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# Disinfection By-Products: Method Optimization for Quantification, Unknown Analysis, and Calculated Toxicity

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# DISINFECTION BY-PRODUCTS: METHOD OPTIMIZATION FOR QUANTIFICATION, UNKNOWN ANALYSIS, AND CALCULATED TOXICITY

by

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# DEDICATION

This is dedicated to my large and loving family who instilled the love of science, and art in me. To my four sisters, whom I can always turn to. To my father, who is here, but not there. And, to my mother, who takes care of us all. Most of all, this is dedicated to my patient and kind husband.

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# ABSTRACT

Disinfected drinking water contains hundreds of disinfection by-products (DBPs) that are formed by the reaction of disinfectants with natural and anthropogenic organic matter, bromide, and iodide. Understanding what these DBPs are is important because millions of people worldwide consume drinking water every day, and human epidemiologic studies have reported cancer, miscarriage, and birth defects from consuming such waters. While more than 700 DBPs are reported in the literature, very few studies quantify complete classes of chlorinated, brominated, and iodinated DBPs. The following document contains five chapters in the format designated for specific scientific journals on this subject. Chapter 1 describes an optimized extraction method that combines 61 disinfection by-products from 7 different chemical classes prioritized by *in vivo* toxicity, and includes regulated trihalomethanes (THMs) and haloacetic acids (HAAs), unregulated iodinated HAAs and THMs, tri-haloacetaldehydes, haloketones, haloacetonitriles, halonitromethanes, and haloacetamides. Chapter 2 describes a novel method developed for the quantification of 10 halobenzoquinones (HBQs), a class of DBPs in drinking water which have been shown to be more toxic than most regulated DBPs. This method allows low ng/L limits of detection, requires minimal sample preparation, and analysis is almost entirely automated.

Chapter 3 discusses a method that simultaneously quantifies DBPs, and analyzes unknown DBPs under full-scan conditions using a new type of time-of-flight (TOF) mass

spectrometer, which combines selected ion monitoring (SIM)-level sensitivity with mass accuracy of ± 0.05 Da. Chapter 4 evaluates the reduction of 70 priority unregulated and regulated DBPs in full-scale chlorinated drinking plants using granular activated carbon (GAC), while assessing calculated cytotoxicity and genotoxicity. Chapter 5 evaluates the reduction of these priority unregulated DBPs, regulated DBPs, and *N*-nitrosodimethylamine (NDMA) in full-scale chloraminated drinking water plants that utilize biological activated carbon, while assessing calculated cytotoxicity and genotoxicity. Chapter 4 and 5 provide valuable insight into how to reduce these toxic unregulated DBPs, and what DBPs are toxicity-drivers in real-world exposure conditions. Chapter 1 is in the format of the journal, *Analytical Chemistry*; Chapter 2: *Journal of Chromatography A*; Chapter 3: *Water Research*; Chapter 4: *Environmental Science & Technology*; Chapter 5: *Water Research*.

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# CHAPTER 1

# TRACE ANALYSIS OF 61 DBPS USING MULTIPLE SALT-ASSISTED LIQUID-LIQUID EXTRACTIONS, AND PRECONCENTRATION WITH GC-MS[1]

[1] Amy A. Cuthbertson, Hannah K. Liberatore, Susana Y. Kimura, Alena Bensussan, and Susan D. Richardson. To be submitted to *Analytical Chemistry.*

**Abstract:** This extraction method combines 61 disinfection by-products (DBPs) from 7 different chemical classes prioritized by *in vivo* toxicity, and includes regulated trihalomethanes (THMs) and haloacetic acids (HAAs), unregulated iodinated HAAs and THMs, tri-haloacetaldehydes, haloketones, haloacetonitriles, halonitromethanes, and haloacetamides. Method optimization included comparison of three gas chromatography (GC) columns, and a comparison of solid phase extraction to salt-assisted liquid-liquid extraction. The final method employs the use of multiple salt-assisted liquid-liquid extractions, coupled with a pre-concentration step under nitrogen with a 500-fold concentration factor. This resulted in a single extraction method for a wide range of DBPs, producing the lowest method detection limits to-date for many compounds, including highly toxic iodinated and brominated DBPs. Extracts were divided for the analysis of the HAAs (including iodinated HAAs) by diazomethane derivatization and analysis using a GC-triple quadrupole mass spectrometer with multiple reaction monitoring, resulting in higher signal-to-noise ratios, greater selectivity, and improved detection of these compounds. The rest of the DBPs were run using a GC-single quadrupole mass spectrometer with selected ion-monitoring, utilizing a multi-mode inlet that allowed for lower injection temperatures, then ramping to higher temperatures. This allowed for the analysis of thermally labile DBPs that decompose at higher inlet temperatures and also for the inlet to be cleaned at higher temperatures between samples, preserving calibrations for longer periods. Finally, the use of a Restek Rtx-200 GC column significantly improved peak shapes, helping to improve separations and lower detection limits.

- **INTRODUCTION**

Chemical disinfection is used world-wide to inactivate pathogenic microorganisms to prevent the spread of infectious water-borne diseases. An unintended, and inevitable consequence of chemical disinfection is the formation of disinfection by-products (DBPs) through the reaction of a chemical oxidant with dissolved organic matter, and inorganic components of water, including bromide, and iodide.[1] Since the 1980s, more than 700 DBPs have been reported in literature for major disinfectants and their combinations (chlorine, ozone, chlorine dioxide, chloramines).[2,3] In 1998, an extensive toxicological review of over 500 DBPs ranked many of these compounds on their carcinogenic potential, which led to an occurrence study in 2002 of the 50 highest ranking unregulated DBPs to target for future studies.[2-5] These priority DBPs were primarily formed by chlorine or chloramine disinfection, and included halomethanes, haloacetonitriles, haloketones, haloacetaldehydes, halonitromethanes, haloketones, and halogenated furanones. Several epidemiological studies since have linked drinking disinfected water to adverse health effects including bladder and colon cancers, miscarriage, and birth defects.[6-13]

More than 100 DBPs, including these priority DBPs, have undergone systematic, and quantitative comparative analyses on the induction of cytotoxicity and genotoxicity using a consistent analytical biological platform and endpoint, Chinese hamster ovary (CHO) cells.[13,14] The top 10 most cytotoxic DBPs in the CHO assay follow the rank order of most potent to least potent: diiodoacetamide > iodoacetamide > bromoacetamide > dibromoacetonitrile > iodoacetic acid > tribromoacetamide > bromoacetonitrile > chloroacetaldehyde > tribromoacetaldehyde > bromoiodoacetamide. The top ten most

genotoxic DBPs in the CHO comet assay follow the rank order of: iodoacetic acid > bromoacetic acid > dibromonitromethane > tribromoacetamide > diiodoacetamide > iodoacetamide > bromoacetamide > bromoacetonitrile > dibromoacetonitrile > dibromochloronitromethane.[14-16] Several toxicity trends emerged from this extensive study, indicating that nitrogenous DBPs (N-DBPs) tend to be more toxic than DBPs without nitrogen, and iodinated DBPs (I-DBPs) are more toxic than brominated DBPs (Br-DBPs), which are much more toxic than chlorinated analogues (I > Br >> Cl).[17-22]

Our new analytical method analyzes the top 10 most cytotoxic and genotoxic compounds, and also includes 4 regulated trihalomethanes (THMs) and 6 haloacetic acids (HAAs), along with unregulated DBPs, 4 tri-haloacetaldehydes (HALs), 9 haloketones (HKs), 6 iodo-THMs (ITHMs), 8 haloacetonitriles (HANs), 7 halonitromethanes (HNMs), 13 haloacetamides (HAMs), and 4 iodo-HAAs (IAAs). CAL is not included in this method because it requires derivatization for analysis. A list of these compounds, and their abbreviations can be found in Table 1.1.

A key aspect of DBP analysis that is different from the analysis of other environmental contaminants is the need to quench the active disinfectant (e.g., chlorine or chloramine) to prevent continued reaction of the DBPs with the disinfectant after sample collection. This is often complicated because some quenchers, including ascorbic acid and sodium thiosulfate will significantly degrade many DBPs.[23,24] While ammonium chloride is often the least destructive quenching agent for most compounds, particularly the N-DBPs,[23] it reacts with hypochlorous acid in chlorinated drinking water to form chloramines, which continue to form DBPs, but at a much slower rate.[25-27] Samples should also be preserved and pH-adjusted at the time of collection to prevent hydrolysis

and degradation.[2,28] Therefore, a stability study was conducted to optimize the reproducibility of the entire sampling, quenching/preservation, and extraction procedure for the 61 DBPs in this method.

Due to the chemical nature of many of these DBPs, a GC-electron ionization (EI)-mass spectrometry (MS) platform was chosen (rather than liquid chromatography (LC)-MS) for analysis. This is because most of these DBPs are volatile or semi-volatile and ionize better with EI than with electrospray ionization. However, developing a single method for this extensive list of compounds is challenging due to their broad range in polarity and volatility, along with thermal instability for some compounds. For example, tribromonitromethane thermally degrades at higher GC inlet temperatures, and hydrogen abstraction occurs at higher GC transfer line temperatures.[29] Optimization of the GC-EI-MS method included testing different GC columns with a variety of stationary phases, (DB-5, Rtx-624, and a Rtx-200), as well as optimization of GC temperature parameters, including the use of a multi-mode inlet (MMI), which allowed for a ramping of the inlet temperatures and prevented degradation of thermally labile DBPs. HAAs, and IAAs were analyzed using an optimized GC tandem mass spectrometry (GC-MS/MS) method, which led to improved signal-to-noise ratios (SNRs) and greater selectivity for these compounds.

Two extraction procedures, liquid-liquid extraction (LLE) and solid phase extraction (SPE), were evaluated due to their widespread use and availability. Previous methods have used LLE for a subset of many of these compounds, usually with only one extraction step.[2,22,29,30-33] To improve sensitivity and lower detection limits, optimization with multiple extractions, along with a pre-concentration step, significantly improved

detection limits. This method includes the most comprehensive list of priority DBPs combined into one cohesive method at the lowest possible limits of quantification (LOQs) currently reported (Table 1.1 and 1.6).

- **EXPERIMENTAL SECTION**

**Chemicals, Reagents, and Standard Solutions.** Analytical standards for priority DBPs were purchased or custom synthesized at the highest purity available from CanSyn Chem. Corp. (Toronto, Ontario), Sigma-Aldrich (St. Louis, MO), and TCI America (Boston, MA). Organic solvents were of the highest purity available. Acetonitrile, ethyl acetate, methyl *tert*-butyl ether (MTBE), methanol, and pure water were purchased from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA). Vendor information for each standard, along with abbreviations can be found in Table 1.1. Individual standards from 1000-5000 ppm were prepared in different solvents based on chemical class. HNMs were prepared in ethyl acetate; HANs, ITHMs, HKs, and HALs were prepared in acetonitrile; HAMs were prepared in methanol; IAAs were prepared in MTBE; and standards from the EPA Method 551.1 mix were purchased in acetone from Millipore-Sigma (DCAN, BCAN, DBAN, TCAN, TCNM, 111TCP, TCAL, and 11DCP). Calibration mixtures were prepared at 100 ppm in methanol, and diluted to 10 ppm in methanol before calibration. Individual standards (1000-5000 ppm) were stored up to one year, and 10 ppm mixes were prepared fresh before calibration. TBNM, BDCNM, DBCNM, and TBAN must be calibrated separately to avoid reactions with other compounds, which reduces sensitivity.

**Sample Collection and Preservation.** Samples were collected in amber borosilicate glass, headspace free. Chlorinated drinking water samples were quenched with ammonium chloride at a molar ratio of 1:1.3 free chlorine to quenching agent. Chloraminated drinking water samples were not quenched. pH was adjusted between 3-5 using 1 M sulfuric acid. Samples were stored at 4 °C until extraction, which occurred within 48 hours of sampling.

**Liquid-Liquid Extraction Procedure.** 100 mL of sample was transferred to a 125-mL amber borosilicate bottle with a polyethylene cap. The pH was dropped using concentrated sulfuric acid to pH <2 to maximize extraction efficiency for all compounds (some have a $pK_a$ <1). Five mL of MTBE was added to the bottle, along with 30 g of sodium sulfate. Samples were shaken for 15 minutes using a Burrell wrist action shaker, and then settled for 10 minutes to allow the organic layer to separate. The organic layer was removed into a separate test tube. This was repeated 2 more times with 5 mL of MTBE each, shaken for 15 minutes, settled for 10 minutes, and the organic layer removed into the same test tube for a total volume of approximately 15 mL. This extract was then dried using small sodium sulfate columns (made using disposable glass Pasteur pipettes filled with sodium sulfate) to remove any excess water, careful not to add water from the base of the test-tube which would plug the drying column. The remaining extract was concentrated under a gentle stream of nitrogen to a final volume of slightly below 200 μL, and then brought up to volume with fresh MTBE using a Hamilton gas tight syringe. The final extract was transferred to an amber GC vial with a cemented insert. It is important to store extracts in a GC vial with a cemented insert to prevent evaporation of volatile compounds. Internal standard 1,2-dibromopropane was added to

the final extract. 100 µL of the final extract was removed to a separate GC vial for the analysis of the HAAs and IAAs using diazomethane derivatization described below.

**Diazomethane Procedure.** 100 µL of the final extract underwent diazomethane derivatization for the analysis of HAAs, and IAAs. Diazomethane was generated following established methods (Richardson, 2009) using an Aldrich® diazomethane generator apparatus. Approximately 0.367 g of Diazald® and 1 mL of CARBITOL™ were added to the inner tube of the apparatus. MTBE (3.0 mL) was added to the outer tube, and the inner and outer tubes were connected together. The apparatus was then placed in an ice bath. Potassium hydroxide (1.5 mL of 37% KOH) solution was injected slowly and carefully dropwise through the top septum into the inner tube. After reacting for 1 hour, the diazomethane generated in the outer tube in MTBE is carefully placed into a vial for immediate use. An aliquot of diazomethane solution (50 µL) is added to each vial of 100 µL sample extract, allowed to react for 30 minutes, and then quenched with approximately 10 mg of silica. Derivatized samples were then transferred to new GC vials to remove silica solids, which can cause mass spectral interferences.

**Solid-Phase Extraction (SPE) Procedure.** SPE extractions were based on a previous published method for a smaller subset of DBPs.[34] Oasis HLB cartridges were used in conjunction with a manual extraction manifold connected to a vacuum pump. The cartridges were conditioned with 6 mL of methanol, followed by 6 mL water, careful to not let the cartridge dry before adding sample. Samples (100 mL) were eluted through the cartridges following conditioning. To avoid volatilization of DBPs, the cartridges were not dried prior to elution. Samples were eluted with 10 mL solvent by gravity, without vacuum. Extracts were dried using sodium sulfate columns, and concentrated under a

slow stream of nitrogen to a final volume 200 µL. Elution solvents acetone, MTBE, and ethyl acetate were evaluated for percent recovery.

**Instrumental analysis.** Instrumental optimization was conducted on a single quadrupole GC-mass spectrometer with electron ionization in selected ion monitoring (SIM) mode (7890 GC, 5977A mass spectrometer, Agilent Technologies, Santa Clara, CA) using a Restek Rtx-200MS column (30 m x 0.25 mm ID x 0.25 µm film thickness; Restek Corporation, Bellefonte, PA). Samples were injected using a multi-mode inlet (MMI) in pulsed splitless mode with the following temperature program: 35°C held for 0.1 minute, ramped to 220°C at a rate of 360°C/min, held for 5 minutes, and then ramped to 280°C at a rate of 720°C/min. The GC oven temperature was held for 5 minutes at 35°C, ramped to 200°C at 9°C/min, then ramped to 280°C and held for 20 minutes. The transfer line temperature was held at 225°C, the source temperature at 200°C, and the quadrupole temperature at 150°C. An ionization energy of 70 eV was used.

In systems that do not contain a programmable temperature vaporization (PTV) inlet or an MMI, two temperature programs can be utilized. A lower temperature program can be used for thermally unstable compounds (TBNM, BDCNM, DBCNM, and TBAN) as follows: inlet temperature 125°C, transfer line temperature 225°C. For the rest of the compounds a higher temperature program can be used: inlet temperature 250°C, and transfer line temperature 280°C. The oven ramp temperature program was the same for all GC programs.

Analysis of derivatized HAAs and IAAs was optimized using a GC-triple quadrupole mass spectrometer (TRACE GC Ultra, Quantum GC$^{TM}$ MS/MS, Thermo Scientific, Waltham, MA) with multiple reaction monitoring (MRM). Two µL of sample

was injected into the inlet at 250°C with a splitless time of 0.80 minutes and split flow of 50 mL/min, onto a Restek Rtx-200MS column (30 m x 0.25 mm ID x 0.25 μm film thickness; Restek Corporation, Bellefonte, PA). The oven temperature program started at 35°C, which was held for 2 minutes, ramped to 280°C at 9°C/min, and held for 20 minutes. The transfer line was held at 280°C, and the source temperature at 200°C. An emission current of 50 μA and ionization energy of 70 eV were used. Two MS/MS transitions were collected, one used for quantification and one used for qualification. SIM and MRM ions are listed in Table 1.1 and 1.2.

**Holding Study.** A holding study was conducted over a week-long period with ammonium chloride, ascorbic acid, and no quench. A previous study[23] indicated that these two quenchers were most suitable for a similar subset of compounds. Chlorinated and chloraminated water was spiked with all analytical standards, and then quenched with respective quenchers. Stability was monitored using the extraction procedure described above. Data was plotted by dividing the internal standard response ratio from each day by the internal standard response ratio on Day 0 (Figure 1.1 and 1.2).

**Method Validation.** Method detection limits (MDLs) were determined with the following parameters: visual confirmation, area response of the instrument over 500 and a signal to noise ratio (SNR) minimum of 3. For limits of quantification (LOQs), six replicate samples were run at the limit of quantification for verification. Quality parameters include an average percent accuracy from the known spiked concentration of ±20%, and a percent relative standard deviation (%RSD) of ±30%. Table 1.7 lists the instrument response and SNR of each compound at the MDL, and Table 1.1 includes the LOQ concentration, the average % accuracy, and %RSD. It should be noted that

instrument performance, and ultimately method performance, will vary from instrument to instrument, and will change over time on the same instrument. Method performance should be verified yearly, or with major maintenance, such as replacing the ion source or other parts in the mass spectrometer.

**Sampling for Method Validation.** Finished drinking water samples were collected from four drinking water plants of varying size and with different types of disinfectants and source waters. For final disinfection, Plants 1 used chloramine, Plants 2 and 3 used gaseous chlorine, and Plant 4 used a combination of chlorine for three groundwater wells and 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) for one groundwater well, which were blended prior to distribution. Plants 1 and 2 uses surface water from the same river, Plant 3 uses surface water from both a reservoir and a river, and Plant 4 uses groundwater. A distribution sample was also collected and evaluated for Plants 1 and 2 for comparison. All sample extraction and analyses were conducted in triplicate. Information on each plant including pH, total organic carbon (TOC), bromide, iodide, disinfectant dose, estimated water age, and number of consumers can be found in Table 1.3. Bromide and iodide were measured using a Dionex 1600 ion chromatograph (Dionex, Sunnyvale, CA).

- **RESULTS AND DISCUSSION**

**Quencher Holding Study.** It was found that ascorbic acid reduced BDCNM, DBCNM, TBNM, and TBAN to undetectable concentrations within hours of contact in chlorinated and chloraminated water quenched with ascorbic acid. This is consistent with previous studies showing similar results on a smaller subset of compounds.[3,23] DBP formation was determined to not be statistically significant (±30%) between 24-48 hours

for both chlorine quenched with ammonium chloride and chloramine samples not quenched. Several compounds degraded, including IAM, which degraded by 30% in 24 hours, and then degraded by 36% in 48 hours. CP and DCNM degraded by 30% within 48 hours in both quenched chlorinated water and non-quenched chloraminated water. Compounds degraded by more than 30% in 72 hours in quenched chlorinated water, including BCAM (32%), DBAM (34%), and 1133TeCP (42%). In non-quenched chloraminated water, compounds that degraded more than 30% in 72 hours included BCAM (37%), DBAM (38%), DIAM (42%), and CP (38%). Stability graphs for all chemical classes can be found in Figures 1.1 and 1.2. To accommodate the maximum number of DBPs, ammonium chloride was chosen as a quencher. Since many compounds degrade relatively rapidly, it is imperative to extract samples with 48 hours of sampling. Samples may also be held up to the estimated distribution system water age as reflective of real-world conditions, which may exceed 48 hours.

**Optimization of Instrumental Conditions.**

**GC Column Tests.** Three columns were tested for peak shape and instrument response for all compounds to maximize sensitivity for the widest group of compounds. An Agilent DB-5 (5% phenyl, 95% dimethyl polysiloxane), a Restek Rtx-642 (6% cyanopropylphenyl, 86% dimethyl polysiloxane), and a Restek Rtx-200 (trifluoropropyl methyl polysiloxane) were chosen because of the range of polarity of each stationary phase. While the DB-5 is by far the most commonly used column in the literature, this column shows significant peak tailing for some emerging haloacetamides, including CAM, BAM, IAM, CIAM, and DIAM (Figure 1.4). These same compounds showed significant peak shape improvement on both the Rtx-624 and the Rtx-200. All HNMs had

sharp peak shape profiles on the DB-5 and Rtx-200, but several compounds had poor peak shape or were destroyed on the Rtx-624 column (Figure 1.4). On the Rtx-624 column, DBNM had reduction in response due to peak tailing, and three compounds were not visible at all including BDCNM, DBCNM, and TBNM. It is postulated that these compounds react with the stationary phase of the Rtx-624 column, which contains a cyano-anion functional group. Rtx-200 was chosen because all compounds showed good or improved peak shape and instrument response in comparison to the other two columns. This is the first method published that uses this column for this set of compounds.

