes.[5–8]

The most abundant DBPs include trihalomethanes (THMs) and haloacetic acids (HAAs), and the scope has been expanded to include nitrosoamines, haloaldehydes and haloamines, to name a few. The pervasiveness of DBP exposure demands limiting exposure on precautionary grounds.[4] The US EPA set the enforceable maximum contaminant level (MCL) for total THMs and total levels of five HAAs. Similarly, Health Canada has also set maximum level guidelines to govern THM and HAA levels.[9] These regulated DBPs do not fully account for the increased risk of disease posed by DBPs in epidemiological studies. Approximately 70% of halogenated DBPs are unaccounted for based on measurements of total organic halogens (TOX). As a consequence, the toxicities of unknown DBPs have not been considered in the overall risk of DBPs.[10]

Quantitative structure toxicity relationship (QSTR) analysis has predicted that halobenzoquinones (HBQs) have lowest observed adverse effect levels (LOAEL) up to 10 000 times lower than some regulated DBPs.[11] Compounds or transformation products with similar structures, such as benzoquinones, interact through multiple pathways such

as redox reactions, alkylation and interactions with a variety of biologically active molecules, such as DNA and proteins, causing hazardous effects.[12]

My thesis research is aimed to fill the knowledge gap on HBQ DBPs. To achieve this, I investigated the occurrence of various HBQs in swimming pool water (Chapter 2), identified HBQ precursors in natural organic matter (NOM) or anthropogenic contaminants (Chapter 3); examined water treatment processes on the control of HBQ formation (Chapter 4); elucidated the fate of HBQs in drinking water distribution systems (DWDSs) (Chapter 5); and studied the conjugation pathways of HBQs with glutathione (Chapter 6). Chapter 7 presents the summaries of the major findings from my research (Chapter 2-6), and conclusions based on my thesis work as a whole and suggestions for future research.

7.2 ADVANCES IN KNOWLEDGE

7.2.1 Discovery of new HBQs in swimming pool waters

DBPs are produced from the reactions of NOM and/or anthropogenic organic compounds in water with the disinfectants such as chlorine. The dissolved organic carbon (DOC) and the chlorine dose are the key factors affecting their formation. Because of the high DOC and chlorine doses in swimming pools, I proposed that more HBQs could be produced in swimming pools than in the tap water. Therefore, my first aim was to discover what HBQs are formed in swimming pool waters, as described in Chapter 2.

To detect and identify trace levels of HBQs in treated water requires an analytical method with high selectivity and sensitivity. I have used solid phase extraction (SPE) to separate and pre-concentrate HBQs from water, high performance liquid chromatography (HPLC) to separate individual HBQs, and tandem mass spectrometry (MS/MS) to quantify

these HBQs. I have developed and optimized a SPE-HPLC-MS/MS method, enabling the determination of eight HBQs. This method can provide reproducible recovery of 67–102% and detection limits (LODs) of 0.03–1.2 ng/L for the eight HBQs.

Water samples collected from 10 swimming pools and their input tap water were analyzed for the eight HBQs. 2,6-DCBQ was detected in all the 10 swimming pools at concentrations of 19–299 ng/L. The concentrations of 2,6-DCBQ in the pools were as much as 100 times higher than its concentration in the input tap water (1–6 ng/L). In addition, TriCBQ, DMDBBQ, and 2,6-DBBQ were identified in some swimming pools at concentrations of <0.1–11.3, <0.05–0.7, and <0.05–3.9 ng/L, respectively, but not in the input tap water. This is the first time that DMDBBQ was confirmed as a DBP and enhanced formation of HBQs in swimming pools was observed. The enhanced formation of HBQs in the pools compared to the input tap water support the human contribution to the precursors of HBQs in the pools. Other factors including higher doses of chlorine and higher temperature in the pools also contributed to the enhanced HBQ formation.[13]

7.2.2 Formation of HBQs from NOM and anthropogenic material

After confirming the presence of various HBQs in swimming pool water and input tap water, my second aim (Chapter 3) was to identify what precursors result in the formation of the HBQs. NOM from source water is widely recognized as the dominant source of DBP precursors.[1] We collaborated with Dr. R. Andrews' group at the University of Toronto to address whether NOM or specific components of NOM can contribute to HBQ formation. They collected three representative source waters (Lake Ontario, Otonabee River and Grand River) and divided each to two aliquots, one of which was treated by coagulation to decrease the amount of NOM. The paired water samples, with or without coagulation, were

then analyzed for specific groups of NOM using liquid chromatography–organic carbon detection–ultraviolet detection (LC-OCD-UVD). The LC-OCD-UVD method could separate NOM as hydrophobic DOC, biopolymers, humic substance, building blocks, low molecular weight acids, and low molecular weight neutrals. I determined the concentrations of HBQs in these paired water samples after the identical chlorination disinfection treatment. Based on the measured concentrations of HBQs and NOM fractions, I performed correlation analysis between specific HBQs and NOM or NOM fractions using multiple linear correlation analysis. 2,6-DCBQ was detected in the chlorinated samples and its concentration was higher in the source water samples without coagulation, demonstrating that the NOM contributes to 2,6-DCBQ formation. Furthermore, the contents of humic substance and the biopolymer fraction were statistically correlated with 2,6-DCBQ formation.[14]

I then explored what additional precursors in swimming pools contributed to the enhanced formation of 2,6-DCBQ and other HBQs, TriCBQ, DMDBBQ, and 2,6-DBBQ. Human activities may increase the contents of some organic components in swimming pools, therefore, I proposed to investigate the formation of HBQs from anthropogenic materials, including urine and PCPs (lotions and sunscreens). Mixed urine samples of 20 healthy subjects and commercial synthetic urine were chlorinated and analyzed for HBQs. No HBQs were detected in the urine samples with or without chlorination at the same content of DOC as the swimming pools. This suggests that urine does not likely contribute to the enhanced HBQ formation in swimming pools. I then examined PCPs as the precursors, selecting four body lotions and four sunscreens commonly available in supermarkets. All chlorinated samples of lotions and sunscreens produced 2,6-DCBQ.

DCMBQ was detected in all four chlorinated lotion samples and two chlorinated sunscreen samples, ranging from <0.1 to 4.0±0.6 ng/L. TetraB-1,4-BQ was detected in all four chlorinated sunscreen samples, ranging from 0.9±1.0 to1.6±1.4 ng/L, but it was not detectable in any lotion samples. TriCBQ was found in two sunscreen samples. These results support that PCPs such as lotions and sunscreens can serve as precursors to form HBQs.[13]

The identification of both biopolymer fractions of NOM and PCPs as important HBQ precursor sources indicate that their common component biomolecules, such as peptides and proteins, should be active HBQ precursors. I plan to carry out a detailed investigation on the formation of HBQs from these biomolecules, and explain the formation mechanism of HBQs from these precursors. The study will initially examine the formation of HBQs from amino acids.

7.2.3 Removal of HBQ precursors during water treatment steps

Following the identification of HBQ precursors in Chapter 3, in Chapter 4, I further investigated the occurrence of HBQ precursors in plant influents and the effects of water treatment processes before chlorination on the removal of HBQ precursors. We collected plant influents and effluents after each treatment step prior to chlorination at nine DWTPs in Canada. Precursors of 2,6-DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ were determined through formation potential (FP) tests. 2,6-DCBQ precursors were most abundant (100% occurrence frequency, 16–205 ng/L 2,6-DCBQ FP). TriCBQ precursors were also present in all nine influents, but at much lower concentrations (3.6–48 ng/L). DCMBQ and 2,6-DBBQ were detected in five of the nine influent FP samples at concentrations ranging from 1.6 to 4.1 ng/L, 1.8 to 8.7 ng/L, respectively. 2,6-DBBQ FP was linearly correlated

with the bromide concentration (R^2 of 0.85). No strong correlation was observed between NOM measurements and HBQ formation.