**Temperature Programming.** As mentioned earlier, some of these compounds, including TBNM, have significant thermal degradation in the GC inlet. TBNM also undergoes hydrogen abstraction at high GC transfer line temperatures (280$^{o}$C) that are typically used with GC-MS methods.[29] In addition, some compounds have lower detection limits with higher temperature programs, i.e. haloacetamides.[24] There are two solutions to this problem. If the system contains a non-programmable inlet, samples can be run consecutively with two different temperature programs, one at higher temperatures and one at lower temperatures. Since TBNM, BDCNM, DBCNM, and TBAN must be calibrated separately due to their reactivity, a calibration curve can be run on a separate GC method with a lower inlet temperature and transfer line temperature. Alternatively, if the system has the capability to program the inlet temperature with either a programmable temperature vaporization (PTV) or a multimode inlet (MMI), then samples can be run using one GC method. Detection limits have been verified using both techniques with this method, and there is no significant difference in sensitivity between running separate GC programs. Running at lower inlet temperatures usually results in

increased need to replace inlet liners because NOM is not being burned off at these lower temperatures. With an MMI or PTV, the inlet temperature can be ramped to higher temperatures (280°C) to self-clean the inlet between samples, and thus minimizing inlet contamination.

If samples are run on a single GC method, the transfer line temperature would need to be lowered to 225°C for a PTV or MMI for improved detection of TBNM, BDCNM, DBCNM, and TBAN. Since there is a temperature drop between the GC oven and transfer line at the highest temperature (280°C) in the GC temperature gradient program, active sites from environmental samples can accumulate on the oven/transfer line interface as the sample condenses. Unfortunately, this leads to increased need for maintenance on the transfer line, as well. One way to clean the system, including the inlet liner and transfer line, between environmental samples is to run a non-polar solvent blank, such as hexane, at higher temperatures between samples, followed by a more polar solvent, or the solvent of your samples (e.g., MTBE). This can clean the system between samples that are running at lower temperature gradients, and prolong the calibration curve stability.

**Comparison of LLE and SPE.** LLE was optimized using MTBE, and included comparing one extraction, two extractions, and three extractions collected into the same vial for concentrating. Comparison of LLE to SPE was conducted by comparing the percent recovery of each compound. Recovery of some compounds was higher using SPE, including BIAM and DIAM, probably due to their high hydrophilic behavior (Table 1.4). SPE methods are promising for some compound classes when using an automated system. Without an automated system, these methods are also laborious, use similar

solvent amounts, and create single-use plastic waste, which often ends up being more expensive than LLE. This comparison of SPE to multiple LLE is particularly important for the HAMs, which are hydrophilic and have high detection limits in previous methods that only use one extraction. We found higher percent recovery using multiple LLE extractions for most HAMs as opposed to SPE (Table 1.4). LLE with multiple extractions and a pre-concentration step was chosen because it resulted in the lowest detection limits for the greatest number of DBPs.

**Problem Compounds.** Four compounds, bromodichloronitromethane (BDCNM), dibromochloronitromethane (DBCNM), tribromonitromethane (TBNM), and tribromoacetonitrile (TBAN), were calibrated separately do to the apparent reactivity of these compounds in mixture with the other compounds. When added to a mixture of analytes, BDCNM, DBCNM, TBNM, and TBAN had reduced sensitivity (below detection), but were easily detected when extracted separately. Compounds that had reduced sensitivity when in mixture with these four compounds include: dibromochloroacetaldehyde, tribromoacetaldehyde, triiodomethane, and 1,1,3,3-tribromopropanone. Further study is need to understand the reactivity of these compounds. The mono-HAMs (CAM, BAM, and IAM) degrade quickly and have poor chromatographic response over time. It is likely that these standards need to be monitored on a more frequent basis, and may benefit from regular checks with nuclear magnetic resonance (NMR) spectroscopy or standardization.

**Drinking Water Results.** Concentrations of DBPs for Plants 1-4 can be found in Table 1.5, and are plotted by class in Figure 1.5. Not surprisingly, the two plants with the highest DBP concentrations were Plant 1 (81,030-81,750 ng/L), and Plant 3 (59,530

ng/L), which chlorinate surface waters. Chlorinated water historically forms higher levels of THMs and HAAs than chloraminated water. Plant 4, which utilizes groundwater, had the lowest formation of DBPs (12,960 ng/L). Generally, surface waters contain higher levels of NOM, which can be indicated by TOC (Table 1.3). Plant 2 has the highest TOC levels, but with chloramination, results in lower total DBP formation than Plant 1, which uses a similar source water. Plant 4 had the lowest TOC levels with groundwater, resulting in the lowest DBP concentrations. THMs constituted 6-40% and HAAs contributed 30-71% to the overall DBP concentrations at Plants 1-4 (Figure 1.5). HKs also formed significant concentrations at Plants 1 and 3, at 18-24% and 35% of total DBP concentration, respectively. While THMs and HAAs often form at higher concentrations than other unregulated DBPs, they are often less toxic than many unregulated N-DBPs, I-DBPs, and Br-DBPs.

In terms of DBP speciation for Plant 1 (chloraminated drinking water), there was no significant variation from the finished water at the plant to the distribution system. In terms of concentration, most compounds were not significantly different or were decreased in the distribution system. Statistically relevant (student's t-test 95% CI) changes in concentration included compounds TCAL, TCAN, 13DCP, BDCNM, and DBCNM, all of which decreased in the distribution system (-10, -17, -74, -21, and -20%, respectively) (Table 1.5). Overall, total DBP formation decreased 22% in the distribution system. Plant 2 (chlorinated) finished water also had a similar DBP profile to the distribution system samples, but had three DBPs forming only in the distribution system, including BCAM, DBAM, and DBAN. From the finished drinking water at the plant to the distribution system, DBCAL decreased 83%, while TCAL increased 49%.

Statistically relevant changes in concentrations for haloacetamides included formation of BCAM and DBAM and a 41% increase of DBCAM in the distribution system. All haloacetonitriles decreased in the distribution system, including DCAN, BCAN, TCAN, and TBAN (-6, -27, -58, -35%, respectively), except DBAN which formed only in the distribution system. It has been shown that haloacetamides may form by the hydrolysis of haloacetonitriles[35], which helps to explain the decrease in HANs and increase in HAMs over time. For haloketones, both chloropropanone and 1,1,1-trichloropropanone decreased in the distribution system (-83 and -84% respectively).

These results highlight how using finished drinking water collected at the plant as a representative sample for DBP levels at the tap is limited in scope in terms of exposure. Chlorinated drinking water samples showed a considerable difference between the finished drinking water from the plant to the distribution system, likely due to the high reactivity of chlorine. These results also illustrate why it is important to measure complete classes of DBPs to better capture DBP exposure and to understand reactions and changes that can happen over time in the distribution system, including conversion of some DBP classes to other DBP classes. DBPs from different classes and with different chlorine/bromine/iodine speciation also have a wide range of different toxicities; thus, having a more comprehensive analytical method to more adequately capture the overall exposure is important when trying to understand and assess the potential risks of DBPs in drinking water.

- **CONCLUSIONS**

This method includes several compounds which have not previously been reported, including iodinated haloacetamides, trihaloacetaldehydes, iodoacetonitrile, and

iodo-trihalomethanes at similar or better limits of quantification than previously published methods. This one cohesive extraction method is easily repeatable by most laboratories because of the accessibility of materials and instrumentation. We believe it is currently the most sensitive method available for this set of compounds (Table 1.6). Other instrumentation which can be coupled to this method include GC-triple quadrupole-MS (GC-MS/MS), which may reduce background noise, thereby decreasing limits of quantification for more than just IAAs and HAAs. Instrumental limitations prevented its use during this method development. Other techniques that may improve the accuracy of this method include using isotopically labeled internal standards that extend over the range of retention times. Due to the current lack of available standards and cost, that was not done in this method. Future research should include finding a quencher that does not destroy any compounds of interest, and does not produce by-products such as chloramines, which can produce more DBPs. Finally, this method was developed specifically for finished treated drinking water samples and would need to be validated for other matrices including treated wastewater, hydraulic fracturing brines, and seawater. It is very likely that detection limits would be significantly higher in samples with more complex matrices.

- **ASSOCIATED CONTENT**

**Supporting Information Available**

Additional data and tables including a comparison of previous methods and data for each drinking water plant with relative standard deviations.

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**Table 1.1. DBP method information for single quadrupole mass spectrometer**

| DBP Class | DBP name | Abbrev. | RT (min.) | Quant Ion (m/z) | Qual Ion (m/z) | LOQ Conc. (ng/L) | Avg.% Accuracy | %RSD |
|---|---|---|---|---|---|---|---|---|
| THM | Trichloromethane[a] | **TCM** | 3.7 | 83 | 85 | 50 | 100 | 6 |
| THM | Bromodichloromethane[a] | **BDCM** | 4.2 | 83 | 129 | 50 | 102 | 12 |
| THM | Dibromochloromethane[a] | **DBCM** | 5.5 | 129 | 127 | 50 | 97 | 14 |
| THM | Tribromomethane[a] | **TBM** | 7.4 | 173 | 252 | 50 | 100 | 5 |
| HAL | Trichloroacetaldehyde[a] | **TCAL** | 3.8 | 82 | 110.9 | 50 | 115 | 13 |
| HAL | Bromodichloroacetaldehyde[b] | **BDCAL** | 5.2 | 83 | 111/163.8 | 100 | 124 | 5 |
| HAL | Dibromochloroacetaldehyde[b] | **DBCAL** | 7.2 | 128.9 | 127.9 | 30 | 71 | 1 |
| HAL | Tribromoacetaldehyde[a] | **TBAL** | 9.1 | 172.8 | 171.8 | 100 | 105 | 13 |
| HK | Chloropropanone[a] | **CP** | 4.7 | 92 | 43 | 100 | 122 | 6 |
| HK | 1,1-Dichloropropanone[a] | **11DCP** | 4.6 | 43 | 83 | 100 | 113 | 10 |
| HK | 1,3-Dichloropropanone[b] | **13DCP** | 9.0 | 77 | 49 | 100 | 115 | 11 |
| HK | 1,1-Dibromopropanone[b] | **11DBP** | 8.2 | 43 | 215.9 | 50 | 104 | 1 |
| HK | 1,1,1-Trichloropropanone[a] | **111TCP** | 6.9 | 43 | 125 | 100 | 99 | 17 |
| HK | 1,1,3-Trichloropropanone[a] | **113TCP** | 10.0 | 77 | 83 | 30 | 74 | 3 |
| HK | 1-Bromo-1,1-dichloropropanone[b] | **1B11DCP** | 8.8 | 43 | 125 | 100 | 95 | 12 |
| HK | 1,1,3,3-Tetrachloropropanone[b] | **1133TeCP** | 10.9 | 83 | 85 | 100 | 115 | 9 |
| HK | 1,1,3,3-Tetrabromopropanone[c] | **1133TeBP** | 16.1 | 200.8 | 119.9 | 100 | 123 | 8 |
| I-THM | Dichloroiodomethane[b] | **DCIM** | 4.3 | 83 | 126.9 | 50 | 129 | 16 |
| I-THM | Bromochloroiodomethane[b] | **BCIM** | 6.0 | 128.9 | 126.9 | 30 | 108 | 11 |
| I-THM | Dibromoiodomethane[b] | **DBIM** | 7.8 | 172.8 | 299.7 | 100 | 93 | 15 |
| I-THM | Chlorodiiodomethane[b] | **CDIM** | 8.3 | 174.9 | 126.9 | 30 | 92 | 3 |
| I-THM | Bromodiiodomethane[b] | **BDIM** | 10.1 | 218.8 | 220.8 | 40 | 81 | 2 |
| I-THM | Iodoform[a] | **TIM** | 12.1 | 393.7 | 266.8 | 100 | 88 | 8 |
| HAN | Chloroacetonitrile[a] | **CAN** | 4.4 | 75 | 48 | 100 | 101 | 8 |
| HAN | Bromoacetonitrile[a] | **BAN** | 6.2 | 118.9 | 120.9 | 75 | 88 | 3 |
| HAN | Iodoacetonitrile[a] | **IAN** | 8.9 | 167 | 126.9 | 100 | 116 | 11 |
| HAN | Dichloroacetonitrile[a] | **DCAN** | 4.2 | 74 | 82 | 30 | 84 | 3 |
| HAN | Bromochloroacetonitrile[a] | **BCAN** | 6.0 | 74 | 155 | 100 | 100 | 13 |
| HAN | Dibromoacetonitrile[a] | **DBAN** | 8.2 | 117.9 | 199 | 100 | 105 | 14 |
| HAN | Trichloroacetonitrile[a] | **TCAN** | 3.4 | 108 | 110 | 40 | 83 | 6 |
| HAN | Tribromoacetonitrile[b] | **TBAN** | 9.1 | 197.8 | 195.8 | 100 | 126 | 16 |
| HNM | Dichloronitromethane[b] | **DCNM** | 4.8 | 83 | 85 | 30 | 83 | 4 |
| HNM | Bromochloronitromethane[b] | **BCNM** | 6.6 | 129 | 127 | 100 | 111 | 14 |
| HNM | Dibromonitromethane[b] | **DBNM** | 8.5 | 172.8 | 171 | 100 | 110 | 16 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| HNM | Trichloronitromethane[a] | **TCNM** | 4.7 | 116.9 | 119 | 15 | 95 | 6 |
| HNM | Bromodichloronitromethane[b] | **BDCNM** | 6.7 | 163 | 161 | 100 | 85 | 24 |
| HNM | Dibromochloronitromethane[b] | **DBCNM** | 8.9 | 206.8 | 209 | 100 | 92 | 10 |
| HNM | Tribromonitromethane[b] | **TBNM** | 10.8 | 251 | 253 | 500 | 76 | 25 |
| HAM | Chloroacetamide[a] | **CAM** | 10.1 | 93 | 44 | 750 | 105 | 29 |
| HAM | Bromoacetamide[a] | **BAM** | 11.7 | 137 | 44 | 5000 | 108 | 7 |
| HAM | Iodoacetamide[a] | **IAM** | 14.1 | 185 | 58 | 750 | 106 | 3 |
| HAM | Dichloroacetamide[d] | **DCAM** | 12.0 | 44 | 127 | 100 | 101 | 5 |
| HAM | Bromochloroacetamide[b] | **BCAM** | 13.4 | 44 | 173 | 100 | 111 | 16 |
| HAM | Dibromoacetamide[b] | **DBAM** | 14.1 | 44 | 217 | 100 | 112 | 10 |
| HAM | Chloroiodoacetamide[b] | **CIAM** | 15.2 | 92 | 219 | 100 | 97 | 6 |
| HAM | Bromoiodoacetamide[b] | **BIAM** | 16.3 | 136 | 138 | 100 | 111 | 16 |
| HAM | Diiodoacetamide[b] | **DIAM** | 17.9 | 184 | 311 | 100 | 108 | 11 |
| HAM | Trichloroacetamide[a] | **TCAM** | 13.9 | 44 | 82 | 100 | 113 | 15 |
| HAM | Bromodichloroacetamide[b] | **BDCAM** | 15.2 | 44 | 128 | 100 | 97 | 7 |
| HAM | Dibromochloroacetamide[b] | **DBCAM** | 16.4 | 44 | 128 | 100 | 106 | 16 |
| HAM | Tribromoacetamide[b] | **TBAM** | 17.6 | 44 | 173 | 100 | 103 | 15 |

[a] Millipore-Sigma. [b] CanSyn Chem. Corp. [c] Aldlab Chemicals. [d] TCI America

**Table 1.2. DBP method information for triple quadrupole mass spectrometer**

| DBP Class | DBP name | Abbrev. | RT (min.) | MRM Transition Quant (m/z) | MRM Transition Qual (m/z) | LOQ Conc. (ng/L) | Avg.% Accuracy | %RSD |
|---|---|---|---|---|---|---|---|---|
| IAA | Iodoacetic acid[a] | **IAA** | 7.5 | 200>73 | 169>141 | 25 | 88 | 22 |
| IAA | Chloroiodoacetic acid[b] | **CIAA** | 9.7 | 234>79 | 234>107 | 25 | 90 | 15 |
| IAA | Bromoiodoacetic acid[b] | **BIAA** | 11.1 | 278>123 | 278>151 | 50 | 82 | 18 |
| IAA | Diiodoacetic acid[b] | **DIAA** | 12.9 | 326 >171 | 326>199 | 50 | 85 | 16 |
| HAA | Chloroacetic acid[a] | **CAA** | 5.3 | 108>76 | 77>49 | 100 | 112 | 7 |
| HAA | Bromoacetic acid[a] | **BAA** | 6.1 | 121>93 | 72>42 | 100 | 94 | 11 |
| HAA | Dichloroacetic acid[a] | **DCAA** | 6.2 | 83>48 | 76>48 | 100 | 87 | 14 |
| HAA | Bromochloroacetic acid[a] | **BCAA** | 7.1 | 157>129 | 129>48 | 100 | 95 | 15 |
| HAA | Dibromoacetic acid[a] | **DBAA** | 8.4 | 173>92 | 120>92 | 100 | 88 | 8 |
| HAA | Trichloroacetic acid[a] | **TCAA** | 7.0 | 117>82 | 141>113 | 100 | 84 | 13 |

**Table 1.3. Drinking water plant information and bulk parameters**

| Drinking Water Plant | Final Disinfectant | pH | TOC (mg/L) | Bromide (μg/L) | Iodide (μg/L) | Disinfectant Dose (mg/L) | Estimated Water Age in Distribution System (Days) | Estimated # of consumers |
|---|---|---|---|---|---|---|---|---|
| Plant 1 | $NH_2Cl$ | 7.8-8.2 | 11 | 28.1 | <10.0 | 3.5-4.0 | 1 | 300,000 |
| Plant 2 | $Cl_2$ | 8.43 | 18 | 19 | <10.0 | 2.0 | 3 | 17,360 |
| Plant 3 | $Cl_2$ | 7.3 | 4.2 | 55.2/38.1 | <10.0 | 3.3 | 6-7 | 400,000 |
| Plant 4 | $Cl_2$/ BCDMH[a] | 7.65 | <1.0 | 15.4 | <10.0 | 2.0 | 2-4 | 5,600 |

[a]1-bromo-3-chloro-5,5-dimethylhydantoin

**Table 1.4. Comparison of multiple liquid-liquid extractions (LLE) to solid phase extraction (SPE) for the haloacetamides.**

| DBP % Recovery | SPE | LLE-2 Extractions | LLE-3 Extractions |
|---|---|---|---|
| CAM | 1 | 5 | 8 |
| BAM | 0 | 10 | 14 |
| DCAM | 13 | 55 | 73 |
| BCAM | 23 | 50 | 65 |
| TCAM | 96 | 143 | 149 |
| IAM | 1 | 16 | 22 |
| DBAM | 40 | 47 | 60 |
| CIAM | 47 | 44 | 51 |
| BDCAM | 99 | 111 | 115 |
| BIAM | 84 | 50 | 57 |
| DBCAM | 105 | 104 | 104 |
| TBAM | 111 | 114 | 111 |
| DIAM | 122 | 62 | 62 |

**Table 1.5. Concentrations of DBPs at Plants 1-4 in ng/L.**

| Class | DBP | Plant 1 (ng/L) | Plant 1 Dist. (ng/L) | Plant 2 (ng/L) | Plant 2 Dist. (ng/L) | Plant 3 (ng/L) | Plant 4 (ng/L) |
|---|---|---|---|---|---|---|---|
| HAA | **TCM** | 3586 | 4571 | 9834 | 11339 | 2898 | 2332 |
| HAA | **BDCM** | 711 | 750 | 3814 | 5602 | 1748 | 532 |
| HAA | **DBCM** | 159 | 163 | 1209 | 2022 | 1078 | 1074 |
| HAA | **TBM** | 66 | 67 | 129 | 180 | 188 | 1073 |
| HAL | **TCAL** | 1171 | 1054 | 2792 | 4168 | 529 | 64 |
| HAL | **BDCAL** | | | | | | 155 |
| HAL | **DBCAL** | 40 | 41 | 130 | 22 | 110 | 93 |
| HK | **CP** | 17599 | 13742 | 1800 | 307 | 17755 | 61 |
| HK | **11DCP** | | | | | 1422 | |
| HK | **13DCP** | 1104 | 283 | | | 710 | |
| HK | **11DBP** | | | | | 99 | |
| HK | **111TCP** | 552 | 526 | 620 | 101 | 161 | |
| HK | **113TCP** | | | | | 137 | |
| HK | **1133TeCP** | | | | | 402 | |
| ITHM | **BCIM** | | | 100 | 117 | 59 | |
| HAN | **DCAN** | 901 | 858 | 959 | 404 | 631 | |
| HAN | **BCAN** | | | 515 | 337 | 514 | 147 |
| HAN | **DBAN** | | | | 113 | 128 | 402 |
| HAN | **TCAN** | 31 | 25 | 47 | 34 | | |
| HAN | **TBAN** | | | 181 | 170 | | |
| HNM | **TCNM** | 624 | 589 | 92 | 78 | 107 | |
| HNM | **BDCNM** | 328 | 258 | 257 | 241 | 420 | |
| HNM | **DBCNM** | 254 | 204 | 311 | 291 | 655 | |
| HNM | **TBNM** | | | | | 1097 | |
| HAM | **CAM** | | | | | | 2996 |
| HAM | **BCAM** | 138 | 131 | 0 | 1057 | 370 | 0 |
| HAM | **DBAM** | | | | 305 | 163 | 153 |
| HAM | **BDCAM** | | | 124 | 175 | | |
| HAM | **TBAM** | | | 86 | 91 | | |
| IAA | **IAA** | 76 | 78 | | 27 | 72 | |
| IAA | **CIAA** | 72 | 90 | 52 | 72 | 66 | |
| IAA | **BIAA** | | | <LOQ | <LOQ | <LOQ | <LOQ |
| HAA | **CAA** | 6190 | 7278 | 1683 | 1334 | 2783 | 192 |
| HAA | **BAA** | 564 | 530 | 339 | 195 | 868 | 296 |
| HAA | **DCAA** | 36339 | 38840 | 16978 | 9455 | 9376 | 947 |
| HAA | **BCAA** | 6734 | 7273 | 7968 | 4650 | 9625 | 873 |
| HAA | **DBAA** | 487 | 539 | 2097 | 1137 | 4035 | 1572 |
| HAA | **TCAA** | 3304 | 3863 | 5053 | 3966 | 1330 | <LOQ |
| | **Total** | 81030 | 81753 | 57169 | 47990 | 59533 | 12962 |