I further evaluated the removal of HBQ precursors by coagulation, sand filtration, GAC, ozonation, and UV. All treatments except UV irradiation reduced HBQ FPs to some extent, but none of them appreciably removed HBQ FPs. Among all of these treatments, coagulation showed the highest removal efficiency of HBQ precursors. But the removal efficiency of HBQ FP was largely lower than that of DOC, indicating that organic matter removed by coagulation had a high proportion of non-HBQ-precursor material. While the reduction of HBQ FPs exceeded the removal of DOC and UV_{254} after GAC treatment, indicating that the HBQ FPs decreased 10–30% after ozonation treatment. Anthracite/sand filtration and UV irradiation had little impact on HBQ precursors (removal efficiencies ≤ 3%). In summary, the results indicated that HBQ precursors cannot be substantially removed with conventional treatment processes. These results are useful for DWTPs when they optimize treatment processes to remove DBP precursors.

7.2.4 Transformation of HBQs in disinfected water

Our previous study showed that HBQs are not stable under neutral or alkaline conditions, which indicate that HBQs are transformed to other products under realistic water treatment and distribution conditions. Therefore, my third aim (Chapter 5) was to explore the mechanisms of the transformation of HBQs in water using laboratory-controlled experiments. To achieve the third objective, I needed to identify and quantify the transformation products of HBQs. I initially focused on the four HBQs (2,6-DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ), because they are confirmed to exist in tap water. I first

used a triple quadrupole time-of-flight mass spectrometer (QTOF) to obtain the accurate mass, full scan, and MS/MS spectra of the transformation products of HBQs. OH-HBQs were identified as the main transformation products of the four HBQs.

To systematically study the mechanism and kinetics of the transformation from HBQs to OH-HBQs, I needed to quantify the formation of OH-HBQs and the decrease of HBQs. Therefore, I have developed an SPE-UHPLC-MS/MS method to determine the four HBQs along with the four OH-HBQs. The method can achieve stable retention times (SD ≤ 0.05 min), complete separation within 30 min, LODs of sub-ng/L levels, and recoveries of 60%–96%. A series of controlled laboratory experiments demonstrate that HBQs first undergo a self-redox reaction to form OH-HBQs. The findings of the laboratory experiments were further validated in the field. I investigated the concentrations of both HBQs and OH-HBQs in five DWDSs of defined locations, including water plant influents and effluents, tap water from halfway or maximum distance in the DWDSs. The samples containing HBQs also contained corresponding OH-HBQs. The concentration of HBQs decreased while that of OH-HBQs increased with the increasing distance from drinking water treatment plants. These results suggested that the transformation process from HBQs to OH-HBQs may occur in the DWDSs.

7.2.5 Conjugation of HBQs with GSH in aqueous solution and HepG2 cells

HBQs have shown high cytotoxicity in mammalian cells through reduction of intracellular glutathione (GSH) level, generation of reactive oxygen species (ROS), and damage of proteins and DNAs in cells.[15] Cellular GSH plays a key role in detoxification of HBQs.[16] Therefore, I investigated the reactions between GSH and HBQs (2,6-DCBQ,

DCMBQ, TriCBQ, and 2,6-DBBQ) both in aqueous solution and in HepG2 cells (Chapter 6).

To identify the conjugation products of HBQs with GSH, I developed an information dependent analysis (IDA) method using UHPLC–QToF MS. Mono, di, tri and tetraSG-HBQ conjugates and glutathione disulfide (GSSG) were identified in the aqueous solution of HBQs and GSH, based on the information of accurate masses, isotope patterns, and fragments obtained from MS and MS/MS spectra. With the increase of the GSH/HBQ ratio, the conjugation ratio of GSH to HBQ is increased. That is, mono and di-SG substituted BQs are further glutathionylated to tri- and tetra-SG BQs. Halosemiquinone (HSQ) free radicals were identified in the aqueous mixture of GSH and HBQs using electron paramagnetic resonance spectroscopy, and were gradually depleted with the increase in GSH levels. These results suggest that three typical reactions between GSH and 2,6-DCBQ were involved: (1) nucleophilic substitution of chlorine on the BQ ring to form glutathionyl BQs; (2) Michael addition of GSH to the BQ ring to form glutathionyl HQs; and (3) reversible redox reaction between HBQ and HSQ or HHQ along with oxidizing GSH to GSSG. The reaction follows the order: formation of HSQ radical > substitution of bromine by GSH > Michael addition of GSH on BQ ring > substitution of chlorine by GSH. In addition, unique desulfurized and disulfide GSH-DBBQ conjugates, and the substitution of methyl group by GSH on DCMBQ were also observed. The conjugates in HBQ-treated HepG2 cells were identified to be the same as those produced in the reaction of (5:1) GSH/HBQs.

7.3 CONCLUSIONS

HBQs are a group of DBPs that were predicted to be potential carcinogens. This work expanded the knowledge on the occurrence, formation, and transformation of HBQs as DBPs. In addition to 2,6-DCBQ, DCMBQ, TriCBQ, and 2,6-DBBQ, DMDBBQ was identified in swimming pool water as a new DBP. The concentrations of 2,6-DCBQ in swimming pools were as much as 100 times higher than the input tap water, which may be due to the high chlorine doses, high DOC content and high water temperature in swimming pools. NOM in surface water contained the precursors of HBQs. 2,6-DCBQ precursors were consistently identified to be the most abundant, of highest occurrence frequency (100%) and concentrations (16–205 ng/L). TriCBQ, DCMBQ, and 2,6-DBBQ precursors were also identified in some source waters with lower concentrations. In addition, anthropogenic materials, i.e., body lotion and sunscreens, contained precursors of 2,6-DCBQ, DCMBQ, TriCBQ and TetraB-1,4-BQ.

The widespread occurrence of HBQ precursors in surface water requires effective strategies to limit HBQ occurrence in disinfected water. Thus we evaluated the efficiency of current water treatment steps on the removal of HBQ precursors using the FP test. We examined the treatment steps used in nine DWTPs. Coagulation removed up to 39% 2,6-DCBQ FP, but that only accounted for a small proportion of the removed DOC. GAC removed 10–20% of HBQ FPs and only 0.2–4.7% of DOC, indicating biodegradation of HBQ precursors may occur. HBQ FPs decreased 10–30% after ozonation treatment, whereas there was negligible change after anthracite/sand filtration and UV irradiation. All of these results indicated that conventional water treatment can only partially reduce

HBQ precursors. Thus HBQs are formed after chlorination and further transformation to OH-HBQs in DWTPs.

7.4 FUTURE RESEARCH

The motivation of research on HBQs is to identify the culprit of the adverse health effects. To evaluate whether HBQs contribute to the adverse health effects, we need to collect large amounts of occurrence data, sufficient toxicity evidence, and well-designed epidemiological observations. My work has confirmed the widespread occurrence of HBQs as DBPs, and provided mechanistic insight into the formation and fate of HBQs. However, the information is far from enough to judge the role of HBQs on the observed cancer risk. Future research needs to be conducted to evaluate the health risk from HBQs in drinking water.

I have analyzed HBQ occurrence in several DWTPs, DWDSs, and swimming pools in Canada (Chapter 2 and 5). The data of global occurrence of HBQs are largely lacking. International surveys will provide comprehensive information on the correlation between the characteristics of source water (e.g., pH, DOC, TOC, specific characteristics of NOM, bromide content) or drinking water (e.g., the concentration of regulated DBPs), with HBQ formation, and accurate estimation of the exposure frequency of HBQs. Other HBQs may be identified as DBPs in addition to the five HBQs and four OH-HBQs.

The identification of both biopolymer fractions of NOM and PCPs as important HBQ precursor sources (Chapter 3) indicate that their common components of biomolecules, such as amino acids, peptides, and proteins may be active HBQ precursors. Detailed investigation should be carried out on the formation of HBQs from these biomolecules to explain the formation mechanism of HBQs from these precursors.

The study in Chapter 4 generally investigated the impacts of water treatment steps on the removal of HBQ precursors and found that these current steps cannot eliminate HBQ precursors. Follow-up study should be conducted to optimize each step to best control HBQ occurrence. The coagulants other than alum salt, such as iron and polymers, should be examined. The mechanisms of GAC function remain unclear, thus the effects of adsorption and biodegradation of GAC on the removal of HBQs should be carefully compared. The operation parameters of ozonation need to be further optimized, including evaluation of the effects of residual ozone concentration, contact time (CT values, milligrams per liter per minute), and pH of zone on HBQ formation. The knowledge could directly help DWTPs on improving treatment technology to minimize the formation of HBQs and assist with regulatory decisions.

Toxicity data is necessary to assess the health effects of HBQs. The concurrent toxicity study in our group confirmed that HBQs were over 1000 times more cytotoxic than some regulated DBPs in Chinese hamster ovary (CHO) cell lines. Furthermore, HBQs can produce reactive oxygen species (ROS), causing oxidative damage to proteins and DNA, and deplete cellular GSH and related enzymes. Future studies on animal toxicity are necessary to understand the target organs and effects of HBQs.

Finally, the search for the DBP responsible for the observed increased bladder cancer risk will continue. The highly sensitive and specific analytical tools for studying HBQs and OH-HBQs in source water and treated water could be applied for discovery of other new DBPs or contaminants in water. A cautious outlook on every potential candidate and controlling their formation will help until the ultimate culprit is determined.

In summary, this research provides mechanistic insights into the formation and fate of new DBP HBQs. The mechanistic understanding of the formation and transformation of HBQs will provide critical information necessary for assessing human exposure to these novel DBPs and for controlling/eliminating the formation of these DBPs at DWTPs. The fundamental understanding, combined with the occurrence data, could direct DWTPs on improving treatment technology to minimize the formation of these DBPs and assist with regulatory decision making in the future. The outcome of my research will also provide highly sensitive and specific analytical tools for studying HBQs and OH-HBQs in source water and treated water. This technical advance for discovery of new DBPs could be applied to studies of other contaminants in water.

7.5 REFERENCES

1. Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.; Sclimenti, M. J.; Onstad, G. D.; Thruston, A. D. Occurrence of a new generation of disinfection byproducts. *Environ Sci Technol* **2006**, *40* (23), 7175–7185.
2. King, W. D.; Marrett, L. D.; Woolcott, C. G., Case-control study of colon and rectal cancers and chlorination by-products in treated water. *Cancer Epidemiol Biomar* **2000**, *9* (8), 813–818.
3. Villanueva, C. M.; Cantor, K. P.; Grimalt, J. O.; Malats, N.; Silverman, D.; Tardon, A.; Garcia-Closas, R.; Serra, C.; Carrato, A.; Castano-Vinyals, G.; Marcos, R.; Rothman, N.; Real, F. X.; Dosemeci, M.; Kogevinas, M. Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *Am J Epidemiol* **2007**, *165* (2), 148–156.
4. Hrudey, S. E., Chlorination disinfection by-products, public health risk tradeoffs and me. *Water Res* **2009**, *43* (8), 2057–2092.
5. Swan, S. H.; Waller, K. Drinking water and reproductive outcomes. *Epidemiology* **1998**, *9* (3), 358–358.

6. Hunter, T. B.; Rice, S. F.; Hanush, R. G. Raman spectroscopic measurement of oxidation in supercritical water. 2. Conversion of isopropyl alcohol to acetone. *Ind Eng Chem Res* **1996**, *35* (11), 3984–3990.
7. Colman, J.; Rice, G. E.; Wright, J. M.; Hunter, E. S.; Teuschler, L. K.; Lipscomb, J. C.; Hertzberg, R. C.; Simmons, J. E.; Fransen, M.; Osier, M.; Narotsky, M. G., Identification of developmentally toxic drinking water disinfection byproducts and evaluation of data relevant to mode of action. *Toxicol Appl Pharm* **2011**, *254* (2), 100–126.
8. Nieuwenhuijsen, M. J.; Martinez, D.; Grellier, J.; Bennett, J.; Best, N.; Iszatt, N.; Vrijheid, M.; Toledano, M. B. Chlorination disinfection by-products in drinking water and congenital anomalies: review and meta-analyses. *Environ Health Perspect* **2009**, *117* (10), 1486–1493.
9. Health Canada. *Guideline for Canadian Drinking Water Quality, Ontario, Canada*, 6th ed.; 1996. http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/sum_guide-res_recom/index-eng.php. Accessed: Jan 5, 2016.
10. Richardson, S. D. Disinfection by-products and other emerging contaminants in drinking water. *Trends Anal Chem* **2003**, *22* (10), 666–684.
11. Bull, R. J.; Reckhow, D. A.; Li, X.-F.; Humpage, A. R.; Joll, C.; Hrudey, S. E., Potential carcinogenic hazards of non-regulated disinfection by-products: haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology* **2011**, *286* (1-3), 1–19.
12. Bolton, J. L.; Trush, M. A.; Penning, T. M.; Dryhurst, G.; Monks, T. J. Role of quinones in toxicology. *Chem Res Toxicol* **2000**, *13* (3), 135–160.
13. Wang, W.; Qian Y.; Boyd, J.M.; Wu, M.; Hrudey, S.E.; Li, X-F. Halobenzoquinones in swimming pool waters and their formation from personal care products. *Environ. Sci. Technol.* **2013**, *47* (7), 3275–3282.
14. Diemert, S.; Wang, W.; Andrews, R. C.; Li, X.-F., Removal of halo-benzoquinone (emerging disinfection by-product) precursor material from three surface waters using coagulation. *Water Res* **2013**, *47* (5), 1773–1782.

15. Li, J. H.; Wang, W.; Moe, B.; Wang, H. L.; Li, X. F., Chemical and toxicological characterization of halobenzoquinones, an emerging class of disinfection byproducts. *Chem Res Toxicol* **2015**, *28* (3), 306–318.
16. Li, J. H.; Wang, W.; Zhang, H. Q.; Le, X. C.; Li, X. F., Glutathione-mediated detoxification of halobenzoquinone drinking water disinfection byproducts in T24 cells. *Toxicol Sci* **2014**, *141* (2), 335–343.

BIBLIOGRAPHY

Aiken, G.R.; McKnight, D.M.; Wershaw, R.L.; MacCarthy, P. An introduction to humic substances in soil, sediment and water. In: *Humic Substances in Soil, Sediment and Water;* John Wiley and Sons: New York, NY, 1985; pp 1.

Alegria, A. E.; Ferrer, A.; Santiago, G.; Sepulveda, E.; Flores, W. Photochemistry of water-soluble quinones. Production of the hydroxyl radical, singlet oxygen and the superoxide ion. *J Photochem Photobio A* **1999**, *127* (1-3), 57–65.

American Public Health Association (APHA); American Water Works Association (AWWA); Water Environment Federation (WEF). *Standard Methods for the Examination of Water and Wastewater*, 21st ed. Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg. A.E., Eds. Washington, USA, 2005.

Amy, G. L.; Kuo, C. J.; Sierka, R. A. Ozonation of humic substances: effects on molecular weight distributions of organic carbon and trihalomethane formation potential. *Ozone- Sci Eng* 1988, *10* (1), 39–54.

Anichina, J.; Zhao, Y.; Hrudey, S. E.; Le, X. C.; Li, X.-F. Electrospray ionization mass spectrometry characterization of interactions of newly identified water disinfection byproducts halobenzoquinones with oligodeoxynucleotides. *Environ Sci Technol* **2010**, *44* (24), 9557–9563.

Anichina, J.; Zhao, Y.; Hrudey, S. E.; Schreiber, A.; Li, X.-F. Electrospray ionization tandem mass spectrometry analysis of the reactivity of structurally related bromo-methyl-benzoquinones toward oligonucleotides. *Anal Chem* **2011**, *83* (21), 8145–8151.

Arumugam, S.; Popik, V. V. Dual reactivity of hydroxy- and methoxy- substituted o-quinone methides in aqueous solutions: hydration versus tautomerization. *J Org Chem* **2010**, *75* (21), 7338–7346.

Baghoth, S. A.; Dignum, M.; Grefte, A.; Kroesbergen, J.; Amy, G.L. Characterization of NOM in a drinking water treatment process train with no disinfectant residual. *Wat Sci Technol: Wat Supply* **2009**, *9* (4), 379–386.

Balmer, M. E.; Buser, H. R.; Muller, M. D.; Poiger, T. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. *Environ Sci Technol* **2005**, *39* (4), 953–962.

Bellar, T. A.; Lichtenberg, J. J.; Kroner, R. C. Occurrence of organohalides in chlorinated drinking waters. *J Am Water Works Assoc* **1974**, *66* (12), 703–706.

Blatchley, E. R.; Cheng, M. M. Reaction mechanism for chlorination of urea. *Environ Sci Technol* **2010**, *44* (22), 8529–8534.