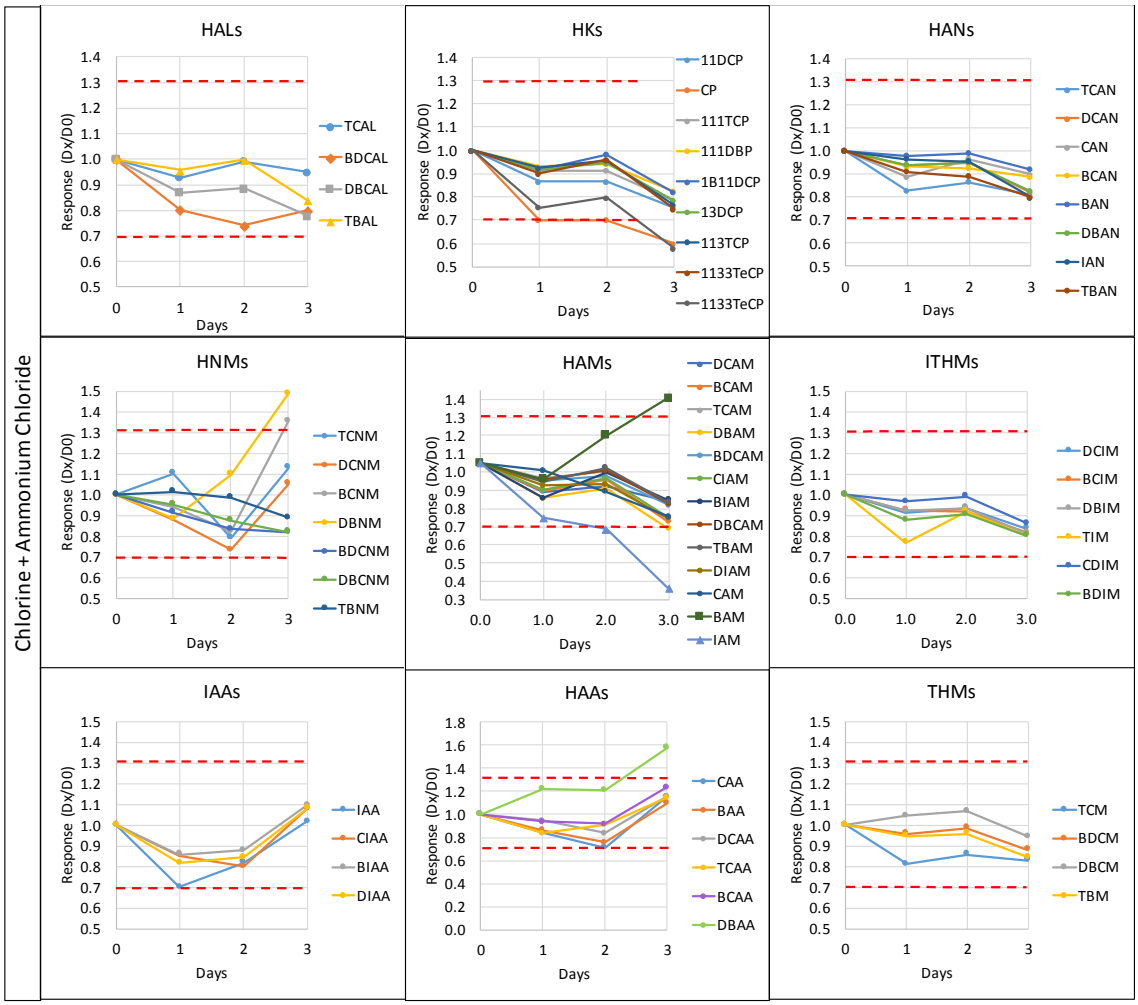


**Figure 1.1.** Holding study results for chlorinated water quenched with ammonium chloride.

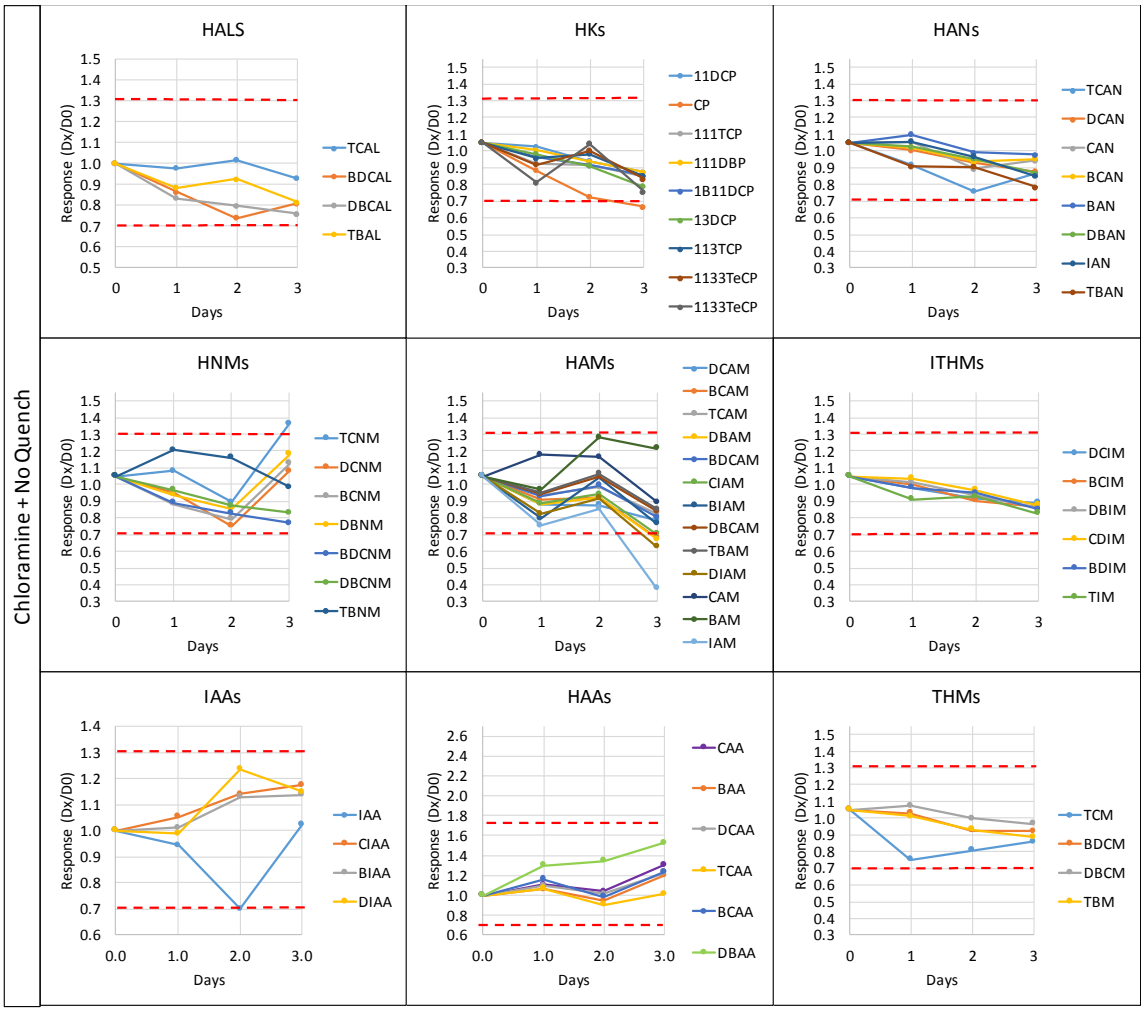


**Figure 1.2.** Holding study results from non-quenched chloraminated water.

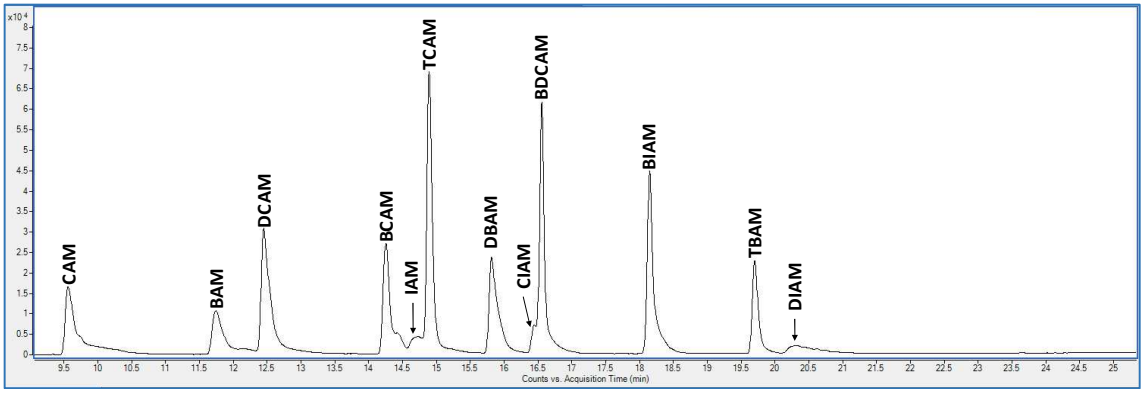


**Figure 1.3.** Chromatography of haloacetamides on a DB-5 column.

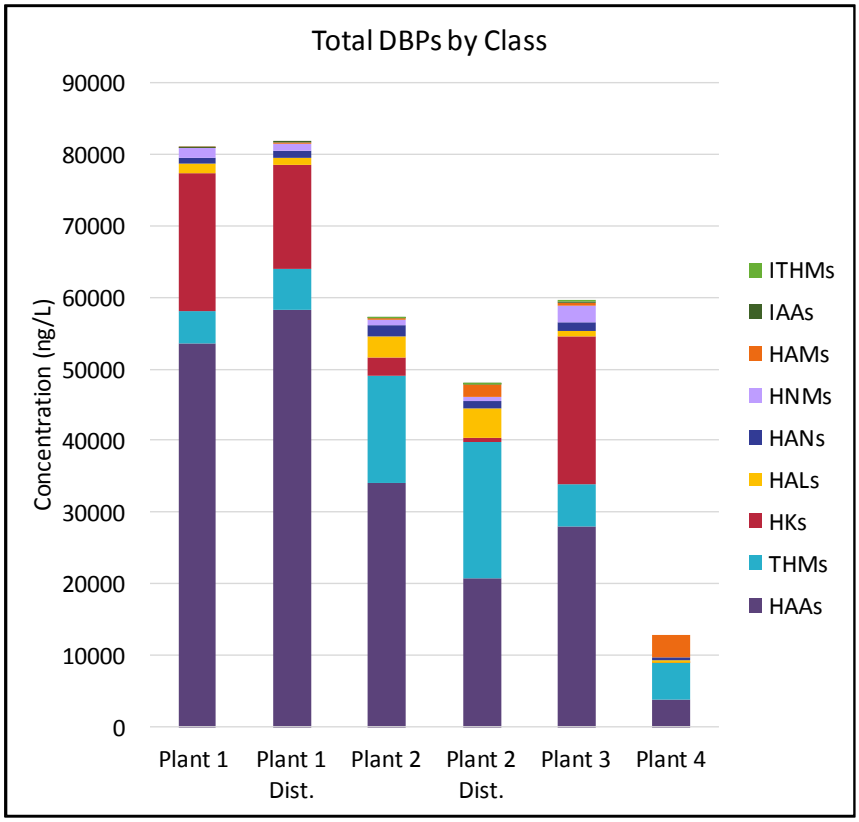


**Figure 1.4.** Drinking water Plants 1-4 total DBP concentrations (ng/L) by class.

**Table 1.6. Literature review for previous methods with minimum reporting levels (MRLs) in µg/L**

| Class | **DBP** | Weinberg et al., 2002, Krasner et al. 2006 | Bougeard et al., 2010 | Carter et al., 2019 | This Method |
|---|---|---|---|---|---|
| HAL | **TCAL** | 0.1 | | | 0.05 |
| HAL | **BDCAL** | | | | 0.10 |
| HAL | **DBCAL** | | | | 0.03 |
| HAL | **TBAL** | 0.1 | | | 0.10 |
| HAM | **CAM** | | | | 0.75 |
| HAM | **BAM** | | | | 2.50 |
| HAM | **DCAM** | | | 1.1 | 0.10 |
| HAM | **BCAM** | | | 1.2 | 0.10 |
| HAM | **TCAM** | | | | 0.10 |
| HAM | **IAM** | | | 1.4 | 0.75 |
| HAM | **DBAM** | | | 1.2 | 0.10 |
| HAM | **CIAM** | | | | 0.10 |
| HAM | **BDCAM** | | | 1.1 | 0.10 |
| HAM | **BIAM** | | | | 0.10 |
| HAM | **DBCAM** | | | 1.2 | 0.10 |
| HAM | **TBAM** | | | 1.0 | 0.10 |
| HAM | **DIAM** | | | | 0.10 |
| HAN | **TCAN** | 0.1 | 0.061 | 1.5 | 0.04 |
| HAN | **DCAN** | 0.1 | 0.057 | 1.6 | 0.03 |
| HAN | **CAN** | 0.1 | | 1.6 | 0.10 |
| HAN | **BCAN** | 0.1 | 0.07 | 1.3 | 0.10 |
| HAN | **BAN** | 0.1 | | 1.3 | 0.08 |
| HAN | **DBAN** | 0.1 | 0.041 | 1.1 | 0.10 |
| HAN | **IAN** | | | | 0.10 |
| HAN | **BDCAN** | | | 0.9 | 0.10 |

| Class | **DBP** | Weinberg et al., 2002, Krasner et al. 2006 | Bougeard et al., 2010 | Carter et al., 2019 | This Method |
|---|---|---|---|---|---|
| HAN | **DBCAN** | | | 0.8 | 0.10 |
| HAN | **TBAN** | 0.5 | | 0.7 | 0.05 |
| HK | **11DCP** | 0.1 | 0.086 | | 0.10 |
| HK | **CP** | 0.1 | | | 0.10 |
| HK | **111TCP** | 0.1 | 0.268 | | 0.10 |
| HK | **11DBP** | 0.5 | | | 0.05 |
| HK | **1B11DCP** | 0.1 | | | 0.10 |
| HK | **13DCP** | 0.1 | | | 0.10 |
| HK | **113TCP** | 0.1 | | | 0.03 |
| HK | **1133TeCP** | 0.1 | | | 0.10 |
| HK | **1133TeBP** | 0.5 | | | 0.10 |
| HNM | **TCNM** | 0.1 | 0.117 | 1.3 | 0.02 |
| HNM | **DCNM** | 0.1 | | 1.7 | 0.03 |
| HNM | **BCNM** | 0.1 | | 1.2 | 0.10 |
| HNM | **BDCNM** | | | 1.2 | 0.10 |
| HNM | **DBNM** | 0.1 | 0.178 | 1.1 | 0.10 |
| HNM | **DBCNM** | | | 0.5 | 0.10 |
| HNM | **TBNM** | | | 0.4 | 0.50 |
| ITHM | **DCIM** | 0.5 | 0.257 | | 0.05 |
| ITHM | **BCIM** | 5 | 0.324 | | 0.03 |
| ITHM | **DBIM** | 0.5 | | | 0.10 |
| ITHM | **CDIM** | 0.1 | | | 0.03 |
| ITHM | **BDIM** | 0.5 | | | 0.04 |
| ITHM | **TIM** | 2 | | | 0.10 |

**Table 1.7. Method detection limits (MDLs) for each compound, including instrument response and signal-to-noise ratio (SNR).**

| Class | DBP | MDL Conc. (ng/L) | Instrument Area | SNR |
|---|---|---|---|---|
| HAL | TCAL | 8 | 789 | 3 |
| HAL | BDCAL | 8 | 499 | 3 |
| HAL | DBCAL | 10 | 893 | 6 |
| HAL | TBAL | 10 | 712 | 8 |
| HAM | CAM | 300 | 1142 | 3 |
| HAM | BAM | 2000 | 2398 | 3 |
| HAM | DCAM | 50 | 8199 | 3 |
| HAM | BCAM | 40 | 675 | 6 |
| HAM | TCAM | 8 | 5208 | 4 |
| HAM | IAM | 500 | 4204 | 3 |
| HAM | DBAM | 50 | 10700 | 3 |
| HAM | CIAM | 50 | 3752 | 3 |
| HAM | BDCAM | 30 | 10980 | 5 |
| HAM | BIAM | 100 | 5385 | 3 |
| HAM | DBCAM | 30 | 9478 | 6 |
| HAM | TBAM | 40 | 33546 | 3 |
| HAM | DIAM | 40 | 965 | 3 |
| HAN | TCAN | 8 | 566 | 5 |
| HAN | DCAN | 2 | 1544 | 3 |
| HAN | CAN | 30 | 1555 | 3 |
| HAN | BCAN | 10 | 1713 | 3 |
| HAN | BAN | 30 | 1472 | 3 |
| HAN | DBAN | 10 | 1076 | 4 |
| HAN | IAN | 10 | 1083 | 3 |
| HAN | TBAN | 100 | 536 | 14 |
| HK | 11DCP | 8 | 3189 | 3 |
| HK | CP | 75 | 602 | 3 |
| HK | 111TCP | 10 | 589 | 3 |
| HK | 11DBP | 18 | 4108 | 3 |
| HK | 1B11DCP | 18 | 2203 | 7 |
| HK | 13DCP | 10 | 4422 | 3 |
| HK | 113TCP | 10 | 2967 | 3 |
| HK | 1133TeCP | 10 | 1164 | 4 |
| HK | 1133TeBP | 18 | 558 | 4 |
| HNM | TCNM | 8 | 503 | 4 |
| HNM | DCNM | 8 | 1491 | 3 |
| HNM | BCNM | 10 | 714 | 4 |
| HNM | BDCNM | 100 | 310 | 4 |
| HNM | DBNM | 8 | 644 | 7 |
| I-THM | DCIM | 40 | 1218 | 3 |
| I-THM | BCIM | 10 | 925 | 3 |
| I-THM | DBIM | 4 | 1626 | 9 |
| I-THM | CDIM | 10 | 934 | 6 |
| I-THM | BDIM | 10 | 738 | 3 |
| I-THM | TIM | 18 | 688 | 3 |

**Table 1.8. Plant 1 ($NH_2Cl$) finished drinking water quantified DBPs with standard deviation and percent relative standard deviation**

| Class | DBP | Conc. (ng/L) | %RSD |
|---|---|---|---|
| HAL | TCAL | 1171 | 0.2 |
| HAL | DBCAL | 40 | 1.8 |
| HAM | BCAM | 138 | 11.2 |
| HAN | DCAN | 901 | 4.6 |
| HAN | TCAN | 31 | 13.1 |
| HK | CP | 17599 | 10.7 |
| HK | 13DCP | 1104 | 12.7 |
| HK | 111TCP | 552 | 4.2 |
| HNM | TCNM | 624 | 3.3 |
| HNM | BDCNM | 328 | 0.5 |
| HNM | DBCNM | 254 | 0.7 |
| IAA | IAA | 76 | 32.9 |
| IAA | CIAA | 72 | 67.5 |
| IAA | BIAA | <LOQ | NA |
| HAA | CAA | 6190 | 19.1 |
| HAA | BAA | 564 | 30.1 |
| HAA | DCAA | 36339 | 27.7 |
| HAA | DBAA | 487 | 45.5 |
| HAA | BCAA | 6734 | 29.7 |
| HAA | TCAA | 3304 | 25.2 |
| THM | TCM | 3586 | 27.1 |
| THM | BDCM | 711 | 10.2 |
| THM | DBCM | 159 | 5.8 |
| THM | TBM | 66 | 0.6 |

**Table 1.9. Plant 1 ($NH_2Cl$) random distribution sample quantified DBPs with standard deviation and percent relative standard deviation**

| Class | DBP | Conc. (ng/L) | %RSD |
|---|---|---|---|
| HAL | TCAL | 1054 | 1.1 |
| HAL | DBCAL | 41 | 2.7 |
| HAM | BCAM | 131 | 7.8 |
| HAN | DCAN | 858 | 3.5 |
| HAN | TCAN | 25 | 3.4 |
| HK | CP | 13742 | 11.6 |
| HK | 13DCP | 283 | 26.1 |
| HK | 111TCP | 526 | 5.7 |
| HNM | TCNM | 589 | 5.8 |
| HNM | BDCNM | 258 | 1.0 |
| HNM | DBCNM | 204 | 2.2 |
| IAA | IAA | 78 | 9.1 |
| IAA | CIAA | 90 | 11.0 |
| IAA | BIAA | <LOQ | NA |
| HAA | CAA | 7278 | 0.8 |
| HAA | BAA | 530 | 3.7 |
| HAA | DCAA | 38840 | 1.7 |
| HAA | DBAA | 539 | 16.0 |
| HAA | BCAA | 7273 | 0.6 |
| HAA | TCAA | 3863 | 5.6 |
| THM | TCM | 4571 | 1.8 |
| THM | BDCM | 750 | 0.2 |
| THM | DBCM | 163 | 1.1 |
| THM | TBM | 67 | 0.2 |