Bolto, B.; Dixon, D.; Eldridge, R.; King, S. Removal of THM precursors by coagulation or ion exchange. *Water Res* **2002**, *36* (20), 5066–5073.

Bolton, J. L.; Trush, M. A.; Penning, T. M.; Dryhurst, G.; Monks, T. J. Role of quinones in toxicology. *Chem Res Toxicol* **2000**, *13* (3), 135–160.

Bond, T.; Heniriet, O.; Goslan, E. H.; Parsons, S. A.; Jefferson, B. Disinfection byproduct formation and fraction behavior of natural organic matter surrogates. *Environ Sci Technol* **2009**, *43* (15), 5982–5989.

Boyd, J. M.; Huang, L.; Xie, L.; Moe, B.; Gabos, S.; Li, X.-F. A cell-microelectronic sensing technique for profiling cytotoxicty of chemicals. *Anal Chim Acta* **2008**, *789* (1), 83–90.

Brausch, J. M.; Rand, G. M. A review of personal care products in the aquatic environment: environmental concentrations and toxicity. *Chemosphere* **2011**, *82* (11), 1518–1532.

Brisson, I. J; Levallois, P.; Tremblay, H.; Serodes, J.; DeBlois, C.; Charrois, J.; Taguchi, V.; Boyd, J. M.; Li, X.-F.; Rodriguez, M. J. Spatial and temporal occurrence of N-nitrosamines in seven drinking water distribution systems. *Environ Monit Assess* **2013**, *185* (9), 7693–7708.

Brown, T. J.; Emelko, M. B. Chitosan and metal salt coagulant impacts on *Cryptosporidium* and microsphere removal by filtration. *Water Res* **2009**, *43* (2), 311–338.

Bull, R. J.; Reckhow, D. A.; Li, X.-F.; Humpage, A. R.; Joll, C.; Hrudey, S. E. Potential carcinogenic hazards of non-regulated disinfection by-products: haloquinones, halo-cyclopentene and cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines. *Toxicology* **2011**, *286* (1-3), 1–19.

Bull, R. J.; Reckhow, D. A.; Rotello, V.; Bull, O. M.; Kim, J. *Use of Toxicological and Chemical Models to Prioritize DBP Research*; American Water Works Association Research Foundation and American Water Works Association: Denver, CO, 2006.

Brunmark, A.; Cadenas, E. Redox and addition chemistry of quinoid compounds and its biological implications. *Free Radic Biol Med* **1989**, *7* (4), 435–477.

Busca, G.; Berardinelli S.; Resini, C.; Arrighi L. Technologies for the removal of phenol from fluid streams: A short review of recent developments. *J Hazard Mater* **2008**, *160* (2-3), 265–288.

Canonica, S.; Meunier, L.; Von Gunten, U. Phototransformation of selected pharmaceuticals during UV treatment of drinking water. *Water Res* **2008**, *42* (1-2), 121–128.

Carter, J. M.; Moran, M. J.; Zogorski, J. S.; Price, C. V. Factors associated with sources, transport and fate of chloroform and three other trihalomethanes in untreated groundwater used for drinking water. *Environ Sci Technol* **2012**, *46* (15), 8189–8197.

Chen, W.; Duan, L.; Zhu, D. Q. Adsorption of polar and nonpolar organic chemicals to carbon nanotubes. *Environ Sci Technol* **2007**, *41* (24), 8295–8300.

Chiu, C. A.; Westerhoff, P.; Ghosh, A. GAC removal of organic nitrogen and other DBP precursors. *J Am Water Works Assoc* **2012**, *104* (7), E406–E405.

Chung, H. S.; Wang, S. B.; Venkatraman, V.; Murray, C. I.; Van Eyk, J. E. Cysteine oxidative posttranslational modifications emerging regulation in the cardiovascular system. *Circ Res* **2013**, *112* (2), 382–392.

Collins, M. R.; Amy, G. L.; Steelink, C. Molecular weight distribution, carboxylic acidity, and humic substances content of aquatic organic matter: implications for removal during water treatment. *Environ Sci Technol* **1986**, *20* (10), 1028–1032.

Colman, J.; Rice, G. E.; Wright, J. M.; Hunter, E. S.; Teuschler, L. K.; Lipscomb, J. C.; Hertzberg, R. C.; Simmons, J. E.; Fransen, M.; Osier, M.; Narotsky, M. G. Identification of developmentally toxic drinking water disinfection byproducts and evaluation of data relevant to mode of action. *Toxicol Appl Pharmacol* **2011**, *254* (2), 100–126.

Condie, L. W., Toxicological problems associated with chlorine dioxide. *J Am Water Works Assoc* **1986**, *78* (6), 73–78.
Craik, S. A.; Weldon, D.; Finch, G. R.; Bolton, J. R.; Belosevic, M. Inactivation of Cryptosporidium parvum oocysts using medium- and low-pressure ultraviolet radiation. *Water Res* **2001**, *35* (6), 1387–1398.
De Laat, J.; Feng, W. T.; Freyfer, D. A.; Dossier-Berne, F. Concentration levels of urea in swimming pool water and reactivity of chlorine with urea. *Water Res* **2011**, *45* (3), 1139–1146.
Deng, L.; Huang, C. H.; Wang, Y. L. Effects of combined UV and chlorine treatment on the formation of trichloronitromethane from amine precursors. *Environ Sci Technol* **2014**, *48* (5), 2697–2705.
Diemert, S.; Wang, W.; Andrews, R. C.; Li, X.-F. Removal of halobenzoquinone (emerging disinfection by-product) precursor material from three surface waters using coagulation. *Water Res* **2013**, *47* (5), 1773–1782.
Du, H. Y.; Li, J. H.; Moe, B.; McGuigan, C. F.; Shen, S. W.; Li, X.-F. Cytotoxicity and oxidative damage induced by halobenzoquinones to T24 bladder cancer cells. *Environ Sci Technol* **2013**, *47* (6), 2823–2830.
El-Najjar, N.; Gali-Muhtasib, H.; Ketola, R. A.; Vuorela, P.; Urtti, A.; Vuorela, H. The chemical and biological activities of quinones: overview and implications in analytical detection. *Phytochem Rev* **2011**, *10* (3), 353–370.
Esbenshade, J. L.; Cardoso, J. C.; Zanoni, M. V. B. Removal of sunscreen compounds from swimming pool water using self-organized TiO_2 nanotubular array electrodes. *J Photochem Photobio A* **2010**, *214* (2-3), 257–263.
Escher, B. I.; Fenner, K. Recent advances in environmental risk assessment of transformation products. *Environ Sci Technol* **2011**, *45* (9), 3835–3847.
Farre, M. J.; Reungoat, J.; Argaud, F. X.; Rattier, M.; Keller, J.; Gernjak, W. Fate of N-nitrosodimethylamine, trihalomethane and haloacetic acid precursors in tertiary treatment including biofiltration. *Water Res* **2011**, *45* (17), 5695–5704.
Farre, M. L.; Perez, S.; Kantiani, L.; Barcelo, D. Fate and toxicity of emerging pollutants, their metabolites and transformation products. *Trends Anal Chem* **2008**, *27* (11), 991–1007.
Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. Electrospray ionization for mass-spectrometry of large biomolecules. *Science* **1989**, *246* (4926), 64–71.
Fonvielle, M.; Mellal, D.; Patin, D.; Lecerf, M.; Blanot, D.; Bouhss, A.; Santarem, M.; Mengin-Lecreulx, D.; Sollogoub, M.; Arthur, M.; Etheve-Quelquejeu, M. Efficient access to peptidyl-RNA conjugates for picomolar inhibition of non-ribosomal FemXWv aminoacyl transferase. *Chem Eur J* **2013**, *19* (4), 1357–1363.
Franzen, S.; Sasan, K.; Sturgeon, B. E.; Lyon, B. J.; Battenburg, B. J.; Gracz, H.; Dumariah, R.; Ghiladi, R. Nonphotochemical base-batalyzed hydroxylation of 2,6-dichloroquinone by H_2O_2 occurs by a radical mechanism. *J Phys Chem B* **2012**, *116* (5), 1666–1676.
Galapate, R. P.; Baes, A. U.; Okada, M. Transformation of dissolved organic matter during ozonation: effects on trihalomethane formation potential. *Water Res* **2001**, *35* (9), 2201–2206.