**Table 1.10. Plant 2 ($Cl_2$) finished water quantified DBPs with standard deviation and percent relative standard deviation**

| Class | DBP | Conc. (ng/L) | %RSD |
|---|---|---|---|
| HAL | TCAL | 2792 | 3.3 |
| HAL | DBCAL | 130 | 0.3 |
| HAM | TBAM | 86 | 8.3 |
| HAM | BDCAM | 124 | 9.4 |
| HAN | DCAN | 959 | 1.1 |
| HAN | BCAN | 515 | 0.1 |
| HAN | TBAN | 181 | 1.6 |
| HAN | TCAN | 47 | 2.9 |
| HK | CP | 1800 | 3.6 |
| HK | 111TCP | 620 | 1.5 |
| HNM | TCNM | 92 | 1.8 |
| HNM | BDCNM | 257 | 5.0 |
| HNM | DBCNM | 311 | 13.6 |
| I-THM | BCIM | 100 | 1.3 |
| IAA | CIAA | 52 | NA |
| IAA | BIAA | <LOQ | NA |
| HAA | CAA | 1683 | NA |
| HAA | BAA | 339 | NA |
| HAA | DCAA | 16978 | NA |
| HAA | DBAA | 2097 | NA |
| HAA | BCAA | 7968 | NA |
| HAA | TCAA | 5053 | NA |
| THM | TCM | 9834 | NA |
| THM | BDCM | 3814 | NA |
| THM | DBCM | 1209 | NA |
| THM | TBM | 129 | NA |

**Table 1.11. Plant 2 ($Cl_2$) random distribution sample quantified DBPs with standard deviation and percent relative standard deviation**

| Class | DBP | Conc. (ng/L) | %RSD |
|---|---|---|---|
| HAL | TCAL | 4168 | 0.3 |
| HAL | DBCAL | 22 | 1.4 |
| HAM | BCAM | 1057 | 9.2 |
| HAM | DBAM | 305 | 21.4 |
| HAM | BDCAM | 175 | 1.7 |
| HAM | TBAM | 91 | 5.8 |
| HAN | DCAN | 404 | 5.1 |
| HAN | DBAN | 113 | 24.8 |
| HAN | BCAN | 337 | 12.3 |
| HAN | TBAN | 170 | 1.4 |
| HAN | TCAN | 34 | 14.8 |
| HK | CP | 307 | 9.2 |
| HK | 111TCP | 101 | 12.1 |
| HNM | TCNM | 78 | 16.3 |
| HNM | BDCNM | 241 | 8.6 |
| HNM | DBCNM | 291 | 6.9 |
| I-THM | BCIM | 117 | 12.6 |
| IAA | IAA | 27 | 45.1 |
| IAA | CIAA | 72 | 29.1 |
| IAA | BIAA | <LOQ | NA |
| HAA | CAA | 1334 | 1.4 |
| HAA | BAA | 195 | 26.6 |
| HAA | DCAA | 9455 | 26.8 |
| HAA | DBAA | 1137 | 46.3 |
| HAA | BCAA | 4650 | 23.5 |
| HAA | TCAA | 3966 | 17.5 |
| THM | TCM | 11339 | 32.5 |
| THM | BDCM | 5602 | 13.4 |
| THM | DBCM | 2022 | 4.3 |
| THM | TBM | 180 | 0.2 |

**Table 1.12. Plant 3 ($Cl_2$) finished water quantified DBPs with standard deviation and percent relative standard deviation**

| Class | DBP | Conc. (ng/L) | %RSD |
|---|---|---|---|
| HAL | TCAL | 529 | 7.1 |
| HAL | DBCAL | 110 | 5.1 |
| HAM | BCAM | 370 | 15.5 |
| HAM | DBAM | 163 | 4.3 |
| HAN | DCAN | 631 | 6.6 |
| HAN | BCAN | 514 | 1.2 |
| HAN | DBAN | 128 | 0.6 |
| HK | CP | 17755 | 12.1 |
| HK | 11DCP | 1422 | 10.4 |
| HK | 13DCP | 710 | 6.7 |
| HK | 11DBP | 99 | 3.6 |
| HK | 111TCP | 161 | 14.2 |
| HK | 113TCP | 137 | 28.9 |
| HK | 1133TeCP | 402 | 10.9 |
| HNM | TCNM | 107 | 11.3 |
| HNM | BDCNM | 420 | 3.4 |
| HNM | DBCNM | 655 | 9.5 |
| HNM | TBNM | 1097 | 3.2 |
| I-THM | BCIM | 59 | 7.2 |
| IAA | IAA | 72 | 119.9 |
| IAA | CIAA | 66 | 4.5 |
| IAA | BIAA | <LOQ | NA |
| HAA | CAA | 2783 | 15.7 |
| HAA | BAA | 868 | 7.9 |
| HAA | DCAA | 9376 | 3.7 |
| HAA | DBAA | 4035 | 4.3 |
| HAA | BCAA | 9625 | 1.7 |
| HAA | TCAA | 1330 | 12.2 |
| THM | TCM | 2898 | 3.3 |
| THM | BDCM | 1748 | 5.8 |
| THM | DBCM | 1078 | 3.0 |
| THM | TBM | 188 | 0.7 |

**Table 1.13. Plant 4 finished water quantified DBPs with standard deviation and percent relative standard deviation**

| Class | DBP | Conc. (ng/L) | % RSD |
|---|---|---|---|
| HAL | BDCAL | 155 | 11.4 |
| HAL | DBCAL | 93 | 6.1 |
| HAL | TCAL | 64 | 8.2 |
| HAM | CAM | 2996 | 6.4 |
| HAM | DBAM | 153 | 10.6 |
| HAN | BCAN | 147 | 8.9 |
| HAN | DBAN | 402 | 10.0 |
| HK | CP | 61 | 3.9 |
| IAA | BIAA | <LOQ | NA |
| HAA | CAA | 192 | 46.8 |
| HAA | BAA | 296 | 9.3 |
| HAA | DCAA | 947 | 18.7 |
| HAA | DBAA | 1572 | 4.5 |
| HAA | BCAA | 873 | 3.6 |
| HAA | TCAA | <LOQ | NA |
| THM | TCM | 2332 | 0.6 |
| THM | BDCM | 532 | 12.5 |
| THM | DBCM | 1074 | 6.0 |
| THM | TBM | 1073 | 3.5 |

# CHAPTER 2

# A NOVEL AUTOMATED METHOD FOR THE QUANTIFICATION OF HALOBENZOQUINONES USING ONLINE SPE COUPLED WITH LC-MS/MS[2]

[2] Amy A. Cuthbertson, Cristina Bach, Xavier Dauchy, Susan D. Richardson. To be submitted to *Journal of Chromatography A*.

**Abstract**

When assessing occurrence and exposure to toxic chemicals in drinking water, developing methods that are sensitive and efficient is paramount. A new method was developed for the quantification of 10 halobenzoquinones (HBQs), a class of disinfection by-products (DBPs) in drinking water which have been shown to be more toxic than most regulated DBPs. This method uses a small sample volume with online solid phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Previous methods used offline SPE coupled with standard addition, and a pre-concentration step. This requires larger volumes of sample, solvent, and standards. The current method has comparable limits of detection, requires minimal sample preparation, and analysis is almost entirely automated. A stability test was conducted over a week period with different disinfectant quenchers including ascorbic acid, sodium thiosulfate, and formic acid. Method optimization included source temperature, SPE size and type, sample volume, and SPE loading time. This method was validated using drinking water collected from four different drinking water plants in France, with spike recoveries of HBQs between 70-100%, and reports the highest concentration of 2,6-dibromo-1,4-benzoquinone ever reported in drinking water (254 ng/L).

## 1. Introduction

An unintended consequence of chemically disinfecting drinking water is the formation of disinfection by-products (DBPs) that form by the reaction of chemical oxidants with organic matter and inorganic constituents of source water (bromide, iodide, etc.) [1-3]. Several epidemiologic studies have found an association with consumption of chlorinated drinking water to negative health consequences, including bladder cancer,

colon cancer, and adverse reproductive outcomes [4-12]. To date, known quantified DBPs only account for less than 50% of the total halogenated material in drinking water, while much of the halogenated DBPs remains unknown [11, 13].

Halobenzoquinones (HBQs) were first identified as a new class of halogenated DBP in 2010 [14], and four of these have been detected in drinking water, including 2,6-dichloro-1,4-benzoquinone (26DCBQ), 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ), 2,3,6-trichloro-1,4-benzoquinone (TriCBQ), and 2,6-dibromo-1,4-benzoquinone (26DBBQ) [15, 16]. It was later discovered that HBQs undergo hydrolysis in water to form halo-hydroxyl-benzoquinones (OH-HBQs), which are the more stable form of HBQ DBPs in chlorinated drinking water [17]. These reactions occur within 12-24 hours of sampling chlorinated drinking water. 2,6-Dichloro-hydroxyl-1,4-benzoquinone (OH-DCBQ) was the most commonly identified of the four OH-HBQs, which is also consistent with DCBQ being the most commonly detected HBQ [16, 17]. OH-HBQs are less toxic than their HBQ counterparts, but have shown to induce greater cytotoxicity and/or greater developmental toxicity than most regulated DBPs [18]. Recent studies have shown that regulated trihalomethanes (THMs) and haloacetic acids (HAAs) are the dominant DBP classes formed upon chlorination, but are not necessarily drivers of toxicity [19-21]. Currently, there are no OH-HBQ standards available, and therefore this method focuses on HBQs.

Early analytical techniques for identifying HBQs include ultraviolet-visible (UV-vis) spectroscopy, which was used to monitor the transformation of HBQs . Electron spin resonance (ESR) spectroscopy confirmed the formation of hydroxyl free radicals ($OH^{\bullet}$) from the reaction of HBQs with $H_2O_2$, which is considered to be an important route of

HBQs' toxicity. HBQs have also been analyzed using gas chromatography (GC)-mass spectrometry (MS) with electron ionization (EI) and chemical ionization (CI). Some non-volatile, and/or thermally unstable HBQs and their benzoquinone analogues have been derivatized to be analyzed using GC-MS, and have also been analyzed using a programmed temperature vaporization (PTV)-GC-MS method with large volume injection [16]. While the use of GC-MS for the analysis of HBQs is promising, the most widely used and most sensitive technique to-date utilizes standard addition with solid phase extraction (SPE) coupled with liquid chromatography (LC)-tandem mass spectrometry (MS/MS) with electrospray ionization (ESI). This method quantifies twelve HBQs, and four OH-HBQs by addressing LC-MS matrix interferences with standard addition and using pre-concentration with nitrogen to increase sensitivity. This resulted in lowest limits of detection (LODs) of 0.03-8.7 ng/L for sixteen HBQs. However, this method requires a calibration curve to be made for each sample, resulting in low sample throughput. It is important to note that LODs are lower than limits of quantification (LOQs), and minimum reporting levels (MRLs) must be at or above the LOQs. These distinctions are especially important for regulatory laboratories.

The need for an analysis that is cost-effective and easy to implement is important for laboratories conducting occurrence studies, particularly on the international level. We developed a method utilizing large-volume injection online SPE coupled to LC-MS/MS with ESI. This method is almost entirely automated and only requires 5 mL of sample acidified with 0.1% formic acid (FA). Method optimization included source temperature, sample volume, sample loop size, SPE cartridge type and size, and loading time on the SPE cartridges. LOQs were validated in bottled drinking water, and were between 2.5

ng/L and 1.0 µg/L for ten HBQs. The method was validated by measuring HBQs in water collected from four drinking water plants located in France.

## 2. Experimental

### *2.1. Chemicals and reagents*

2,5-Dichloro-1,4-benzoquinone (25DCBQ) (98%); 2,3,5,6-tetrachloro-1,4-benzoquinone (TetraCBQ) (99%); and 3,4,5,6-tetrachloro-1,2-benzoquinone (TetraC12BQ) (97%) were purchased from Sigma-Aldrich (St. Louis, MO). 2,6-Dichloro-1,4-benzoquinone (26DCBQ) (98%) was purchased from Carbosynth (Berkshire, U.K.). 2,3,5,6-Tetrabromo-1,4-benzoquinone (TetraBBQ); 2,5-dibromo-1,4-benzoquinone (25DBBQ); 2,6-dibromo-1,4-benzoquinone (26DBBQ); 3,4,5,6-tetrabromo-1,2-benzoquinone (TetraB12BQ); 2,6-dibromo-5,6-dimethyl-1,4-benzoquinone (26DBDMBQ); and 2,6-dibromo-3-chloro-5-methyl-1,4-benzoquinone (26DBCMBQ) were purchased from Sigma-Aldrich$^{CPR}$ (St. Louis, MO) and did not have verified purity. Compounds without verified purity were calculated with an assumption of 100% purity.

### *2.2. Preparation of stock solutions and samples*

Stock solutions were prepared by dissolving HBQs in acetonitrile in the range of 500-5000 mg/mL. A working solution was prepared with concentrations between 1-100 mg/mL for all HBQs, which was used for subsequent dilution into water for calibration. Standard mixes were made monthly and monitored for changes in sensitivity. Calibration curves were made using bottled water with 0.1% formic acid.

*2.3. Instrumental analysis*

LC-MS/MS analysis was performed using a UFLC XR (Shimadzu Corp., Kyoto, Japan) coupled to an SCIEX Qtrap® 5500 mass spectrometer (SCIEX, Framingham, MA, USA) using a Uptisphere® Strategy C18 100 Å reversed phase (3.0 x 50 mm, 2.6 µm) analytical column by Interchim® (Montluçon, France) which was maintained at 30°C. The CTC Pal autosampler (Eksigent, Dublin, CA, USA) temperature was set at 10°C and was coupled to Oasis® HLB dual online solid phase extraction (SPE) columns (2.1 x 20 mm x 25 µm) (Waters Corporation, Milford, MA, USA). The LC instrument was equipped with three LC-20AD pumps (A, B, and C). The pumps A and B delivered solvents onto the chromatographic column and pump C was used for SPE extraction. SPE and LC steps were done online using a six-port switching valve. The mobile phase consisted of 0.1% formic acid in water (A & C) and 0.1% formic acid in acetonitrile (B).

Water samples (4 mL) were carried from the sample loop into the SPE column with water and 0.1% formic acid at 1 mL/min for 12 minutes (3 times the volume of the sample loop). The LC gradient then passed through the SPE cartridge into the mass spectrometer with the following program: 0 min, 20% B; 40 min, 90% B (hold for 5 min); 20% B, 45.1 min (hold for 5 min). The flow rate of the mobile phase was 300 µL/min. To prevent cross-contamination, the syringe and the sample loop were flushed twice with 5 mL of a solvent mixture (ultrapure LC-MS water 25%, acetonitrile 25%, methanol 25%, isopropanol 25%) and then twice with 5 mL of ULC-MS.The MS system was operated using ESI in negative mode. Nitrogen was used as a nebulizer, heater, curtain gas (CUR), and collision activation dissociation (CAD) gas. MS conditions were

as following: CUR 30 psi; collision CAD, high; ion spray voltage (IS), 4500 V; heated nebulizer temperature, 400 °C; ion source gas 1, 40 psi; and ion source gas 2, 40 psi. Two or three multiple reaction monitoring (MRM) transitions were chosen for each analyte, with one MRM transition chosen for quantification. MRM transitions, retention times, and conditions are shown in Table 2.1. Data was processed using SCIEX MultiQuant 3.0 software

*2.4. Sample quenching and preservation*

A stability study was conducted over a 5-day period with the quenching agents ascorbic acid, sodium thiosulfate, and formic acid in chlorinated tap water. A control experiment with tap water and no quenching agent was also conducted. Quenching agents were spiked into samples at a 1.3:1 molar ratio of quenching agent to free chlorine. Concentrations were based on a worst-case scenario of 4 mg/L free chlorine. The experiment was conducted in triplicate in tap water. Blanks monitored included tap water alone and tap water plus respective quencher (ascorbic acid, sodium thiosulfate, or formic acid). A control in non-chlorinated bottled water was also monitored. HBQ stability was monitored using peak areas with the respective quantifying MRM transitions. A ratio of areas from the initial peak area was calculated. Days monitored include 0, 1, 2, and 5 days (Figure 2.1).

*2.5. Method Validation*

Analytical method parameters were validated according to performance criteria established by the European Commission (SANTE/11813/2017). Briefly, calibration ranges were verified to have less than 20% error between three separate calibration curves' calculated concentrations. Limits of quantification (LOQs) were determined by 6

replicates at 3-5 times the method detection limits (MDLs) which were determined by area counts greater than 1000. Signal to noise ratio (SNR) was deemed to be an inappropriate measure on the AB SCIEX Qtrap® 5500 mass spectrometer because SNR was calculated using the average noise across the entire chromatogram, as opposed to a small window next to the analyte peak. Because of this, LOQs used in this method are conservative and highly reproducible. LOQs were required to have less than 20% error between replicates and were verified to have 10 times the area of any blank interference. The coefficient of determination ($R^2$) values for calibration curves were required to be a minimum of 0.990 with a quadratic regression line weighted with the inverse concentration (1/X). Calibration curves and LOQs were made and determined using Evian bottled water. These results are summarized in Table 2.2.

*2.6. Quality assurance and quality control*

Calibrations were verified by mid-point calibration verification before and after each sample batch, not exceeding 12 hours. Calibration verification could not exceed 20% relative standard deviation of the mid-point of the calibration curve. A method blank was analyzed to verify that blank interferences were at least 10 times lower than the LOQ.

*2.7. Sample collection and method validation*

Samples were collected from four chlorinated drinking water plants in France and included a raw untreated influent sample and a finished drinking water sample. Samples were collected in borosilicate amber glass bottles with no headspace, and acidified with 0.1% formic acid. Samples were analyzed for HBQs within 48 hours of sample

collection. Sample information including source water type, final disinfectant type, contact time, temperature, conductivity, residual chlorine concentrations, and total organic carbon (TOC) can be found in Table 2.3.

## 3. Results and Discussion

### *3.1. Standard Purity*

Although no other peaks were observed by LC-MS when compounds were analyzed individually, it is possible that compounds that did not have verified purity were not 100% pure (25DBBQ, 26DBBQ, 26DBDMBQ, 26DBCMBQ, TetraBBQ, and TetraB12BQ). Purity can impact calculations for LOQs and have subsequent impacts on the calculations in real samples. If standards are significantly less pure (50% or less), LOQ calculations can be significantly lower, and calculated concentrations in real samples would be over-estimated. For example, if a standard that was originally calculated to have 100% purity, but was discovered to have a purity of only 50%, the concentration in a real sample would be half of what was originally reported. These calculations can have significant impact on occurrence and exposure data. It is highly suggested to verify the purity of standards by nuclear magnetic resonance (NMR) spectroscopy when available, in conjunction with gas and liquid chromatography-mass spectrometry.

### *3.2. Instrumental Optimization*

#### *3.2.1. Source temperature*

Peak areas were monitored for HBQs directly injected into the MS source at 200, 400, and 500°C (Figure 2.2). All compounds showed optimal performance, or the highest peak areas, at a source temperature of 400°C, except 26DBDMBQ. Previous published methods have used a source temperature of 450°C [17], and as high as 700°C [22]. It is likely that 26DBDMBQ would have improved sensitivity at a higher temperatures, but as seen in Figure 2.2, all other HBQs decrease in sensitivity at 500°C.

#### *3.2.2. On-line SPE optimization*

Selection of the online SPE cartridge is essential to analysis, and important factors to consider are stationary phase selection and cartridge length (capacity). Three SPE cartridge types were tested by comparing corresponding peak areas for each HBQ: C18 online SPE columns (2.1 x 20 mm x 25 μm) and Oasis HLB SPE columns at two different lengths (2.1 x 20 mm x 25 μm, and 2.1 x 40 mm x 25 μm) (Waters Corporation, Milford, MA, USA). The Oasis HLB SPE cartridge showed improved sensitivity over the C18 SPE cartridge, but sensitivity was not significantly improved using an Oasis HLB SPE cartridge of longer length. For trace analysis of HBQs, concentrations are very low, and with relatively clean matrices such as drinking water, a longer SPE cartridge with higher capacity may not be necessary. For more complex matrices such as wastewater, a longer/higher capacity column may prove to be beneficial, but would require additional optimization.

Once an SPE cartridge type and length was selected, optimization of loading flow rate and charge time was conducted in scanning mode, to monitor compounds with shifting retention times. The loading flow rate into the SPE cartridge impacts adsorption efficiency, and too high of a flow rate can decrease sensitivity. Charge time refers to the amount of time flow is allowed through the column, and must be optimized by the length of the column. We conducted experiments with different sample volumes, flow rates, and charge times (Figure 2.3). This required adding a larger sample loop and syringe onto the online SPE autosampler. It was found that optimization of HBQ peak areas occurred with a 4.2 mL volume sample at a flow rate of 1 mL/min, and a charge time of 11.2 minutes, which results in a loading volume of approximately three times the sample loop volume. While it appears that a charge time of 16 minutes has the highest overall instrument response, too long of a charge time can negatively impact more polar compounds because they will start to desorb off the SPE cartridge. For example, 26DBDMBQ, 23DBDMBQ, and 26DBCDMBQ had 10 times lower instrumental responses with increased charge time to 16 minutes (Figure 2.3).

With dual online SPE, as one sample is being loaded onto one cartridge, the other cartridge is being eluted onto the LC column. Each sample rotates back-and-forth between two different cartridges. Ideally, the two cartridges would have minimal error between them, but practically, to minimize error, it is suggested to run two calibration curves in duplicate for each cartridge and to apply each curve to the samples run on each specific cartridge. This should significantly reduce standard error between sample replicates.

Since the same cartridges are being used repeatedly, carryover can be a concern. It is essential to verify carryover with the highest calibration point, and show that carryover is at least ten times lower than the LOQ. MRLs must be adjusted to reflect this rule, and may have to be raised in order to meet these criteria. Any samples that have positive results above the highest calibration concentration must be diluted and re-analyzed, and any samples run after this sample on the same cartridge must be re-analyzed. Otherwise, false positives may occur.

*3.3. Stability study with quenching agents*

A stability study was conducted over a 5-day period with the quenching agents ascorbic acid, sodium thiosulfate, and formic acid in chlorinated tap water. A control experiment in bottled spring water, and chlorinated tap water with no quenching agent was also conducted. The results of this holding study can be found in Figure 2.1. In bottled spring water, half of the HBQs were stable over a 48-hour period, with peak areas not decreasing by more than 30%. Compounds that degraded by more than 30% in bottled spring water by 24 hours included 25DCBQ, 26DCBQ, 25DBBQ, 26DBBQ, and TetraCBQ. These compounds appear to be undergoing hydrolysis reactions, since no disinfectant or reducing agent was present. It is likely that there is transformation of 25DCBQ, 26DCBQ, 25DBQ, and 26DBBQ to their hydroxyl analogues. In non-quenched chlorinated drinking water with a chlorine residual of 0.2 mg/L, these same compounds degrade much more rapidly, to less than 15% or undetectable within 24 hours. Chlorine oxidation can catalyze the transformation of HBQs to OH-HBQs. Figure 2.4(A), shows the degradation of 26DBBQ and the subsequent formation of OH-DBBQ

in chlorinated drinking water at neutral pH. It was found that sodium thiosulfate degraded compounds 25DCBQ, 25DCBQ, TetraCBQ, 25DBBQ, 26DBBQ, and TetraBBQ to below detection limits within 5 hours of contact. Sodium thiosulfate is a powerful reducing agent, so it is likely degrading several of these compounds. Compounds that were stable with sodium thiosulfate after 24 hours were 26DBCMBQ, TetraC12BQ, and TetraB12BQ, with the latter stable after 48 hours. These same compounds were stable with an ascorbic acid quench after 5-days, except 26DBCMBQ, which degraded by 30% within 48 hours. 26DBDMBQ, TetraC12BQ, and TetraB12BQ were stable in chlorinated drinking water over a 5-day period, with no increases in concentration over this time frame. They were also stable with ascorbic acid and sodium thiosulfate, although TetraB12BQ increased approximately 40% with both quenchers within 24 hours. 26DBCMBQ was stable in chlorinated drinking water for 48 hours, then reduced by 60% by day 5. 26DBCMBQ, 26DBDMBQ, TetraC12BQ, and TetraB12BQ show significantly less capacity for oxidation or reduction. Tetra-HBQs are less electron rich, due to the effects of electron withdrawing groups, and steric hindrance from bulky groups likely slows hydrolysis reactions [23]. In Figure 2.4 (B), chlorinated water acidified with 0.1% formic acid stabilizes 26DBBQ and limits the formation of OH-DBBQ over a 48-hour period. This is consistent with previous literature showing that HBQs are stable for longer periods in water at lower pH [15]. At pH 2, HOCl is the dominant form of chlorine, and is less reactive generally than $OCl^-$. It should be noted that the formation of OH-HBQs occurs rather rapidly (Figure 2.4), and therefore direct exposure would likely be in the OH-HBQ form for DCBQ, and DBBQ.

*3.4. Analytical performance and validation of the method*

Sample results for Plants 1-4 can be found in Table 2.4. For all four plants, spike recoveries for each HBQ averaged between 70-111% (Table 2.5). No HBQs were measured in the raw untreated source water. Only two HBQs, 26DCBQ and 26DBBQ, were measured out of the ten HBQs analyzed in the finished drinking waters. These two compounds are the most commonly measured of all HBQs reported in environmental samples [16]. Interestingly, 26DCBQ is the most commonly measured compound in the literature, but was only found in Plants 1 and 4 in this study, whereas 26DBBQ is less common measured, but was found in all four plants. This study reports the highest concentration of 26DBBQ ever reported (254.3 ng/L). This may be due to lower chlorine residuals, which were between 0.35-0.8 mg/L as free $Cl_2$. Chlorine residuals are regulated at lower concentrations in France compared to Canada and the U.S. where the other occurrence studies were conducted. In the U.S. and Canada, chlorine residuals can be as high as 4 mg/L, the regulatory limit. Bromide-to-HOCl ratios play an important role in bromine incorporation for other DBPs studied, and it has been shown that brominated THMs and HAAs increased substantially with increasing initial bromide-to-chlorine consumption ratios [24-26]. Brominated DBPs are generally more toxic than chlorinated DBPs [27], and in this case, 26DBBQ is more cytotoxic than 26DCBQ in Chinese Hamster Ovary (CHO) cells [17]. Sample duplicates had percent relative standard deviations between 8-27%, highlighting the reproducibility of this method in drinking water. Other sample matrices, such as swimming pool water and wastewater would need to be validated, and it is likely that LOQs for most HBQs will be higher in more complex matrices.

## 4. Conclusions

This new automated online SPE-LC-MS/MS method allowed rapid measurement of ten HBQ species that can be formed in chlorinated drinking water, with LOQs as low as 2.5 ng/L, comparable to detection limits in previous reported methods, especially for the most commonly measured HBQs, DCBQ and DBBQ. This is a completely automated method, which saves a tremendous amount of labor and time, and increases sample throughput. This is particularly important for regulatory agencies interested in conducting occurrence studies on these compounds. Samples were validated using stringent regulatory compliance set by the European Commission, France. This resulted in conservative LOQs that are highly reproducible. The highest recorded concentration of 26DBBQ found to-date is reported here for a chlorinated drinking water sample from France.

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**Table 2.1. HBQ abbreviations, multiple reaction monitoring (MRM) transitions for quantification and qualification, retention times, and collision energies**

| Compounds | Q1 Mass (Da) | Q3 Mass (Da) | Declustering Potential (volts) | Entrance Potential (volts) | Collision Energy (volts) | Collision Cell Exit Potential (volts) |
|---|---|---|---|---|---|---|
| 2,5-DCBQ-1 | 176.868 | 112.800 | -95.000 | -10 | -24.000 | -15.000 |
| 2,5-DCBQ-2 | 176.868 | 35.000 | -95.000 | -10 | -28.000 | -15.000 |
| 2,5-DCBQ-3 | 176.868 | 140.900 | -95.000 | -10 | -16.000 | -9.000 |
| 2 6-DCBQ-1 | 176.888 | 112.700 | -90.000 | -10 | -22.000 | -7.000 |
| 2 6-DCBQ-2 | 176.888 | 141.000 | -90.000 | -10 | -14.000 | -21.000 |
| TetraCBQ-1 | 244.799 | 208.700 | -20.000 | -10 | -22.000 | -37.000 |
| TetraCBQ-2 | 244.799 | 180.900 | -20.000 | -10 | -22.000 | -13.000 |
| TetraC-1,2-BQ-1 | 246.762 | 35.000 | -95.000 | -10 | -56.000 | -15.000 |
| TetraC-1,2-BQ-2 | 246.762 | 210.700 | -95.000 | -10 | -20.000 | -15.000 |
| TetraC-1,2-BQ-3 | 246.762 | 208.800 | -95.000 | -10 | -22.000 | -15.000 |
| 25-DBBQ-1 | 266.750 | 80.800 | -95.000 | -10 | -24.000 | -11.000 |
| 25-DBBQ-2 | 266.750 | 78.800 | -95.000 | -10 | -22.000 | -11.000 |
| 26-DBBQ-1 | 266.750 | 156.700 | -95.000 | -10 | -28.000 | -11.000 |
| 26-DBBQ-2 | 266.750 | 184.800 | -95.000 | -10 | -22.000 | -13.000 |
| 26-DBBQ-3 | 266.750 | 186.800 | -95.000 | -10 | -20.000 | -13.000 |
| 2,3-DB-5,6-DMBQ-1 | 293.064 | 236.100 | -85.000 | -10 | -18.000 | -17.000 |
| 2,3-DB-5,6-DMBQ-3 | 293.064 | 221.000 | -85.000 | -10 | -24.000 | -17.000 |
| 3,5-DB-2,5-DMBQ-1 | 293.797 | 80.700 | -30.000 | -10 | -24.000 | -9.000 |
| 3,5-DB-2,5-DMBQ-2 | 293.797 | 78.800 | -30.000 | -10 | -44.000 | -13.000 |
| 3,5-DB-2,5-DMBQ-3 | 293.797 | 237.200 | -30.000 | -10 | -22.000 | -23.000 |
| 2 6-DB-3C-5MBQ-1 | 313.719 | 78.800 | -45.000 | -10 | -44.000 | -9.000 |
| 2 6-DB-3C-5MBQ-2 | 313.719 | 80.700 | -45.000 | -10 | -50.000 | -13.000 |
| TetraBBQ-1 | 424.605 | 78.800 | -85.000 | -10 | -68.000 | -9.000 |
| TetraBBQ-2 | 424.605 | 80.800 | -85.000 | -10 | -74.000 | -11.000 |
| TetraB12BQ-1 | 424.603 | 78.800 | -200.000 | -10 | -40.000 | -16.000 |
| TetraB12BQ-2 | 424.603 | 80.800 | -200.000 | -10 | -40.000 | -16.000 |

**Table 2.2. Compound information including abbreviation, limits of quantification (LOQs), linearity range, $R^2$ values, and percent relative standard deviation for 6 replicates**

| Compound | Abbreviation | LOQ (ng/L) | Linearity (ng/L) | $R^2$ | % RSD |
|---|---|---|---|---|---|
| 2,5-Dichloro-1,4-benzoquinone | 25DCBQ | 2.5 | 2.5-100 | 0.998 | 13 |
| 2,6-Dichloro-1,4-benzoquinone | 26DCBQ | 2.5 | 2.5-100 | 0.997 | 7 |
| 2,5-Dibromo-1,4-benzoquinone | 25DBBQ | 2.5 | 2.5-100 | 0.999 | 8 |
| 2,6-Dibromo-1,4-benzoquinone | 26DBBQ | 5.0 | 5.0-500 | 0.998 | 12 |
| 2,3,5,6-Tetrachloro-1,4-benzoquinone | TetraCBQ | 5.0 | 5.0-500 | 0.999 | 8 |
| 3,4,5,6-Tetrachloro-1,2-benzoquinone | TetraC12BQ | 1000 | 1000-10000 | 0.996 | 7 |
| 2,6-Dibromo-5,6-dimethyl-1,4-benzoquinone | 26DBDMBQ | 50 | 50-2500 | 0.999 | 5 |
| 2,6-Dibromo-3-chloro-5-methyl-1,4-benzoquinone | 26DBCMBQ | 100 | 100-2500 | 0.998 | 11 |
| 2,3,5,6-Tetrabromo-1,4-benzoquinone | TetraBBQ | 100 | 100-1000 | 0.998 | 9 |
| 3,4,5,6-Tetrabromo-1,2-benzoquinone | TetraB12BQ | 1000 | 1000-10000 | 0.998 | 5 |

**Table 2.3. Water treatment plant (WTP) information including source water type, disinfection type, contact time, temperature, pH, conductivity, free chlorine, and total organic carbon (TOC)**

| WTP | Water resource | Water Type | Post-disinfection treatment | Contact time[a] | Temp. °C | pH | Conductivity (µS/cm) | Free $Cl_2$ (mg/L) | TOC (mg/L C) |
|---|---|---|---|---|---|---|---|---|---|
| WTP1 | surface water (83%); groundwater (17%) | Surface water | | | 13.7 | 7.8 | 561 | - | 3.1 |
| | | Alluvial water | | | 14.0 | 7.1 | 446 | - | 1.5 |
| | | Treated water | Chlorine gas | 24 h | 14.2 | 7.3 | 59 | 0.80 | 1.9 |
| WTP2 | surface water (>85%); groundwater (<15%) | Raw water | | | 12.6 | 7.1 | 208 | - | 2.5 |
| | | Treated water | Chlorine gas | | 14.3 | 8.0 | 556 | 0.73 | 1.1 |
| WTP3 | groundwater (100%) | Raw water | | | 24.0 | 7.5 | 829 | - | 0.3 |
| | | Treated water | Chlorine gas | | 24.5 | 7.5 | 879 | 0.35 | 0.3 |
| WTP4 | surface water (>85%); groundwater (<15%) | Raw water | | | 14.4 | 7.8 | 355 | - | 5.6 |
| | | Treated water | Sodium hypochlorite | 2 h | 13.9 | 7.8 | 502 | 0.47 | 2.8 |

[a]Contact time corresponds to the time between the chlorination treatment and the selected sampling point.

**Table 2.4. HBQ sample results for water treatment plants (WTP) 1-4 in ng/L**

| HBQs ng/L | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **WTP** | **Water Type** | **25-DCBQ** | **26-DCBQ** | **Tetra-CBQ** | **Tetra-C12BQ** | **25-DBBQ** | **26-DBBQ** | **26-DBDMBQ** | **26-DBCMBQ** | **Tetra-BBQ** | **Tetra-B12BQ** |
| **WTP1** | Treated water | < 2.5 | 16<br>11* | < 5 | < 1000 | < 2.5 | 292<br>217* | < 50 | < 100 | < 100 | < 1000 |
| **WTP2** | Treated water | < 2.5 | < 2.5 | < 5 | < 1000 | < 2.5 | 42.5<br>35* | < 50 | < 100 | < 100 | < 1000 |
| **WTP3** | Treated water | < 2.5 | < 2.5 | < 5 | < 1000 | < 2.5 | 14<br>16* | < 50 | < 100 | < 100 | < 1000 |
| **WTP4** | Treated water | < 2.5 | 14<br>12* | < 5 | < 1000 | < 2.5 | 4<br>6* | < 50 | < 100 | < 100 | < 1000 |

*When a sample had positive results for HBQs, a second analysis was conducted indicated by the asterisk.

**Table 2.5. Average percent recovery of samples from plants 1-4 spiked with HBQs**

| Compound | Spiked concentration (ng/L) | % Recovery |
|---|---|---|
| 25DCBQ | 25 | 72 |
| 26DCBQ | 25 | 79 |
| TetraCBQ | 125 | 74 |
| TetraC12BQ | 2500 | 111 |
| 25DBBQ | 25 | 74 |
| 26DBBQ | 125 | 70 |
| 26DBDMBQ | 1250 | 92 |
| 26DBCMBQ | 2500 | 87 |
| TetraBBQ | 2500 | 95 |
| TetraB12BQ | 2500 | 103 |

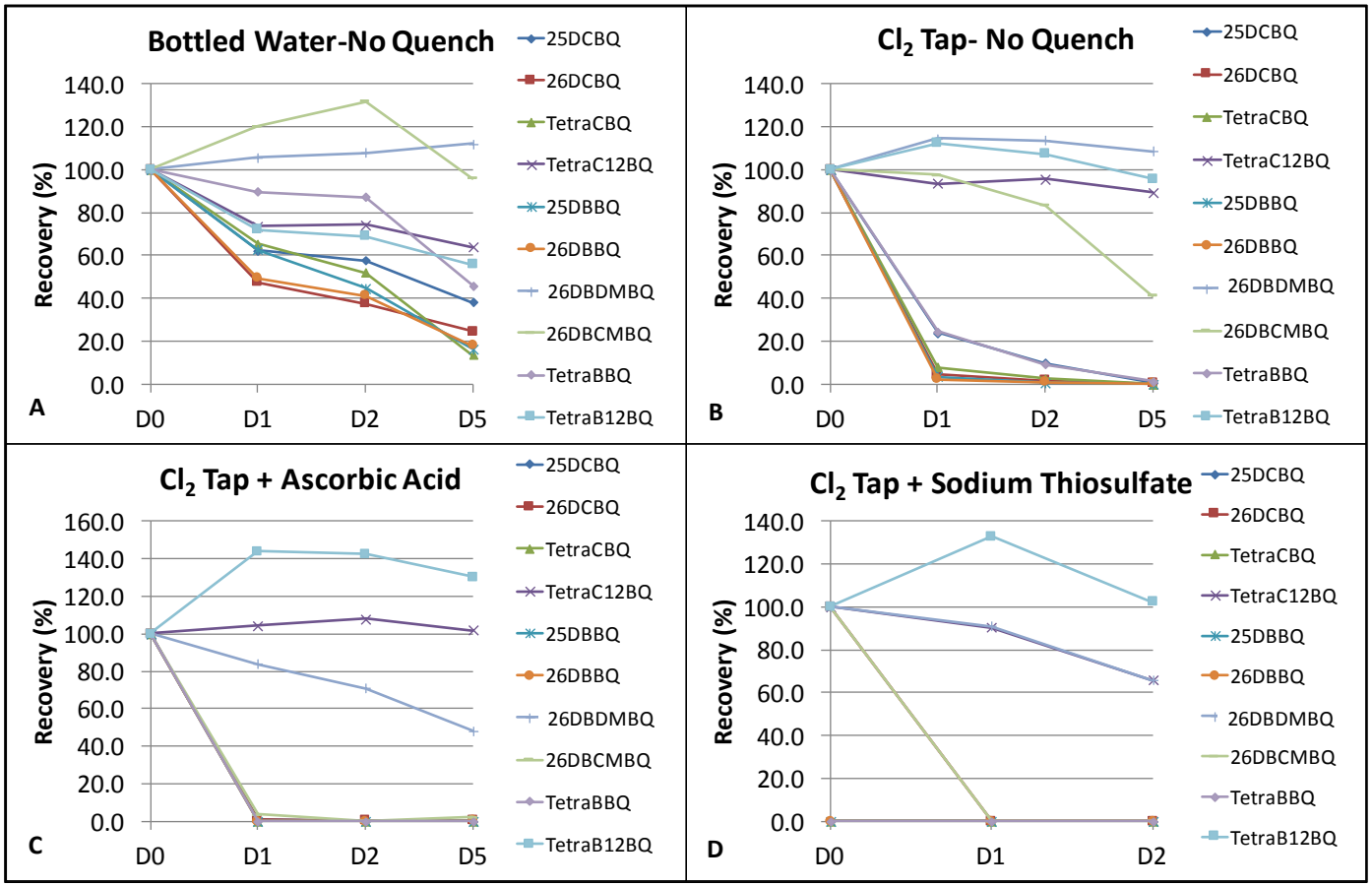


**Figure 2.1.** Holding study for HBQs in (A) bottled water with no quench; (B) chlorinated tap water with no quench; (C) chlorinated tap water quenched with ascorbic acid; and (D) chlorinated tap water quenched with sodium thiosulfate, monitored at 1 day, 2 days, and 5 days