Gallard, H.; von Gunten, U. Chlorination of phenols: Kinetics and formation of chloroform. *Environ Sci Technol* **2002**, *36* (5), 884–890.
Gan, D.; Jia, M.; Vaughan, P. P.; Falvey, D. E.; Blough, N. V. Aqueous photochemistry of methyl-benzoquinone. *J Phys Chem A* **2008**, *112* (13), 2803–2812.
Giokas, D. L.; Slvador, A; Chisvert, A. UV filters: From sunscreens to human body and the environment. *Trends Anal Chem* **2007**, *26* (5), 360-374.
Glezer, V.; Harris, B.; Tai, N.; Lev, L. O. Hydrolysis of haloacetonitriles: linear free energy relationship, kinetics and prducts. *Water Res* **1999**, *33* (8), 1938–1948.
Gorner, H. Photoprocesses of p-benzoquinones in aqueous solution. *J Phys Chem A* **2003,** *107* (51), 11587–11595.
Gunner, M. R.; Madeo, J.; Zhu, Z. Modification of quinone electrochemistry by the proteins in the biological electron transfer chains: examples from photosynthetic reaction centers. *J Bioenerg Biomembr* **2008**, *40* (5), 509–519.
Hanigan, D.; Zhang, J. W.; Herckes, P.; Krasner, S. W. Chen, C.; Westerhoff, P. Adsorption of N-nitrosodimethylamine precursors by powdered and granular activated carbon. *Environ Sci Technol* **2012**, *46* (22), 12630–12639.
Health C*anada. Guideline for Canadian Drinking Water Quality, Ontario, Canada*, 6th ed.; 1996. http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/sum_guide-res_recom/index-eng.php. Accessed: Jan 5, 2016.
Heasley, V. L.; Fisher, A. M.; Herman, E. E.; Jacobsen, F. E.; Miller, E. W.; Ramirez, A. M.; Royer, N. R.; Whisenand, J. M.; Zoetewey, D. L.; Shellhamer, D. F. Investigations of the reactions of monochloramine and dichloramine with selected phenols: examination of humic acid models and water contaminants. *Environ Sci Technol* **2004**, *38* (19), 5022–5029.
Hijnen, W. A. M.; Beerendonk, E. F.; Medema, G. J. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Res* **2006**, *40* (1), 3–22.
Hoq, M. F.; Indu, B.; Ernst, W. R.; Neumann, H. M. Kinetics of the reaction of chlorine with formic acid in aqueous sulfuric acid. *J Phys Chem* **1991**, *95* (2), 681–683.
Hrudey, S. E. Chlorination disinfection by-products, public health risk tradeoffs and me. *Water Res* **2009,** *43* (8), 2057–2092.
Hrudey, S. E., Backer, L. C., Humpage, A. R., Krasner, S. W., Michaud, D. S., Moore, L. E., Singer, P. C., Stanford, B. D. Evaluating evidence for association of human bladder cancer with drinking-water chlorination disinfection by-products. *J Toxicol Environ Health B Criti Rev* **2015**, *18* (5), 213–241.
Hrudey, S. E.; Charrois, J. W. A. *Disinfection by-Products: Relevance to Human Health,* IWA Publishing: London, 2012.
Hu, J. Y.; Aizawa, T.; Ookubo, S. Products of aqueous chlorination of bisphenol A and their estrogenic activity. *Environ Sci Technol* **2002**, *36* (9), 1980–1987.
Hu, J.; Song, H.; Karanfil, T. Comparative analysis of halonitromethane and trihalomethane formation and speciation in drinking water: the effects of disinfectants, pH, bromide, and nitrite. *Environ Sci Technol* **2010**, *44* (2):794–799.
Hu, M. Q.; Wang, Y.; Xiong, Z. G.; Bi, D. Q.; Zhang, Y. H.; Xu, Y. M. Iodine-sensitized degradation of 2,4,6-trichlorophenol under visible light. *Environ Sci Technol* **2012**, *46* (16), 9005–9011.

Hua, G.; Reckhow, D. A., Kim, J. Effect of bromide and iodide ions on the formation and speciation of disinfection byproducts during chlorination. *Environ Sci Technol* **2006**, *40* (9), 3050–3056.
Huang, R.; Wang, W.; Qian, Y.; Boyd, J. M.; Zhao, Y.; Li, X.-F. Ultra pressure liquid chromatography-negative electrospray ionization mass spectrometry determination of twelve halobenzoquinones at ng/L levels in drinking water. *Anal Chem* **2013**, *85* (9), 4520–4529.
Huber, M. M.; Korhonen, S.; Ternes, T. A.; von Gunten, U. Oxidation of pharmaceuticals during water treatment with chlorine dioxide. *Water Res* **2005**, *39* (15), 3607–3617.
Huber, S. A.; Balz, A.; Abert, M.; Pronk, W. Characterization of aquatic humic and non-humic matter with size-exclusion chromatography – organic carbon detection – organic nitrogen detection (LC-OCD-OND). *Water Res* **2011**, *45* (2), 879–885.
Hubig, S. M.; Kochi, J. K. Electrom-transfer mechanisms with photoactivated quinones. The encounter complex versus the Rehm-Weller paradigm. *J Am Chem Soc* **1999**, *121* (8), 1688–1694.
Hunter, T. B.; Rice, S. F.; Hanush, R. G. Raman spectroscopic measurement of oxidation in supercritical water. 2. Conversion of isopropyl alcohol to acetone. *Ind Eng Chem Res* **1996**, *35* (11), 3984–3990.
Hunter, E. S.; Rogers, E. H.; Schmid, J. E.; Richard, A. Comparative effects of haloacetic acids in whole embryo culture. *Teratology* **1996**, *54* (2), 57–64.
Itoh, S.; Gordon, B. A.; Callan, P.; Bartram, J. Regulations and perspectives on disinfection by-products: importance of estimting overall toxicity. *J Water Supply Res T* **2011**, *60* (5), 261–274.
Jacangelo, J. G.; Demarco, J.; Owen, D. M.; Randtke, S. J. Selected processes for removing NOM–an overview. *J Am Water Works Assoc* **1995**, *87* (1), 64–77.
Joshi, D. K.; Gold, M. H. Degradation of 2,4,5-trichlorophenol by the lignin-degrading basidiomycete phanerochaete-chrysosporium. *Appl Environ Microbiol* **1993**, *59* (6), 1779–1785.
Kalmykova, Y.; Moona, N.; Stromvall, A. M.; Bjorklund, K. Sorption and degradation of petroleum hydrocarbons, polycyclic aromatic hydrocarbons, alkylphenols, bisphenol A and phthalates in landfill leachate using sand, activated carbon and peat filters. *Water Res* **2014**, *56* (16), 246–257.
Kanan, A.; Karanfil, T. Formation of disinfection by-products in indoor swimming pool water: the contribution from filling water natural organic matter and swimmer body fluids. *Water Res* **2011**, *45* (2), 926–932.
King, W. D.; Marrett, L. D.; Woolcott, C. G. Case-control study of colon and rectal cancers and chlorination by-products in treated water. *Cancer Epidemiol Biomarkers Prev* **2000**, *9* (8), 813–818.
Kot, M.; Olech, Z. Influence of 2,5-dichloro-1,4-benzoquinone on jack bean urease activity. Inhibitory effect, total reducing capacity and DPPH radical scavenging activity. *Acta Biochim Pol* **2011**, *58* (4), 627–633.
Krasner, S. W.; Mitch, W. A.; Westerhoff, P.; Dotson, A. Formation and control of emerging C- and N-DBPs in drinking water. *J Am Water Works Assoc* **2012**, *104* (11), E582–E595.
Krasner, S. W.; Sclimenti, M. J.; Guo, Y. C.; Hwang, C. J.; Westerhoff, P. Development of DBP and nitrosamine formation potential tests for treated wastewater, reclaimed