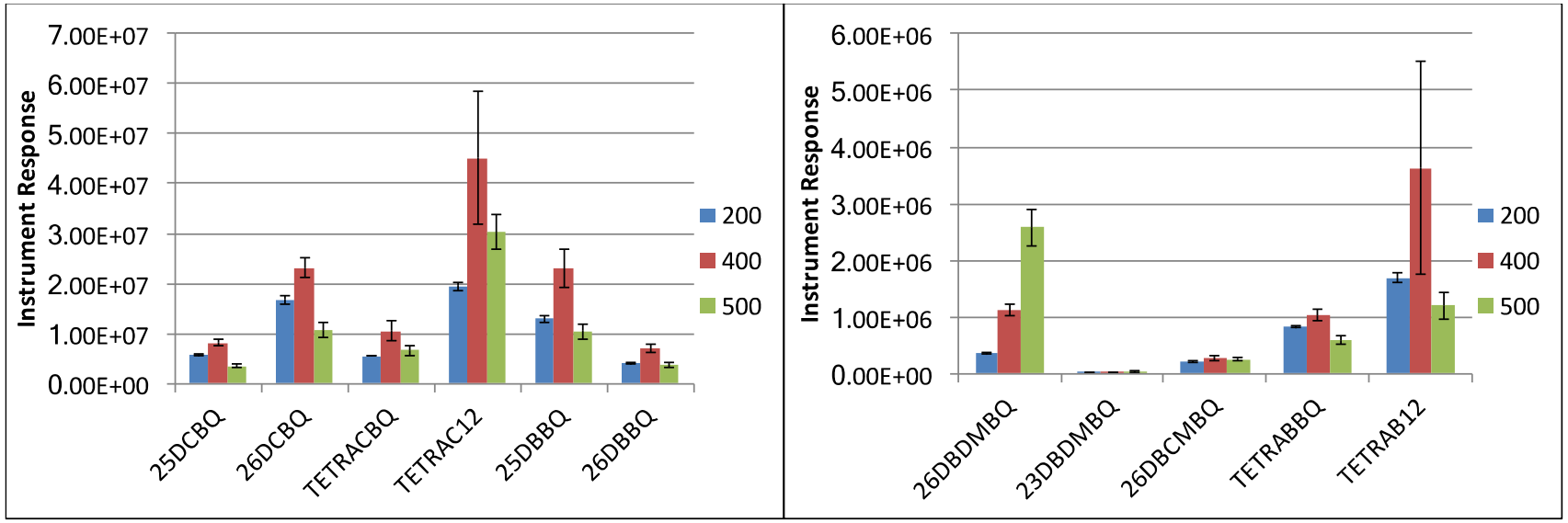


**Figure 2.2.** HBQs tested by direct injection at different source temperatures (200, 400, and 500°C).

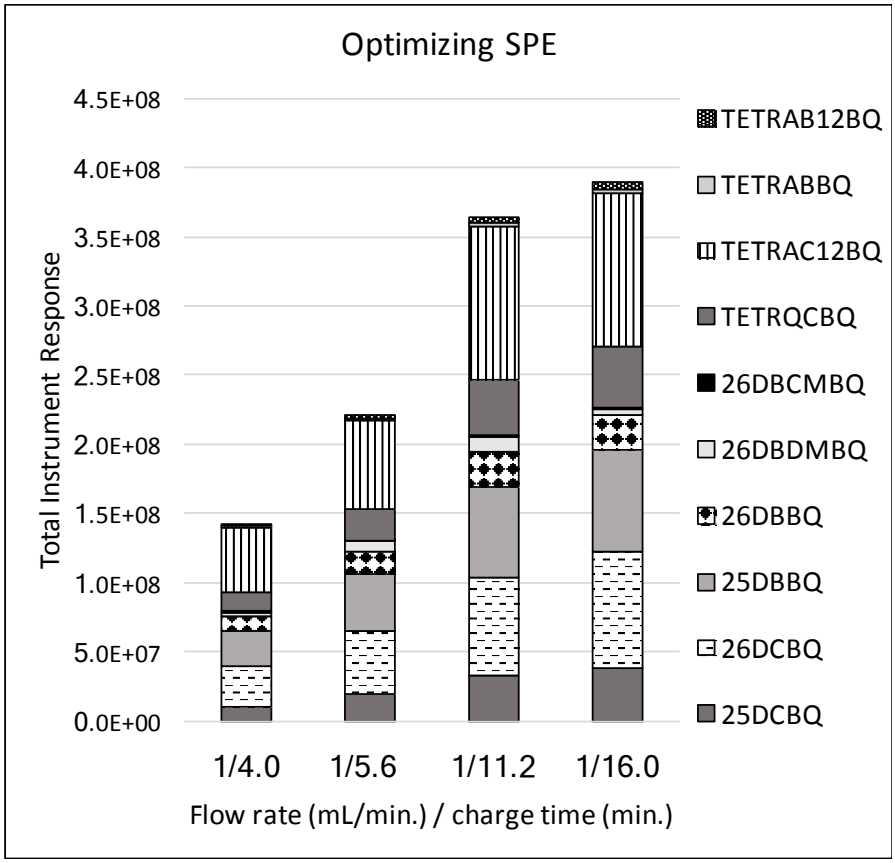


**Figure 2.3.** Peak areas for optimizing flow rate vs charging time through the SPE cartridge.

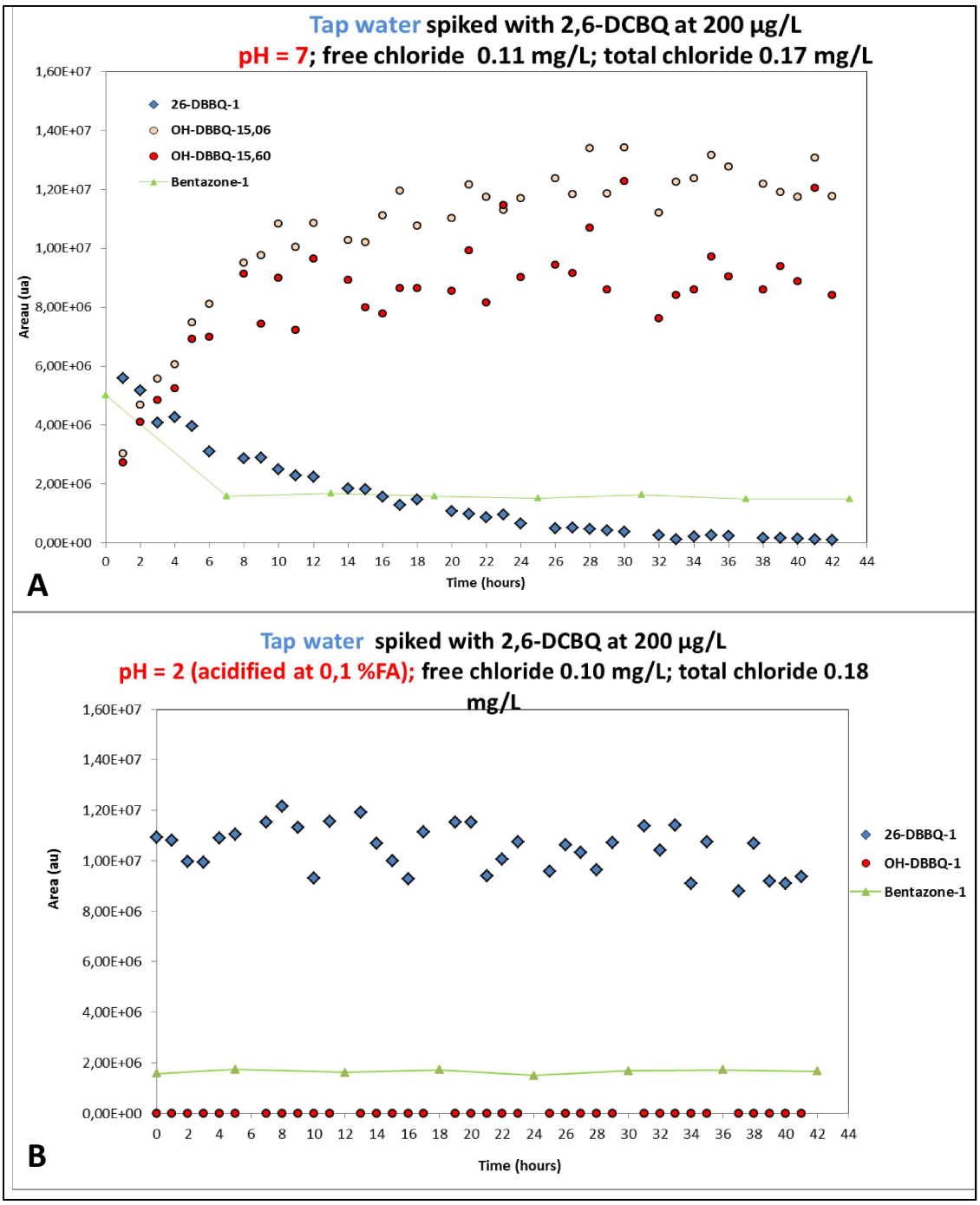


**Figure 2.4.** 2,6-DBBQ and hydroxy-2,6-DBBQ (OH-DBBQ) monitored by direct injection in chlorinated water at (A) pH 7 and (B) acidified to pH 2 with 0.1% formic acid.

# CHAPTER 3

# THE DBP EXPOSOME: DEVELOPMENT OF A NEW METHOD TO SIMULTANEOUSLY QUANTIFY PRIORITY DBPS AND COMPREHENSIVELY IDENTIFY UNKNOWNS[3]

[3] Susana Y. Kimura, Amy A. Cuthberston, Jonathan D. Byer, Susan D. Richardson. 2019. *Water Research.* 148: 324-333.

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Abstract

Disinfected drinking water contains hundreds of disinfection by-products (DBPs) that are formed by the reaction of disinfectants with natural and anthropogenic organic matter, bromide, and iodide. Understanding what these DBPs are is important because millions of people worldwide consume drinking water every day, and human epidemiologic studies have reported cancer, miscarriage, and birth defects from consuming such waters. While more than 600 DBPs are reported in the literature, very few studies quantify complete classes of chlorinated, brominated, and iodinated DBPs. Also, very few studies conduct comprehensive non-target analyses of unknown DBPs to characterize the complete DBP exposure (the exposome). We developed a new gas chromatography (GC)-mass spectrometry (MS) method that simultaneously quantifies 39 priority unregulated DBPs from six different chemical classes (haloacetaldehydes, haloketones, haloacetamides, haloacetonitriles, halonitromethanes, and iodinated-trihalomethanes) and analyzes unknown DBPs with mass accuracy <600 ppm under full-scan conditions. Using a new type of time-of-flight (TOF) mass spectrometer, which combines selected ion monitoring (SIM)-level sensitivity with mass accuracy of ±0.05 Da, method detection limits of 3–61 ng/L were achieved. These levels were found to be quite comparable to those of a widely used single quadrupole mass spectrometer (2–90 ng/L) operated in SIM mode. However, analysis using this TOF mass spectrometer offers two additional advantages over traditional quadrupole-MS: (1) full-scan data, which provides additional confidence for target analytes, as well as complete mass spectra for unknown analysis, and (2) two decimal place mass accuracy, which allows additional confidence for target analytes and importantly, molecular formula indication for unknowns. High resolution

accurate mass TOF was also used to validate identification of selected compounds. This new method was demonstrated on finished drinking waters from three different drinking water plants, where target quantification and non-target unknown analyses were performed simultaneously during the same run. This enabled the quantification of 39 DBPs, along with the non-target identification of many other drinking water contaminants, including two additional non-target DBPs: *N,N*-dimethylacetamide and *N*-nitrosodibutylamine.

Keywords (6)

DBPs, disinfection by-products, drinking water, non-target analysis, quantification

1. Introduction

Water disinfection is used worldwide to protect public health against harmful pathogens that cause waterborne diseases. However, disinfectants can also react with constituents found in natural waters (i.e., natural and anthropogenic organic matter, bromide, and iodide) to unintentionally form DBPs that have been associated with several adverse health effects from long-term exposure, including bladder and colorectal cancer and adverse birth outcomes (Bove et al. 2007, Costet et al. 2011, Grazuleviciene et al. 2013, Nieuwenhuijsen et al. 2013, Nieuwenhuijsen et al. 2000, Righi et al. 2012, Savitz et al. 2005, Smith et al. 2016, Villanueva et al. 2004, Villanueva et al. 2007, Waller et al. 1998). DBPs represent one class of chemical exposure amongst many other chemicals that humans are exposed through water, air, and their environment (Richardson and Ternes 2018, Dai et al. 2017), and possibly being one of the most important exposure chemicals because of the relatively high DBP levels in drinking water. While many water contaminants, such as pesticides, pharmaceuticals, per- and polyfluorinated alkyl substances (PFASs), brominated flame retardants, and UV filters are often present in environmental waters at ng/L levels (Richardson and Ternes 2011, 2014, 2018, Richardson and Kimura 2016), they are generally not detected in finished drinking water. In comparison, DBPs are *always* present in disinfected drinking water, and usually at μg/L levels (Richardson 2011, Richardson et al. 2007).

Although more than 600 DBPs have been reported in the literature (Richardson 2011), more than 50% of the total organic halogen resulting from water chlorination has not been identified (Weinberg et al. 2002, Krasner et al. 2006), and even less is known

for alternative disinfectants like ozone and chloramines. As a result, the complete picture of DBP exposure (the exposome) is not yet known. Measurement of all chloro-, bromo-, and iodo-DBP species of different DBP chemical classes can be intensive and laborious (Richardson and Kimura 2016, Richardson and Ternes 2011, 2014, 2018) because generally they are analyzed with separate analytical methods, due to their different physical and chemical properties. Additionally, DBPs and other environmental contaminants of concern are limited by the technological advancement of analytical instruments and methods that can detect and quantify them. Therefore, DBPs and water contaminants are emerging as research and technology advances.

In recent years, simultaneous target and non-target analysis of water contaminants is an increasing trend with liquid chromatography (LC)-MS, due to improvements in technology that facilitates this analysis and mass spectrometers that offer high resolution (Acena et al. 2015, Krauss et al. 2010, Leendert et al. 2015, Pico and Barcelo 2015, Richardson and Kimura 2016). However, low-level quantification of target compounds is still a common tradeoff to obtaining a well-resolved mass spectrum for qualitative identification of non-target compounds. This is especially true when quantifying small molecules using GC-MS. Most DBP analytical methods focus on one type of analysis (Richardson 2011, Richardson and Kimura 2016, Richardson and Ternes 2011, 2014, 2018). Typically for GC-single quadrupole (SQ) mass spectrometers, target compounds are monitored in SIM mode, where only one or two ions are monitored for each analyte at a specific retention time. While samples are analyzed for trace levels of target compounds, information that pertains to non-target compounds is lost. Samples would

require re-analysis in full-scan mode, with the downside of lower sensitivity compared to SIM mode.

Additionally, non-target analysis is a rigorous process that typically involves careful background subtraction, library searching with standard or user-defined libraries, manual interpretation of mass spectra not present in library databases, exact mass and molecular formula determination, and confirmation of tentative identifications with authentic chemical standards (match of mass spectrum and retention time) (Krauss et al. 2010, Weinberg et al. 2002, Krasner et al. 2006, Richardson et al. 2008, Postigo et al. 2016, Schymanski et al. 2014, Gong and Zhang 2015, Pan et al. 2016). Often, DBPs are difficult to confirm due to the lack of commercially available standards; synthesis of these standards can be time and resource consuming. A higher resolved mass spectrum can narrow the list of possible candidate analytes and minimize false positives. For DBP analysis, a GC-mass spectrometer with high resolving power (e.g., >10,000) can provide accurate mass data with low mass error. However, an increase in resolving power is typically associated with a substantial decrease in sensitivity.

In this study, a simultaneous quantification of 39 target unregulated DBPs and non-target analysis of other unknown DBPs and contaminants was achieved at high sensitivity and increased mass accuracy (<600 ppm) using a newly developed TOF mass spectrometer. This mass spectrometer has several new developments compared to other instrumentation: significantly faster ion pulse frequency (35 kHz) to improve the duty

cycle, full mass spectral profile acquisition (not centroided), and a redesigned ion source to support the increased pulse frequency and mass profile characteristics. Quantitative results achieved in full-scan were compared to SIM analyses carried out using a GC-SQ mass spectrometer. Target DBPs from six different complete chemical classes, including haloacetaldehydes (HALs), halonitromethanes (HNMs), haloacetamides (HAMs), haloacetonitriles (HANs), haloketones (HKs), and iodinated trihalomethanes (ITHMs), were quantified in one single analytical method. These 39 DBPs were chosen because most of them are much more cytotoxic or genotoxic than the DBPs currently regulated, and recent studies have shown that they can be the overall drivers of toxicity in drinking water samples, despite their lower concentrations relative to regulated THMs and HAAs (Plewa et al 2017, Krasner et al 2016). This quantitative and qualitative method was used to evaluate finished drinking waters with different source waters and disinfection treatments in which sensitive quantitative results were achieved, along with comprehensive identification of non-target, unknown DBPs and other chemical contaminants.

## 2. Material and Methods

### 2.1. Water Samples

Drinking water and source water samples were collected headspace-free in 1 L amber glass bottles that contained 5 mg of ammonium chloride (to quench the active disinfectant) and were preserved by adjusting the pH to 3.5-4 (with 1.5 mL of 1 M $H_2SO_4$). Prior to extraction and GC-MS analysis, samples were stored at 4°C with

holding times between <24 h to 3 days. Water quality parameters are shown in Table 3.1. Samples 1 and 2 were from water treatment plants ~40 miles apart that treat the same source water (Lake Michigan). Both use conventional treatment (flocculation, sedimentation, filtration, disinfection) with the difference that Site 1 uses anthracite filtration followed by free chlorine disinfection, and Site 2 uses granular activated carbon (GAC) filtration followed by pre-formed chloramine as a disinfectant. Sample 3 was from a city that uses a deep aquifer as their source water and conventional treatment with free chlorine.

## 2.2. Reagents and Solutions

Analytical standards for priority DBPs were purchased or custom synthesized at the highest purity available from CanSyn Chem. Corp. (Toronto, Ontario), Sigma Aldrich (St. Louis, MO), Aldlab Chemicals (Boston, MA), and TCI America (Boston, MA), as shown in Table 3.2. Organic solvents were of the highest purity available. Acetonitrile, methyl *tert*-butyl ether (MTBE), methanol, and pure water were purchased from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA).

Standards were prepared in anhydrous acetonitrile or methanol. Five 100-200 ppm sub-stock solutions containing a mix of compounds from each DBP group (HANs, HKs, HALs, HNMs, and ITHMs) were prepared in acetonitrile and were found to be stable for 12 months. For example, a HAN sub-stock solution contained a 100 mg/L mixture of

chloroacetonitrile, bromoacetonitrile, dichloroacetonitrile, bromochloroacetonitrile, dibromoacetonitrile, and iodoacetonitrile. A 10 mg/L master sub-stock solution that contained all of the DBPs (except HAMs because they were stable up to one month only) was prepared daily by mixing aliquots of each of the five sub-stocks together. HAM stock solutions were prepared individually every month from pure standards in methanol and combined into a 10 mg/L sub-stock solution that only contained HAMs.

## 2.3. Instrumentation

Two GC-mass spectrometers were used to quantify and analyze priority DBPs. The first system was an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA) coupled to a Pegasus BT TOF mass spectrometer (LECO Corp., Saint Joseph, MI) (mass accuracy of <600 ppm). The second system was an Agilent 7890B GC coupled to a 5977A single quadrupole (SQ)-mass spectrometer (Agilent Technologies, Santa Clara, CA). Both systems use electron ionization (EI). A 200-Rtx GC column (Restek, State College, PA) was used for both systems with the same injection and oven temperature program. The 200-Rtx GC column provided improved peak shape and separation of analytes compared to a more commonly used DB-5 GC column. Samples were introduced into the GC multi-mode inlet (MMI) as a cold-pulsed splitless injection at 40°C and ramped at 360°C/min to 170°C, then ramped at 720°C/min to 250°C and held for 44 min. The inlet was pulsed at a pressure of 25 psi for 90 s and purged immediately after the pulse at 30 mL/min. The oven temperature program was as follows: hold for 5 min at 35°C, then ramped at 9

°C/min to 220°C, followed by a final ramp of 20 °C/min to 280°C and hold for 20 min. Transfer line and ion source temperatures were 250°C and 200°C, respectively.

Mass spectrometer methods differed for each system because each instrument has a different design to select and quantify ions. The BT GC-TOF-MS was programmed at a 30 kHz frequency extraction rate with a scan range of m/z 30-645. The Agilent GC-SQ-MS was programmed using SIM for 2-3 ions that pertained to the quantification and qualifier ions for each target analyte, with a dwell time of 100 ms for the quantification ion and 50-75 ms for each qualifier ion (Table 3.2).

Pure water samples spiked with 39 target analytes were extracted and analyzed using these two GC-MS methods. A specific analyte was quantified using the same ion in both systems with the difference that the Agilent GC-SQ-MS has nominal mass resolution, and the BT GC-TOF-MS has a slightly higher resolution, with mass accuracy to two decimal places (~600 ppm).

High-resolution mass spectrometry (HRMS) using a Pegasus GC-HRT TOF-mass spectrometer (25,000 resolution, 1 ppm mass accuracy) (LECO Corp., Saint Joseph, MI) was used to provide further supporting evidence for the structures of unknown DBPs. GC parameters and the analytical column were identical to those used for the BT GC-TOF-

MS and Agilent GC-MS instruments. The Pegasus GC-HRT TOF-MS was programmed at a frequency extraction rate of 1 kHz with scan range of m/z 30-650.

## 2.4. Extraction method

Water samples (100 mL) were extracted using liquid-liquid extraction (LLE) with 5 mL of MTBE and 30 g of sodium sulfate (salting out) to increase the extraction efficiency of DBPs. Samples were shaken with a wrist action shaker (Burrell, Pittsburg, PA) for 15 min and held for 10 min for the two phases to separate. The MTBE top layer was recovered, and the extraction process was repeated twice. A total extract of about 15 mL was dried through an anhydrous sodium sulfate column to remove any remaining water in the extract. Dried extracts were concentrated under a gentle flow of nitrogen to 200 µL, the internal standard 1,2-dibromopropane was added to the extract (8 µL of 30 mg/L), and the extract was analyzed immediately.

## 2.5. Calibration curves and method detection limits

Water samples used for calibration curves and method detection limits (MDLs) were prepared by spiking 10 mg/L master stock solutions and HAM sub-stock solutions into 100 mL of pure water, which were extracted immediately. Water samples with concentrations of 0.010, 0.05, 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, and 10.0 µg/L were used for

calibration curves, which had a linear range of three orders of magnitude with ($r^2 > 0.99$). Seven replicates for each concentration level of 0.010, 0.050, 0.10, 0.50, and 1.0 µg/L were used to determine MDLs. The instruments' minimum detection limits (MDLs) were calculated from the standard deviation (n=7 replicates) multiplied by the one-sided Student's t-value. MDLs are defined as the minimum concentration of an analyte that can be detected with 99% confidence that the concentration is greater than zero (Harris 2003, Wells et al 2011). MDLs are obtained with the following equation:

$$MDL = S_{\overline{X}} t_{N-1,1-\alpha=0.99}$$

where, $t_{N-1,1-\alpha=0.99}$ is the one-sided Student's t-value (99% confidence level of n-1) and

$S_{\overline{X}}$ is the standard deviation of the replicates. In this study, we used the instrument signal (area counts), so the mean of the instrument signal was multiplied by the analyte concentration level (CL) and divided by the calibration mean ($\overline{X}$) for each analyte (Wells 2011).

$$MDL = S_{\overline{X}} t_{N-1,1-\alpha=0.99} \frac{CL}{\overline{X}}$$

2.6. Data processing software

Water extracts processed with the Agilent GC-SQ-MS were analyzed with Mass Hunter software for quantification of target analytes. Water extracts processed with the BT GC-TOF-MS were analyzed with ChromaTOF software for target and non-target quantification. NIST 2014 and a user mass spectral library were used for non-target

analysis. The user mass spectral library was built by analyzing pure DBP standards with a LECO Pegasus GC-HRT mass spectrometer (25,000 resolution).

# 3. Results and Discussion

## 3.1. Method detection limits for target DBPs

Method detection limits for 39 target DBPs were determined on both BT and Agilent instruments as shown in Table 3.2. The same quantification ion was used for direct comparison. Results show that the MDLs for haloacetaldehydes, halonitromethanes, haloacetonitriles and haloketones obtained on the BT GC-TOF-MS (3-36 ng/L) in full-scan mode were comparable to the Agilent GC-SQ-MS (3-43 ng/L) in SIM mode. The MDLs for ITHMs were slightly higher on the Agilent GC-SQ-MS (12-90 ng/L) compared to the BT GC-TOF-MS (3-26 ng/L). However, haloacetamides had slightly higher MDLs for the BT GC-TOF-MS system (10-324 ng/L) compared to the Agilent GC-SQ-MS (7-50 ng/L), which was mainly driven by dibromochloroacetamide (MDL of 324 ng/L). These results show that a TOF-MS system in full-scan mode can have high sensitivity comparable to a SQ-MS system in SIM mode. Typically, there is a trade-off between mass accuracy and sensitivity, but in this case, the BT GC-TOF-MS was able to provide two-decimal place mass accuracy while providing parts-per-trillion level sensitivity for quantification of low molecular weight DBPs. This is advantageous because of the capacity to simultaneously perform quantification of target analytes and

identify non-target analytes in complex environmental samples. Moreover, the acquisition of full-scan data allows for retroactive analysis of data, providing the possibility to identify other compounds at a later time and also re-analyze other quantification ions without the need to rerun samples or standards.

Of the 39 target DBPs, seven were further evaluated (post-run) for an additional quantifying ion because these compounds had an ion with higher signal-to-noise ratio (S/N) compared to the ion with the highest abundance (Table 3.2), including bromochloroacetamide, dibromoacetamide, bromodichloroacetamide, dibromochloroacetamide, tribromoacetamide, 1-bromo-1,1-dichloropropanone, and iodoform. Quantifying ions were selected based on the highest abundance or on the highest S/N. While it is preferred that the quantifying ion have both criteria, it is not always the case. For example, most haloacetamides have m/z 44.01 as their base peak, which is often used as the quantifying ion. However, m/z 44.01 is a common ion that is shared among many compounds, which lowers the S/N ratio as shown for bromochloroacetamide in Figure 3.1a. Even though the abundance for base peak at m/z 44.01 was ~2 x $10^7$ (a significantly high signal), the S/N ratio was only 9.1. On the other hand, m/z 172.91 had a higher S/N of 56 with a lower abundance of 1.5 x $10^4$. When comparing MDLs between ions, m/z 172.91 had a lower MDL of 6.0 ng/L than the base peak at m/z 44.01 (13.0 ng/L). Bromodichloroacetamide, dibromochloroacetamide, and 1-bromo-1,1-dichloropropanone also had lower MDLs (61, 19, and 25 ng/L, respectively) using ions with higher S/N ratios. Dibromoacetamide, tribromoacetamide, and iodoform had lower MDLs (10.0, 48.0, and 24.0 ng/L, respectively) with the most abundant ions.

As a result, ions that provided the lowest MDLs were chosen and used for quantification of drinking water samples. Final MDLs used for the Agilent GC-SQ-MS and the BT-GC-TOF-MS for quantification of drinking water samples are summarized in Table 3.2.

Mass resolution also plays a major role in the selection of quantifying ions. Mass analyzers measure the mass-to-charge (m/z) ratios of ions based on different principles (Hoffman and Stroobant 2007). A SQ-mass spectrometer is a scanning analyzer that transmits specific ions consecutively over a period of time at low mass resolution. TOF-MS transmits all ions at once through a flight tube, and their m/z ratios are determined by the time the ions take to reach the detector. The advantage of using a mass accuracy of ± 0.05 Da compared to other instruments with lower mass resolution is illustrated for tribromoacetaldehyde (Figure 3.2). A mass accuracy of 0.5 Da for the quantifying ion m/z 172.8 as shown in Figure 3.2a, similar to a SQ-mass spectrometer, captures a wide mass range that can overlap with ions from analytes other than tribromoacetaldehyde. Significantly higher noise is observed in chromatogram 2a compared to 2b (Figure 3.2). The TOF-mass spectrometer (Figure 3.2b) has a higher mass accuracy of 172.84 ±0.05 that is more specific to tribromoacetaldehyde's base peak, with a lower mass error of 10.4 ppm (calculated exact mass of 172.84179). Additionally, mass tolerances can also be used as a filtering parameter for target quantification and identification of non-target compounds.

## 3.2. Target priority DBPs in treated drinking waters

DBPs are composed of many different classes of compounds with different chemical and physical properties, which make it difficult to analyze them with a single method. However, in this study, a new analytical method with very low MDLs was used to simultaneously quantify six different classes of DBPs in chlorinated and chloraminated drinking water samples.

All quantified DBPs grouped by chemical class and individually stacked are shown in Figure 3.3. HALs were the most predominant of the DBP classes quantified in all three sites, which agrees with a previous DBP Nationwide Occurrence Study that found HALs to be the third largest DBP class by weight, followed by THMs and HAAs, which were the highest in concentration (Krasner et al. 2006, Weinberg et al. 2002). For example, median levels of the sum of the four regulated THMs (THM4) and the sum of the nine bromo/chloro-HAAs (HAA9, including the five regulated HAAs) were 31 μg/L and 34 μg/L, respectively, while the median level of HALs was 4 μg/L in the Nationwide Occurrence Study (Krasner et al. 2006). HALs were 3, 0.5, and 3.7 μg/L in three disinfected waters from our current study. The two water utilities that use chlorine as the disinfectant, Site 1 and Site 3, produced the highest concentration of total HALs (3.0 and 3.7 μg/L, respectively) and total HANs (1.9 and 1.8 μg/L, respectively). Trichloroacetaldehyde and bromodichloroacetaldehyde were the major contributors to total HAL concentrations, and dichloroacetonitrile and bromochloroacetonitrile to total

HAN concentrations (Figure 3.4a and 3.4b). Chloraminated waters from Site 2 formed 0.49 and 0.21 µg/L for total HALs and HANs, respectively. HKs were the third largest DBP group (of the DBP classes measured in this study) for Site 1 (1.4 µg/L), driven by 1,1,1-trichloropropanone (Figure 3.4d). Sites 2 and 3 had significantly lower HK formation, with concentrations of 0.20 and 0.17 µg/L, respectively. In addition to differences in source water and disinfectants, another factor in DBP concentrations between the different sites is the use of GAC filtration at Site 2, whereas Site 1 and 3 use sand/anthracite filtration. It is likely that GAC filters at Site 2 improved the removal of DBP precursors (natural organic matter), and therefore, DBP levels were lower. In fact, Site 2 had lower concentrations of HALs, HANs, and HKs than both Site 1 and 3, but the use of chloramination likely also played a role. A previous study (Chiu et al. 2012) has shown that GAC removes dissolved organic carbon, carbon-based DBP (C-DBP) precursors, resulting in lower C-DBPs, which explains lower HALs and HKs for Site 2. Although HANs are nitrogen-containing DBPs, they are known to have organic carbon precursors (Kimura et al. 2013) that can also be removed by GAC.

Site 3 had the highest total HNM (0.29 µg/L) and I-THM (0.49 µg/L) concentrations by weight as shown in Figure 3.3. HNM formation for Site 1 and 2 was 0.21 and 0.09 µg/L, respectively. I-THM formation was 0.12 and 0.05 µg/L for Site 1 and 2, respectively. ITHMs usually form at lower levels in chlorinated waters compared to chloraminated waters (Postigo et al. 2017, Richardson et al. 2008). However, I-THM and HNM formation can increase at higher pH (Karanfil et al. 2011), which may explain the higher concentrations at Site 3 (pH 8.9) vs. the other two sites (pH 7.1-7.4). Therefore,

pH might have a significant effect on the formation of both ITHMs and HNMs in finished drinking water. HNM and I-THM speciation are largely driven by dichloroiodomethane and trichloronitromethane, closely followed by dichloronitromethane and bromochloronitromethane (Figure 3.4e and 3.4f). I-THM and halonitromethane concentrations were significantly higher in finished water with high pH (8.9) compared to neutral pH (~7.3).

Total HAMs were detected at similar concentrations (0.15-0.27 μg/L) in all three sites. Dichloroacetamide predominantly formed, followed by bromochloroacetamide (Figure 3.4c) and dibromoacetamide. Despite a somewhat higher total organic carbon (TOC) in the groundwater of Site 3, higher levels of HAMs were found at Sites 1 and 2, which treated surface waters.

## 3.3. Non-target priority DBPs in treated drinking waters

For non-target analysis or unknown compound identification, it is important to obtain a clean mass spectrum specific to a compound of interest that can be further interpreted. This requires a good chromatographic separation of analytes and software that can deconvolute overlapping mass spectra of coeluting compounds. The final mass spectrum of a compound can then be compared to standard (e.g., NIST) or user-defined libraries that rank the compound of interest to possible library matches (typically a score of 0 to 1000). Compounds found in samples analyzed with low-resolution mass spectrometers are usually tentatively identified using a library match (Biemann 1962, McLafferty

1966). However, a higher mass accuracy, provides another level of compound validation by selecting library matches within a specific mass tolerance.

In this study, a second post-run data processing method was used for non-target analysis. The criteria and confidence levels used in this study for non-target analyte identification are shown in Figure 3.6 and follow the procedure published by Schymanski et al. (2014), which assigns confidence levels based on evidence obtained through determination of accurate mass and molecular formula, isotopic patterns, library database match, manual interpretation of MS fragment ions. Highest confidence levels are when no other structure fits the experimental information (Level 2), and when structures are confirmed by the match of the mass spectrum and retention time with an authentic standard (Level 1). Results were processed with ChromaTOF software, which deconvolutes all the collected data and selects peaks that have a minimum S/N of 10 and at least three m/z ions to be considered for further compound identification. Deconvoluted peaks were compared to the NIST library and a user mass spectral library database and then filtered by similarity (score >700, out of 1000). Non-target compounds with a similarity score >700 are shown in Table 3.3. A total of 91 compounds were identified as non-target analytes from the analysis of three raw and finished water samples. By including a mass tolerance of 600 ppm, the non-target list was reduced to 58 possible candidates (Table 3.4). Using both library similarity and mass accuracy aids the analyst in choosing potential candidate analytes more accurately. Candidate analytes were individually inspected with their corresponding library matches, compared simultaneously with method blanks, and identified in both replicates of each sample.

Potential molecular formulas of candidate analytes from Table 3.4 were supported with accurate mass data from a high resolution (25,000 resolution) TOF mass spectrometer (Level 2 confidence). Candidate analytes were narrowed down based on the molecular ion (mass accuracy < 2 ppm) and similarity >700 as shown in Table 3.5. Retention time, library match, accurate mass data, mass error, and confidence levels for each sample analyzed by HRMS and with the BT GC-TOF mass spectrometer are shown in Tables 3.5-3.8. Several candidate analytes initially found using the BT GC-TOF-MS instrument were not observed with the high resolution (HR)-TOF-MS, due to lower sensitivity on the high resolution instrument. In one example, most ions in the mass spectrum of 1-bromo-1-chloropropan-2-one were observed in the HR-TOF-MS data, but because the molecular ion and accompanying isotope pattern had low abundance and was not detected with the HR-TOF-MS, the accurate mass of that particular analyte could not be confirmed. The exact molecular formulas for twelve non-target compounds were obtained with HR-TOF-MS, including two THMs (dibromochloromethane and bromoform), a ketone (3-pentanone), two aldehydes (furfural, benzeneacetaldehyde), an acetamide (*N*,*N*-dimethylacetamide), an ester (ethylbenzoate), a nitrile (benzyl nitrile), two aromatic compounds (benzothiazole, quinoline), and a nitrosamine (*N*-nitrosodibutylamine). Analytical standards were acquired for the twelve Level 2 analytes for further confirmation of retention time. Five analytes were confirmed at Level 1, including dibromochloromethane, bromoform, *N,N*-dimethylacetamide, *N*-nitrosodibutylamine, and benzothiazole.

*N*-Nitrosodibutylamine (NDBA) is an unregulated DBP that was detected as a non-target analyte in finished drinking waters in this study. Nitrosamines are suspected human carcinogens and are of health concern (IARC 1978). NDBA has been detected in finished and sources water in China, the U.K., Canada and the U.S. (Wang et al. 2016, Wang et al. 2011, Russell et al. 2011, Templeton and Chen 2010). In a recent study of 54 finished drinking water in 30 cities in China, NDBA was found in 51.9% of the analyzed samples, with concentrations between 0.4-25.3 ng/L in finished drinking waters and 0.8-48.2 ng/L in source waters (Wang et al. 2016). Higher levels of NDBA in source waters compared to finished waters was also observed in an earlier study by the same authors (Wang et al. 2011). NDBA was detected in only five water systems (0.4% of the total monitored water systems) in the U.S. that chlorinated groundwater (Russell et al. 2012). However, NDBA was detected in 32 water systems in Ontario, Canada (20% of the total monitored water systems).

Several compounds were found in both raw waters and finished drinking waters. For example, *N,N*-dimethylacetamide (DMA), a water soluble compound commonly used as an industrial solvent (Snyder 1990) and in pharmaceuticals (Ghayor et al. 2017) was identified in lake water (raw water for Site 2) and for the first time in finished drinking waters. DMA was previously detected in environmental samples, including seawater, river water, rain water, and wastewater effluents in Japan (Kadokami et al. 1993), with mean concentrations of 0.052, 0.046, 0.64, and 0.59 μg/L, respectively. Benzothiazole, which is used in the production of rubber, as an herbicide and fungicide, and as a stabilizer in the photo industry (Bahnmuller et al. 2015, Engels et al. 1993, Hartley and

Kidd 1987, Bugby et al. 1990, Kennedy 1986, Wik and Dave 2009), was detected in all of the finished drinking water samples and most raw water samples. Because of its low degradability in wastewater treatment (Reemtsma et al. 2006), it has been found in multiple aquatic environments (van Leerdam et al. 2009). Benzothiazole metabolites are of particular concern because they are carcinogens that have been associated with human bladder cancer (Gingsberg et al. 2011). Dibromochloromethane and bromoform are regulated DBPs, are commonly found in finished drinking waters (Richardson 2011), and were identified in all three disinfected water samples. Dibromochloromethane was also detected in the raw waters of Site 2.

Identification of unknowns not present in libraries without the use of accurate mass data remains quite a challenging effort. With only low resolution mass spectrometry, there are numerous different molecular formulas possible for unknown DBPs (typically 10-20 possible formulas for compounds the 200-500 molecular weight range), and each molecular formula can have many structural possibilities. High resolution-MS provides accurate masses (typically 3 or 4 decimal places), which significantly narrows the choices of formulas (often to a single formula for the molecular ion), and it also assists with determining the molecular composition of fragment ions, which helps in determining the overall structure of the molecule.

To assist with unknown identification, the BT-GC-TOF-MS software contains filters that can be programmed to identify specific isotope patterns and pinpoint halogen-

containing molecules. Filters were programmed to identify peaks that contained m/z fragment patterns of one, two, or three bromine and chlorine atoms as shown in Table 3.11 and 3.12. Using these filters, *trans*-2,3,4-trichloro-2-butenenitrile was tentatively identified from its two and three chlorine isotope patterns (m/z 133.95/135.95/137.95 and m/z 168.91/170.91/172.91) as shown in Figure 3.5. This analyte co-eluted with other compounds, making it difficult to identify. To overcome this, all fragment ions were graphed and only those that overlay with each other were identified (highlighted in blue in Figure 3.5) and are specific to one analyte, in this case, *trans*-2,3,4-trichloro-2-butenenitrile. HRMS analysis did not detect the molecular ion, possibly due to its low intensity (and lower sensitivity of the HR-MS instrument). *Trans*-2,3,4-trichloro-2-butenenitrile has been predicted to be a carcinogen, based on a structure-activity relationship model (Woo, et al 2002). This halonitrile has only been reported once in finished chlorinated waters (Richardson 2011), which used a large-volume extraction and a concentration factor of >10,000x. However, in our study, *trans*-2,3,4-trichloro-2-butenenitrile was detected with a concentration factor of 500x, which is 20 times lower than the previous study. These results show that the BT-GC-TOF-MS is capable of non-target analysis and is complementary to HR-MS.

The combined high sensitivity and mass accuracy of ± 0.05 Da of the BT provides a powerful tool for initial non-target screening analysis. Potential analyte lists are narrowed down and resources may be focused on a condensed candidate list for further confirmation and analysis with HRMS and analytical standards. Furthermore, filters may

be used to search for analytes that have specific isotope patterns of interest and may be used for the identification of unknowns, thus reducing the time for interpretation.

## 4. Conclusions

- A new analytical method using a GC-TOF-MS with high sensitivity, mass accuracy of 0.05 Da, and software tools was developed to quantify six different classes of DBPs at trace levels and simultaneously performed non-target analysis.
- Method detection limits of 3-61 ng/L were achieved using the GC-TOF-MS. These levels were comparable to those of a widely used single quadrupole mass spectrometer (2-90 ng/L).
- Finished and raw waters from three drinking water utilities were analyzed, and chlorinated and chloraminated waters produced different DBP speciation.
- Chlorinated waters formed haloacetaldehydes predominantly, followed by haloacetonitriles and haloketones.
- Chloraminated water also produced primarily haloacetaldehydes, but at lower concentrations than chlorinated waters, followed by haloacetamides and haloacetonitriles.
- I-THM and halonitromethane concentrations were significantly higher in finished water with high pH (8.9) compared to neutral pH (~7.3).
- The nitrosamine, *N*-nitrosodibutylamine, was detected in all three sampled waters, and *N,N*-dimethylacetamide was identified for the first time in source water (lake water) and finished drinking waters.

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## Table and Figures

**Table 3.1**. Water parameters of samples collected from four sites with different surface water sources and disinfection treatments. *Site 1 and 2 obtain their water source from Lake Michigan and are located 40 miles apart. Water parameters were obtained directly from the water treatment facility.

| | **Site No.** | **1*** | **2*** | **3** |
|---|---|---|---|---|
| Raw | Water Source | Lake Water | Lake Water | Groundwater |
| | Turbidity (NTU) | 0.38 | 1.3 | n.m. |
| | TOC (mg/L) | 2.1 | 2.1 | 2.7 |
| | Alkalinity (mg/L as $CaCO_3$) | n.m. | 110 | n.m. |
| | pH | 8.1 | 7.8 | 7.8 |
| Finished | Turbidity (NTU) | 0.062 | 0.09 | 0.06 |
| | TOC (mg/L) | 2.7 | 1.6 | 1.0 |
| | Alkalinity (mg/L as $CaCO_3$) | 99 | 98 | n.m. |
| | pH | 7.4 | 7.1 | 8.9 |
| | Residual (mg/L as $Cl_2$) | 1.4 | 1.6 | 2.4 |
| | Disinfectant | $Cl_2$ | $NH_2Cl$ | $Cl_2$ |

n.m.: not measured

**Table 3.2**. Summary of 39 disinfection byproducts (DBPs) analyzed in this study with DBP and internal standard information (vendor information and purity), retention time (RT), and quantification ions, and method detection limits by instrument.

| | | | | | Single Quadrupole | | Time of Flight | |
|---|---|---|---|---|---|---|---|---|
| **DBP Class** | **DBP** | **Abb.** | **Purity** | **RT (min)** | **Quant Ion (m/z)** | **MDL (ng/L)** | **Quant Ion (m/z)** | **MDL (ng/L)** |
| I-THM | Dichloroiodomethane | DCIM | 95%[a] | 4.34 | 83.0 | 12 | 82.94 | 25 |
| I-THM | Bromochloroiodomethane | BCIM | 95%[a] | 5.95 | 128.9 | 27 | 128.89 | 20 |
| I-THM | Dibromoiodomethane | DBIM | 90%[a] | 7.83 | 172.8 | 15 | 172.84 | 19 |
| I-THM | Chlorodiiodomethane | CDIM | 90%[a] | 8.33 | 174.9 | 39 | 174.84 | 3.0 |
| I-THM | Bromodiiodomethane | BDIM | ≥90%[a] | 10.09 | 218.8 | 90 | 218.82 | 20 |
| I-THM | Iodoform | TIM | 99%[b] | 12.06 | 393.7 | 47 | 393.73<br>266.81 | 26<br>24 |
| HAN | Chloroacetonitrile | CAN | 99%[b] | 4.36 | 75.0 | 8.0 | 74.99 | 5.0 |
| HAN | Bromoacetonitrile | BAN | 97%[b] | 6.16 | 118.9 | 43 | 118.94 | 6.0 |
| HAN | Dichloroacetonitrile | DCAN | 98%[b] | 4.22 | 74.0 | 5.0 | 73.98 | 21 |
| HAN | Bromochloroacetonitrile | BCAN | 97.2%[b] | 6.00 | 74.0 | 5.0 | 73.98 | 24 |
| HAN | Dibromoacetonitrile | DBAN | 90%[b] | 8.17 | 117.9 | 38 | 117.93 | 24 |
| HAN | Iodoacetonitrile | IAN | 98.7%[b] | 8.85 | 167.0 | 31 | 166.91 | 18 |
| HAM | Dichloroacetamide | DCAM | 97%[c] | 12.03 | 44.0 | 16 | 44.03 | 23 |
| HAM | Bromochloroacetamide | BCAM | 99%[a] | 13.38 | 44.0 | 22 | 44.01<br>172.91 | 13<br>6.0 |
| HAM | Dibromoacetamide | DBAM | 99%[a] | 14.08 | 44.0 | 41 | 44.01<br>216.86 | 10<br>23 |
| HAM | Chloroiodoacetamide | CIAM | 99%[a] | 15.15 | 92.0 | 38 | 91.99 | 67 |
| HAM | Bromoiodoacetamide | BIAM | 85%[a] | 16.28 | 136.0 | 50 | 135.94 | 34 |
| HAM | Diiodoacetamide | DIAM | 99%[a] | 17.91 | 184.0 | 23 | 183.93 | 20 |
| HAM | Trichloroacetamide | TCAM | 99%[b] | 13.92 | 44.0 | 7.0 | 44.03 | 18 |
| HAM | Bromodichloroacetamide | BDCAM | 99%[a] | 15.21 | 44.0 | 30 | 44.01<br>125.93 | 67<br>61 |
| HAM | Dibromochloroacetamide | DBCAM | 99%[a] | 16.44 | 44.0 | 23 | 44.01<br>128.89 | 324<br>19 |
| HAM | Tribromoacetamide | TBAM | 99%[a] | 17.59 | 44.0 | 40 | 44.01<br>172.84 | 48<br>90 |
| HNM | Dichloronitromethane | DCNM | 95%[a] | 4.77 | 83.0 | 28 | 82.96 | 22 |
| HNM | Bromochloronitromethane | BCNM | 85%[a] | 6.64 | 129.0 | 24 | 128.90 | 26 |
| HNM | Dibromonitromethane | DBNM | 90%[a] | 8.51 | 172.8 | 4.0 | 172.85 | 22 |
| HNM | Trichloronitromethane | TCNM | 95%[b] | 4.67 | 116.9 | 6.0 | 116.91 | 3.0 |
| HAL | Bromodichloroacetaldehyde | BDCAL | 90%[a] | 5.17 | 83.0 | 3.0 | 82.95 | 6.0 |
| HAL | Dibromochloroacetaldehyde | DBCAL | 90%[a] | 7.17 | 128.9 | 3.0 | 128.89 | 15 |
| HAL | Trichloroacetaldehyde | TCAL | 99%[b] | 3.82 | 82.0 | 3.0 | 81.93 | 5.0 |
| HAL | Tribromoacetaldehyde | TBAL | 99%[b] | 9.10 | 172.8 | 3.0 | 172.84 | 19 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| HK | Chloropropanone | CP | 96.8%[b] | 4.73 | 92.0 | 24 | 92.00 | 28 |
| HK | 1,1-Dichloropropanone | 11DCP | 98.2%[b] | 4.63 | 43.0 | 26 | 43.03 | 20 |
| HK | 1,3-Dichloropropanone | 13DCP | 95%[a] | 8.95 | 77.0 | 35 | 76.98 | 14 |
| HK | 1,1-Dibromopropanone | 11DBP | 90%[a] | 8.17 | 43.0 | 20 | 43.03 | 7.0 |
| HK | 1,1,1-Trichloropropanone | 111TCP | 98.7%[b] | 6.88 | 43.0 | 6.0 | 43.03 | 16 |
| HK | 1,1,3-Trichloropropnanone | 113TCP | 87.7%[b] | 10.02 | 77.0 | 2.0 | 76.98 | 12 |
| HK | 1-Bromo-1,1-dichloropropanone | 1B11DCP | 90%[a] | 8.80 | 43.0 | 3.0 | 43.03<br>124.96 | 36<br>25 |
| HK | 1,1,3,3-Tetrachloropropanone | 1133TeCP | 90%[a] | 10.92 | 83.0 | 9.0 | 82.95 | 13 |
| HK | 1,1,3,3-Tetrabromopropanone | 1133TeBP | 95%[d] | 16.13 | 200.8 | 22 | 200.83 | 30 |
| IS | 1,2-Dibromopropane | IS | 97%[b] | 5.82 | 121.0 | n.d. | 120.96 | n.d. |

DBP classes are haloacetaldehydes (HALs), haloacetamides (HAMs), haloacetonitriles (HANs), haloketones (HKs), halonitromethanes (HNMs), and iodinated trihalomethanes (I-THMs); IS = internal standard.