water, and drinking water. In *Proceedings of the 2004 AWWA Water Quality Technology Conference;* AWWA: Denver, CO, 2004.
Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.; Sclimenti, M. J.; Onstad, G. D.; Thruston, A. D. Occurrence of a new generation of disinfection byproducts. *Environ Sci Technol* **2006**, *40* (23), 7175–7185.
Krasner, S. W.; Westerhoff, P.; Chen, B. Y.; Rittmann, B. E.; Nam, S. N.; Amy, G. Impact of wastewater treatment processes on organic carbon, organic nitrogen, and DBP precursors in effluent organic matter. *Environ Sci Technol* **2009**, *43* (8), 2911–2918.
Kruve, A.; Auling, R.; Herodes, K.; Leito, I. Study of liquid chromatography/electrospray ionization mass spectrometry matrix effect on the example glyphpste analysis from cereals. *Rapid Commun Mass Spectrom* **2011**, *25* (21), 3252–3258.
Kutyrev, A. A. Nucleophilic reactions of quinones. *Tetrahedron* **1991**, *47* (38), 8043–8065.
Kutyrev, A. A.; Moskv, V. V. Nucleophilic reactions of quinones. *Russ Chem Rev* **1991**, *47* (38), 134–158.
Lafis, S.; Konidari, C. N.; Veltsistas, P. G.; Tzerpos, N.; Karayannis, M. I. Kinetic study of the fast step of the alkaline hydrolysis of *p*-chloranil using stopped flow technique. *Int. J. Chem. Kinet.* **1997**, *29* (5), 385–391.
Lakind, J. S.; Richardson, S. D.; Blount, B. C. The good, the bad, and the volatile: can we have both healthy pools and healthy people? *Environ Sci Technol* **2010**, *44* (9), 3205–3210.
Li, J. H.; Wang, W.; Zhang, H. Q.; Le, X. C.; Li, X.-F. Glutathione-mediated detoxification of halobenzoquinone drinking water disinfection byproducts in T24 cells. *Toxicol Sci* **2014**, *141* (2), 335–343.
Li, J. H.; Wang, W.; Moe, B.; Wang, H. L.; Li, X. F. Chemical and toxicological characterization of halobenzoquinones, an emerging class of disinfection byproducts. *Chem Res Toxicol* **2015**, *28* (3), 306–318.
Li, Q.; Osborne, M.C.; Smith, I.W.M. Rate constants for the reactions of Cl atoms with HCOOH and with HOCO radicals. *Int J Chem Kinet* **2000**, *32* (2), 85-91.
Lim, D.; Ikeda, A.; Vu, K. K. T.; Yamaguchi, K. T.; Tyner, T. R.; Hasson, A. S. Method development for the measurement of quinone levels in urine. *J Chromatogr B* **2011**, *879* (30), 3592–3598.
Lin, P. H.; Waidyanatha, S.; Pollack, G. M.; Rappaport, S. M. Dosimetry of chlorinated quinone metabolites of pentachlorophenol in the livers of rats and mice based upon measurement of protein adducts. *Toxicol Appl Pharmacol* **1997**, *145* (2), 399–408.
Lin, P. H.; Waidyanatha, S.; Pollack, G. M.; Swenberg, J. A.; Rappaport, S. M. Dose-specific production of chlorinated quinone and semiquinone adducts in rodent livers following administration of pentachlorophenol. *Toxicol Sci* **1999**, *47* (1), 126–133.
Lin, P. H.; Waidyanatha, S.; Rappaport, S. M. Investigation of liver binding of pentachlorophenol based upon measurement of protein adducts. *Biomarkers* **1996**, *1* (4), 232–243.
Liu, H. Z.; Korshin, G. V.; Ferguson, J. F. Interactions of Pb(II)/Pb(IV) solid phases with chlorine and their effects on lead release. *Environ Sci Technol* **2009**, *43* (9), 3278–3284.

Liu, J.-L.; Li, X.-Y. Biodegradation and biotransformation of wastewater organics as precursors of disinfection by-products in water. *Chemosphere* **2010**, *81* (9), 1075–1083.
Madrasi, K.; Joshi, M. S.; Gadkari, T.; Kavallieratos, K.; Tsoukias, N. M. Glutathiyl radical as an intermediate in glutathione nitrosation. *Free Radic Biol Med* **2012**, *53* (10), 1968–1976.
Mao, Y.; Wang, X.; Yang, H.; Wang, H.; Xie, Y. F. Effects of ozonation on disinfection byproduct formation and speciation during subsequent chlorination. *Chemosphere* **2014**, *117*, 515–520
Matilainen, A.; Vepsalainen, M.; Sillanpaa, M. Natural organic matter removal by coagulation during drinking water treatment: a review. *Adv Colloid Interfac* **2010**, *159* (2), 189–197.
Mawhinney, D. B.; Young, R. B.; Vanderford, B. J.; Borch, T.; Snyder, S. A. Artificial sweetener sucralose in U.S. drinking water systems. *Environ Sci Technol* **2011**, *45* (20), 8716–8722.
Metz, D. H.; Meyer, M.; Dotson, A.; Beerendonk, E.; Dionysiou, D. D. The effect of UV/H_2O_2 treatment on disinfection by-product formation potential under simulated distribution system conditions. *Water Res* **2011**, *45* (13), 3969–3980.
Mitch, W. A.; Sedlak, D. L. Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environ Sci Technol* **2002**, *36* (4), 588–595.
Monks, T. J.; Hanzlik, R. P.; Cohen, G. M.; Ross, D.; Graham, D. G. Quinone chemistry and toxicity. *Toxicol Appl Pharmacol* **1992**, *112* (1), 2–16.
Monroe, S.; Eaton, S. S. Photo-enhanced production of the spin adduct 5,5-dimethyl-1-pyrroline-N-oxide/center dot OH in aqueous menadione solutions. *Arch Biochem Biophys* **1996**, *329* (2), 221–227.
Namazian, M.; Coote, M. L. Accurate calculation of absolute one-electron redox potentials of some para-quinone derivatives in acetonitrile. *J Phys Chem A* **2007**, *111* (30), 7227–7232.
Neter, J.; Wasserman, W.; Kutner, M. H. *Applied Linear Statistical Models: regression, Analysis of Variance, and Experimental Designs*, 3rd ed.; Richard, D., Ed.; Irwin Inc.: Homewood, IL. 1990.
Nieuwenhuijsen, M. J.; Martinez, D.; Grellier, J.; Bennett, J.; Best, N.; Iszatt, N.; Vrijheid, M.; Toledano, M. B. Chlorination disinfection by-products in drinking water and congenital anomalies: review and meta-analyses. *Environ Health Perspect* **2009**, *117* (10), 1486–1493.
Nieuwenhuijsen, M. J.; Toledano, M. B.; Eaton, N. E.; Fawell, J.; Elliott, P. Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review. *Occup Environ Med* **2000**, *57* (2), 73–85.
Olkowski, A. A.; Aranda-Osorio, G.; McKinnon, J. Rapid HPLC method for measurement of vitamin D-3 and 25(OH)D-3 in blood plasma. *Int J Vitam Nutr Res* **2003**, *73* (1), 15–18.
O'Melia, C. R. Coagulation and flocculation. In *Physicochemical Processes for Water Treatment Control*; W. J. Weber, Ed.; Wiley: New York 1972; 61–109.
Pan, Y.; Zhang, X. Four groups of new aromatic halogenated disinfection byproducts: effect of bromide concentration on their formation and speciation in chlorinated drinking water. *Environ Sci Technol* **2013**, *47* (3), 1265–1273.

Pedersen, J. A. *Handbook of EPR Spectra from Quinones and Quinols*. Boca Raton, Fla.: CRC Press, 1985.
Plewa, M. J.; Simmons, J. E.; Richardson, S. D.; Wagner, E. D. Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products. *Environ Mol Mutagen* **2010**, *51* (8-9), 871–878.
Plewa, M. J.; Wagner, E. D. *Mammalian Cell Cytotoxicity and Genotoxicity of Disinfection By-products*. Water Research Foundation: Denver, 2009; pp 107–108.
Poiger, T.; Buser, H. R.; Balmer, M. E.; Bergqvist, P. A.; Muller, M. D. Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. *Chemosphere* **2004**, *55* (7), 951–963.
Poole, C. F. New trends in solid-phase extraction. *Trends Anal Chem*. **2003**, *22* (6), 362–373.
Qian, Y.; Wang, W.; Boyd. J. M.; Wu M.; Hrudey, S. E.; Li, X.-F. UV-induced transformation of four halobenzoquinones in drinking water. *Environ Sci Technol* **2013**, *47* (9), 4426–4433.
Qin, F.; Zhao, Y. Y.; Zhao, Y. L.; Boyd, J. M.; Zhou, W. J.; Li, X.-F. A toxic disinfection by-product, 2,6-dichloro-1,4-benzoquinone, identified in drinking water. *Angew Chem Int Ed* **2010**, *49* (4), 790–792.
Qin, H.; Huang, C.-H.; Mao, L.; Xia, H.-Y.; Kalyanaraman, B.; Shao, J.; Shan, G.-Q.; Zhu, B.-Z. Molecular mechanism of metal-independent decomposition of lipid hydroperoxide 13-HPODE by halogenated quinoid carcinogens. *Free Radical Bio Med* **2013**, *63*, 459–466.
Quimby, B. D.; Delaney, M. F.; Uden, P. C.; Barnes, R. M. Determination of the aqueous chlorination products of humic substances by gas-chromatography with microwave emission detection. *Anal Chem* **1980**, *52* (2), 259–263.
Ribes, A.; Grimalt, J. O.; Garcia, C. J. T.; Cuevas, E. Temperature and organic matter dependence of the distribution of organochlorine compounds in mountain soils from the subtropical Atlantic (Teide, Tenerife Island). *Environ Sci Technol* **2002**, *36* (9), 1879–1885.
Richardson, S. D. The role of GC-MS and LC-MS in the discovery of drinking water disinfection by-products. *J Environ Monit* **2002**, *4* (1), 1–9.
Richardson, S. D. Disinfection by-products and other emerging contaminants in drinking water. *Trends Anal Chem* **2003**, *22* (10), 666–684.
Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; DeMarini, D. M. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat Res-Rev Mutat Res* **2007**, *636* (1-3), 178–242.
Richardson, S. D.; Ternes, T. A. Water analysis: emerging contaminants and current issues. *Anal Chem* **2011**, *83* (12), 4614–4648.
Richardson, S. D. Environmental mass spectrometry: emerging contaminants and current issues. *Anal Chem* **2012**, *84* (2), 747–778.
Richardson, S. D.; Ternes, T. A. Water analysis: emerging contaminants and current issues. *Anal Chem* **2014**, *86* (6), 2813–2848.
Rhieu, S. Y.; Urbas, A. A.; Bearden, D. W.; Marino, J. P.; Lippa, K. A.; Reipa, V. Probing the intracellular glutathione redox potential by in-cell NMR spectroscopy. *Angew Chem Int Edit* **2014**, *53* (2), 447–450.

Roccaro, P.; Chang, H. S.; Vagliasindi, F. G. A.; Korshin, G. V. *Water Res* **2008**, *42* (8-9), 1879–1888.
Rodil, R.; Quintana, J. B.; Basaglia, G.; Pietrogrande, M. C.; Cela, R. Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography-mass spectrometry. *J Chromatogr A* **2010**, *1217* (41), 6428–6435.
Rodil, R.; Quintana, J. B.; Cela, R. Oxidation of synthetic phenolic antioxidants during water chlorination. *J Hazard Mater* **2012**, *199*, 73–81.
Rook, J. J. Formation of haloforms during chlorination of natural waters. *J Water Treat Exam* **1974**, *23*, 234–243.
Sarr, D. H.; Kazunga, C.; Charles, M. J.; Pavlovich, J. G.; Aitken, M. D. Decomposition of tetrachloro-1,4-benzoquinone (*p*-chloranil) in aqueous-solution. *Environ Sci Technol* **1995**, *29* (11), 2735–2740.
Sedlak, D. L.; von Gunten, U. The chlorine dilemma. *Science* **2011**, *331* (6013), 42–43.
Shah, A. D.; Dotson, A. D.; Linden, K. G.; Mitch, W. A. Impact of UV disinfection combined with chlorination/chloramination on the formation of halonitromethanes and haloacetonitriles in drinking water. *Environ Sci Technol* **2011**, *45* (8), 3657–3664.
Song, Y.; Wagner, B. A.; Witmer, J. R.; Lehmler, H. J.; Buettner, G. R. Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones. *Proc Natl Acad Sci USA* **2009**, *106* (24), 9725–9730.
Song, Y.; Buettner, G. R. Thermodynamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide. *Free Radical Bio Med* **2010**, *49* (6), 919–962.
Swan, S. H.; Waller, K. Drinking water and reproductive outcomes. *Epidemiology* **1998**, *9* (3), 358–358.
Symons, J. M.; Bellar, T. A.; Carswell, J. K.; Demarco, J.; Kropp, K. L.; Robeck, G. G.; Seeger, D. R.; Slocum, C. J.; Smith, B. L.; Stevens, A. A. National organics reconnaissance survey for halogenated organics. *J Am Water Works Assoc* **1975**, *67* (11), 634–647.
Tan, K.H. *Humic Matter is Soil and the Environment*. Marcel Dekker: New York, NY, 2003.
Terasaki, M.; Makino, M. Determination of chlorinated by-products of parabens in swimming pool water. *Int J Environ An Ch* **2008**, *88* (13), 911–922.
Treguer, R.; Couvert, A.; Wolbert, D.; Suty, H.; Randon, G. Particulate products and new polymers for a more efficient removal of dissolved organic matter in drinking water resources. *Environ Technol* **2007**, *28* (8), 861–869.
Tsai, C. H.; Lin, P. H.; Troester, M. A.; Rappaport, S. M. Formation and removal of pentachlorophenol-derived protein adducts in rodent liver under acute, multiple, and chronic dosing regimens. *Toxicol Sci* **2003**, *73* (1), 26–35.
Tsai, C. H.; Lin, P. H.; Waidyanatha, S.; Rappaport, S. M. Characterization of metabolic activation of pentachlorophenol to quinones and semiquinones in rodent liver. *Chem Biol Interact* **2001**, *134* (1), 55–71.
Uchimiya, M.; Stone, A. T. Reversible redox chemistry of quinones: impact on biogeochemical cycles. *Chemosphere* **2009**, *77* (4), 451–458.

United States Environmental Protection Agency (US EPA). N*ational Primary Drinking Water Regulations: Stage 2 Disinfectants and Disinfection Byproducts Rule; Final Rule*. 2006; Vol. 71, pp 388–493.

Urbansky, E. T. The fate of the haloacetates in drinking water- chemical kinetics in aqueous solution. *Chem Rev* **2001**, *101* (11), 3233–3243.

Valli, K.; Wariishi, H.; Gold, M. H. Degradation of 2,7-dichlorodibenzo-para-dioxin by the lignin-degrading basidiomycete phanerochaete-chrysosporium. *J Bacteriol* **1992**, *174* (7), 2131–2137.

Villacanas, F; Pereira, M. F. R.; Orfao, J. J. M.; Figueiredo, J. L. Adsorption of simple aromatic compounds on activated carbons. *J Colloid Interface Sci* **2006**, *293* (1), 128–136.

Villanueva, C. M.; Cantor, K. P.; Grimalt, J. O.; Malats, N.; Silverman, D.; Tardon, A.; Garcia-Closas, R.; Serra, C.; Carrato, A.; Castano-Vinyals, G.; Marcos, R.; Rothman, N.; Real, F. X.; Dosemeci, M.; Kogevinas, M. Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. *Am J Epidemiol* **2007**, *165* (2), 148–156.

von Gunten, U. Ozonation of drinking water: Part I. Oxidation kinetics and product formation. *Water Res* **2003**, *37* (7), 1443–1467.

von Sonntag, J.; Mvula, E.; Hildenbrand, K.; von Sonntag, C., Photohydroxylation of 1,4-benzoquinone in aqueous solution revisited. *Chem-Eur J* **2004**, *10* (2), 440–451.

Waidyanatha, S.; Lin, P. H.; Rappaport, S. M. Characterization of chlorinated adducts of hemoglobin and albumin following administration of pentachlorophenol to rats. *Chem Res Toxicol* **1996**, *9* (3), 647–653.

Waller, K.; Swan, S. H.; DeLorenze, G.; Hopkins, B. Trihalomethanes in drinking water and spontaneous abortion. *Epidemiology* **1998**, *9* (2), 134–140.

Wang, W.; Qian, Y.; Boyd, J. M.; Wu, M.; Hrudey, S. E.; Li, X.-F. Halobenzoquinones in swimming pool waters and their formation from personal care products. *Environ Sci Technol* **2013**, *47* (7), 3275–3282.

Wang, W.; Qian, Y.; Li, J.; Moe, B.; Huang, R.; Zhang, H.; Hrudey, S. E.; Li, X.-F. Analytical and toxicity characterization of halo-hydroxylbenzoquinones as stable halobenzoquinone disinfection byproducts in treated water. *Anal Chem* **2014**, *86* (10), 4982–4988.

Wardman, P. Evaluation of the "radical sink" hypothesis from a chemical-kinetic viewpoint. *J Radioanal Nucl Chem* **1998**, *232* (1-2), 23–27.

Wassink, J.K.; Andrews, R.C.; Peiris, R.H.; Legge, R.L. Evaluation of fluorescence excitation-emission and LC-OCD as methods of detecting removal of NOM and DBP precursors by enhanced coagulation. *Wat Sci Technol: Wat Supply* **2011**, *11* (5), 621–630.

Wei, Y.-Y.; Liu, Y.; Zhang, Y.; Dai, R.-H.; Liu, X.; Wu, J.-J.; Zhang, Q. Influence of soluble microbial products (SMP) on wastewater disinfection by-products: trihalomethanes and haloacetic acid species from the chlorination of SMP. *Environ Sci Pollut Res* **2011**, *18* (1), 46-50.

Weng, S. C.; Li, J.; Blatchley, E. R. Effects of UV_{254} irradiation on residual chlorine and DBPs in chlorination of model organic-N precursors in swimming pools. *Water Res* **2012**, *46* (8), 2674–2682.

Willson, R. L. Free radical repair mechanisms and the interactions of glutathione and vitamins C and E. In *Radioprotectors and Anticarcinogens*, Academic Press: London, 1982; pp 1–23.
Workman, J. Jr. *The Handbook of Organic Compounds: NIR, IR, Raman, and UV-Vis Spectra Featuring Polymers and Surfactants*. Academic Press: San Diego, CA, 2001.
World Health Organization (WHO). *Guidelines for drinking-water quality*, 4th ed.;.2011. http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/. Accessed: Jan 5, 2016.
World Health Organization (WHO). Progress on sanitation and drinking water: 2015 update and MDG assessment. New York, NY, 2015. http://www.unicef.org/publications/index_82419.html. Accessed: Jan 5, 2016.
World Health Organization (WHO). Water-related diseases. http://www.who.int/water_sanitation_health/diseases/en/. Accessed: Jan 5, 2016.
World Health Organization (WHO). *Guidelines for Safe Recreational Water Environments. volume 2, Swimming Pools and Similar Environments*; WHO Press: Geneva, 2006.
Wu, M. H.; Qian, Y. C.; Boyd, J. M.; Leavey, S.; Hrudey, S. E.; Krasner, S. W.; Li, X.-F. Identification of tobacco-specific nitrosamines as disinfection byproducts in chloraminated water. *Environ Sci Technol* **2014**, *48* (3), 1828–1834.
Wu, Z.; Gao, W.; Phelps, M. A.; Wu, D.; Miller, D. D.; Dalton, J. T. Favorable effects of weak acids on negative-ion electrospray ionization mass spectrometry. *Anal Chem* **2004**, *76* (3), 839–847.
Xiao, F.; Zhang, X. R.; Zhai, H. Y.; Lo, I. M. C.; Tipoe, G. L.; Yang, M. T.; Pan, Y.; Chen, G. H. New Halogenated disinfection byproducts in swimming pool water and their Permeability across skin. *Environ Sci Technol* **2012**, *46* (13), 7112–7119.
Xiong, R.; Siegel, D.; Ross, D. Quinone-induced protein handling changes: implications for major protein handling systems in quinone-mediated toxicity. *Toxicol Appl Pharmacol* **2014**, *280* (2), 285–295.
Yu, H.-G.; Francisco, J.S.; Muckerman, J.T. Ab initio and direct dynamics study of the reaction of Cl atoms with HOCO. *J Chem Phys* **2008**, *129* (064301), 1–7.
Zeller, K.-P. Mass spectra of quinones. In *The Chemistry of Quinonoid Compounds*, Wiley Interscience: 1974; Vol. 1, pp 231.
Zhao, H.; Hu, C. Z.; Liu, H. J.; Zhao, X.; Qu, J. H. Role of aluminum speciation in the removal of disinfection byproduct precursors by a coagulation process. *Environ Sci Technol* **2008**, *42* (15), 5752–5758.
Zhai, H.; Zhang, X. Formation and decomposition of new and unknown polar brominated disinfection byproducts during chlorination. *Environ Sci Technol* **2011**, *45* (6), 2194–2201.
Zhai, H.; Zhang, X.; Zhu, X.; Liu, J.; Ji, M. Formation of brominated disinfection byproducts during chloramination of drinking water: new polar species and overall kinetics. *Environ Sci Technol* **2014**, *48* (5), 2579–2588.
Zhao, Y.; Anichina, J.; Lu, X. F.; Bull, R. J.; Krasner, S. W.; Hrudey, S. E.; Li, X.-F. Occurrence and formation of chloro- and bromo-benzoquinones during drinking water disinfection. *Water Res* **2012**, *46* (14), 4351–4360.
Zhao, Y.; Biggs, T. D.; Xian, M. Hydrogen sulfide (H_2S) releasing agents: chemistry and biological applications. *Chem Commun* **2014**, *50* (80), 11788–11805.

Zhao, Y. L.; Qin, F.; Boyd, J. M.; Anichina, J.; Li, X.-F. Characterization and determination of chloro- and bromo-benzoquinones as new chlorination disinfection byproducts in drinking water. *Anal Chem* **2010**, *82* (11), 4599–4605.

Zhao, Y.; Wang, H.; Xian, M. Cysteine-activated hydrogen sulfide (H_2S) donors. *J Am Chem Soc* **2011**, *133* (1), 15–17.

Zhou, W.; Boyd, J. M.; Qin, F.; Hrudey, S. E.; Li, X.-F. Formation of N-nitrosodiphenylamine and two new N-containing disinfection byproducts from chloramination of water containing diphenylamine. *Environ Sci Technol* **2009**, *43* (21), 8443–8448.

Zhu, B. Z.; Har-El, R.; Kitrossky, N.; Chevion, M. New modes of action of desferrioxamine: scavenging of semiquinone radical and stimulation of hydrolysis of tetrachlorohydroquinone. *Free Radic Biol Med* **1998**, *24* (2), 360–369.

Zhu, B.-Z.; Kalyanaraman, B.; Jiang, G.-B. Molecular mechanism for metal-independent production of hydroxyl radicals by hydrogen peroxide and halogenated quinones. *Proc Natl Acad Sci USA* **2007**, *104* (45), 17575–17578.

Zhu, B. Z.; Mao, L.; Huang, C. H.; Qin, H.; Fan, R. M.; Kalyanaraman, B.; Zhu, J. G. Unprecedented hydroxyl radical-dependent two-step chemiluminescence production by polyhalogenated quinoid carcinogens and H_2O_2. *Proc Natl Acad Sci USA* **2012**, *109* (40), 16046–16051.

Zhu, B.-Z.; Shan, G.-Q. Potential mechanism for pentachlorophenol-induced carcinogenicity: a novel mechanism for metal-independent production of hydroxyl radicals. *Chem Res Toxicol* **2009**, *22* (6), 969–977.

Zhu, B.-Z.; Zhu, J.-G.; Mao, L.; Kalyanraman, B.; Shan, G.-Q. Detoxifying carcinogenic polyhalogented quinones by hydroxamic acids via an unuaual double lossen rearrangement mechanism. *Proc Natl Acad Sci USA* **2010**, *107* (48), 20686–20690.

Zwiener, C.; Richardson, S. D.; De Marini, D. M.; Grummt, T.; Glauner, T.; Frimmel, F. H. Drowning in disinfection byproducts? Assessing swimming pool water. *Environ Sci Technol* **2007**, *41* (5), 363–372.