[a] CanSyn Chem Corp.

[b] Sigma Aldrich

[c] TCI America

[d] Aldlab Chemicals

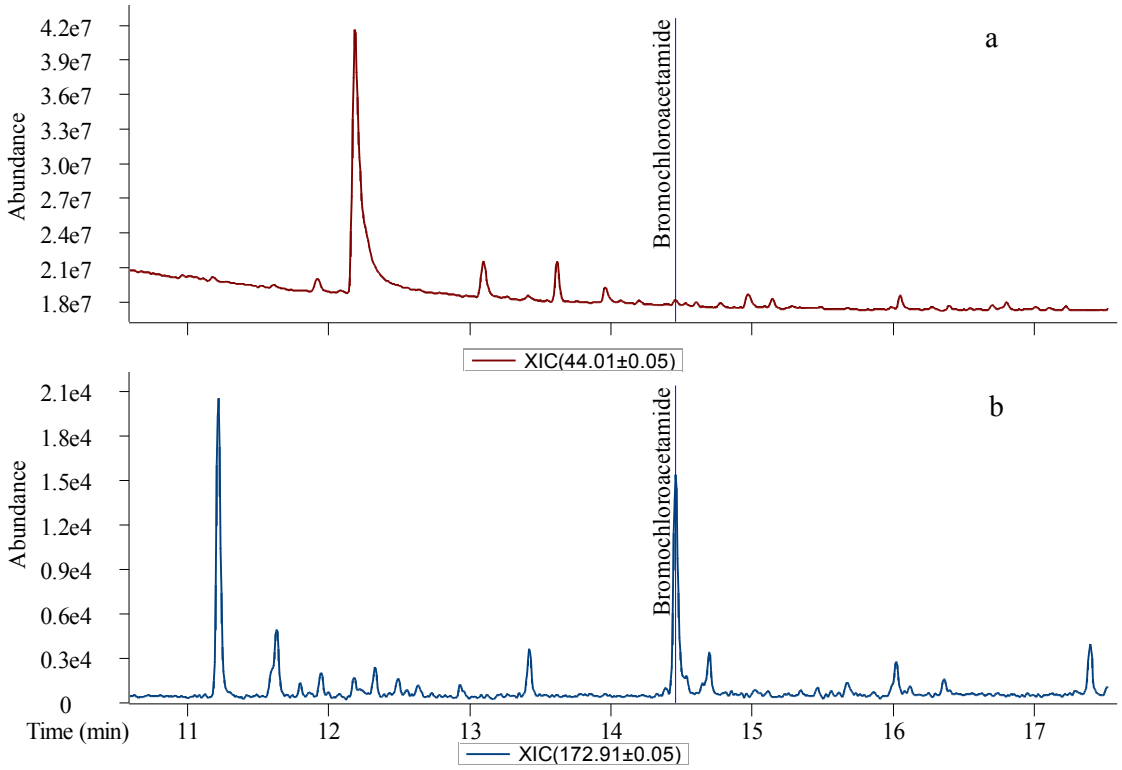


**Figure 3.1.** BT GC-TOF-MS extracted ion chromatograms of (a) m/z 44.01 and (b) m/z 172.91 for a 50 ng/L standard mix of all target DBPs. Ions 44.01 and 172.91 were used for bromochloroacetamide quantitation with method detection limits of 13 and 6 ng/L, respectively. Tradeoff between (a) higher abundance with low signal-to-noise ratio and (b) low abundance with high signal-to-noise ratio.

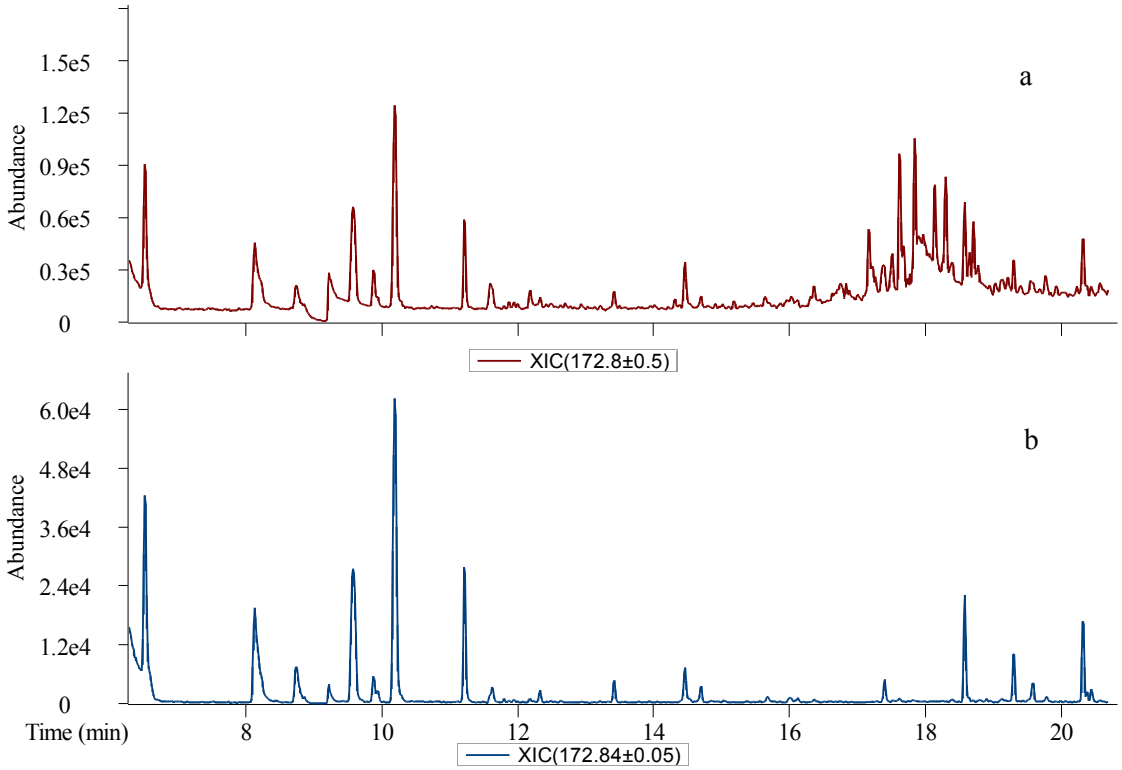


**Figure 3.2.** BT GC-TOF-MS extracted ion chromatograms. Comparison between (a) low and (b) mass accuracy of ± 0.05 Da using narrow extracted ion chromatogram widths for quantification ions for tribromoacetaldehyde. Extracted ion chromatograms (m/z 172.8 and m/z 172.84, respectively) of finished drinking waters of Site 1.

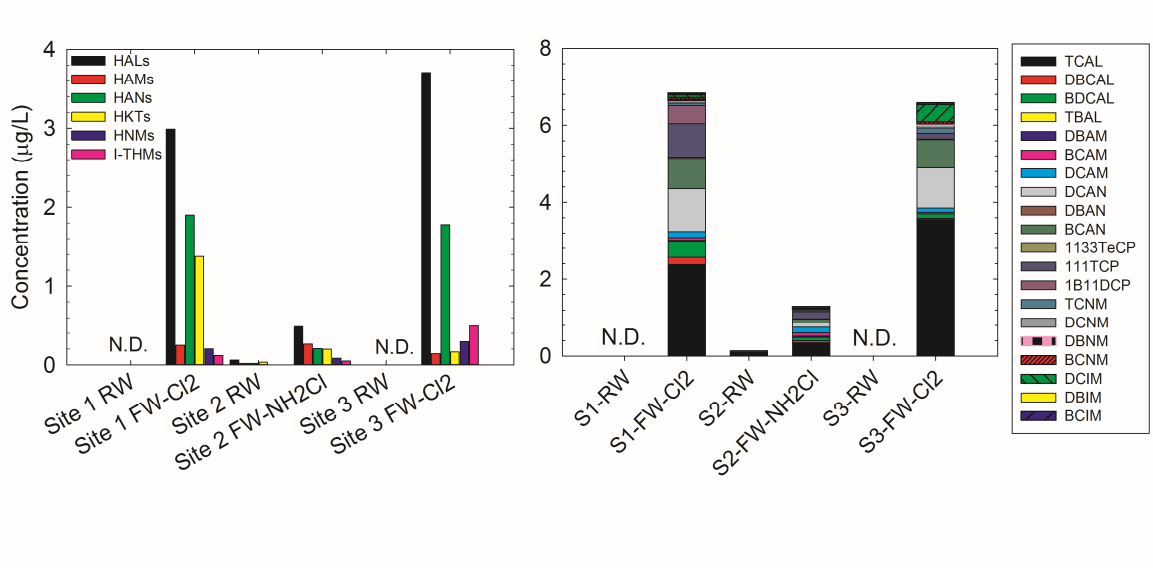


**Figure 3.3**. DBP concentrations measured with TOF-MS in raw (RW) and finished drinking (FW) waters from Sites 1, 2, and 3 that were treated with either free chlorine ($Cl_2$) or chloramines ($NH_2Cl$). Total DBPs are shown by (a) chemical class and (b) individually stacked. DBPs not detected in samples are shown as N.D.

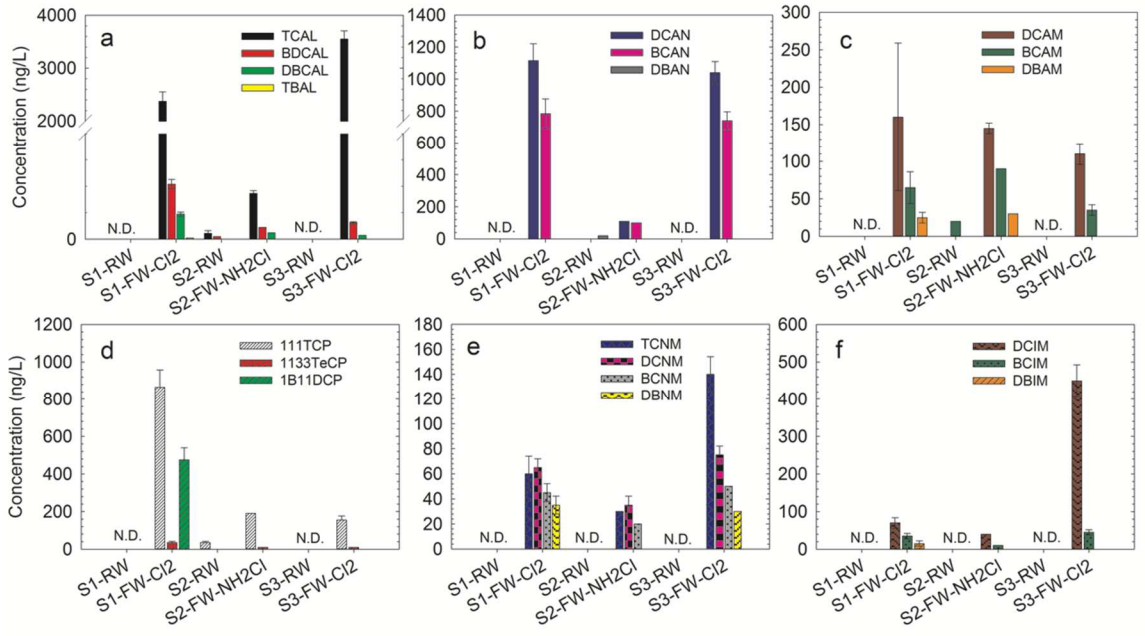


**Figure 3.4.** DBP speciation by chemical class quantified with TOF-MS in raw (RW) and finished drinking (FW) waters from three sites (S1, S2, S3) that were treated with either free chlorine ($Cl_2$) or chloramines ($NH_2Cl$). DBP speciation include a) haloacetaldehydes, b) haloacetonitriles, c) haloacetamides, d) haloketones, e) halonitromethanes and f) iodinated trihalomethanes. Samples were measured in duplicate, error bars represent the range (min, max) of the reported values. DBPs not detected in samples are shown as N.D.

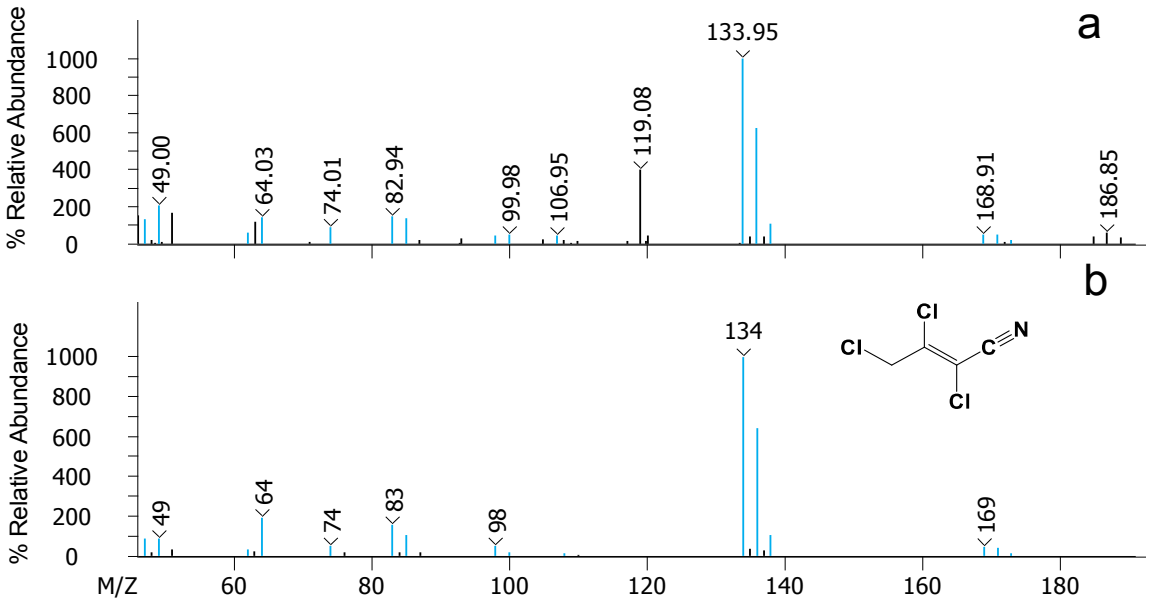


**Figure 3.5.** Mass spectra of deconvoluted peak for finished water from Site 3 (a) and *trans*-2,3,4-trichloro-2-butenenitrile library mass spectra (b).

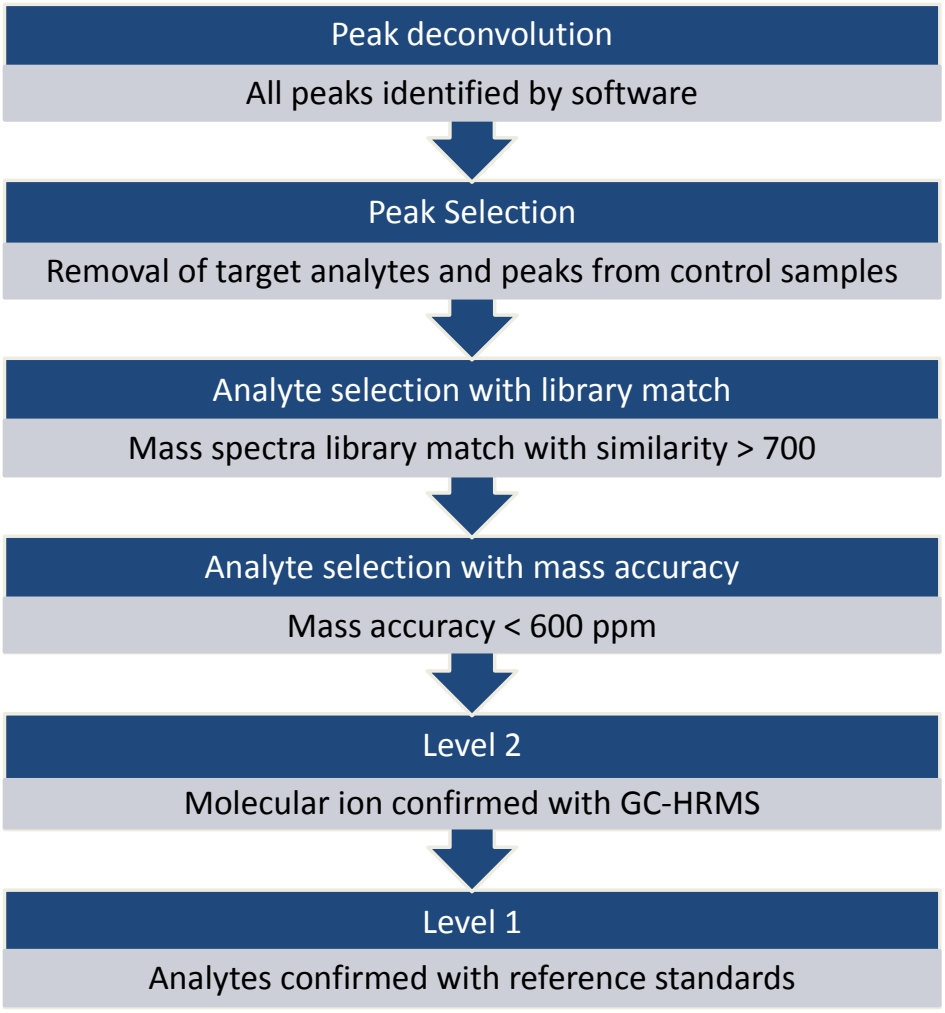


**Figure 3.6.** Criteria used for analyte identification with its corresponding level of confidence (Schymanski et al. 2014, Postigo et al. 2016). Gas chromatography-high resolution mass spectrometry (GC-HRMS) was used to obtain molecular formulas of analyte candidates with a mass accuracy of <10 ppm.

Table 3.3. Non-target compounds tentatively identified with library match > 700 score in duplicate (Level 4)[1]

| No | Compound Name | S1 RW | S1 FW $Cl_2$ | S2 RW | S2 FW $NH_2Cl$ | S3 RW | S3 FW $Cl_2$ |
|---|---|---|---|---|---|---|---|
| | ***Halogenated*** | | | | | | |
| 1 | Dibromochloromethane | | X | X | X | | X |
| 2 | Bromochloroacetaldehyde | | X | | X | | |
| 3 | Bromodichloroacetonitrile | | X | | | | X |
| 4 | Bromoform | | X | X | X | | X |
| 5 | 1-Bromo-1-chloropropan-2-one | | X | | X | | |
| 6 | Dibromochloroacetonitrile | | X | | | | X |
| 7 | Methanesulfonyl chloride | | | | | | X |
| 8 | 2-Chloropropionamide | X | | | | | |
| 9 | 1-Bromooctane | | X | | X | | |
| 10 | 1,2,3-Tribromopropane | | | X | | | |
| 11 | 2-Chloro-2-methylpropanal | | X | | | | |
| | ***Non-halogenated*** | | | | | | |
| 12 | 3-Ethyl-2-methylpentane | | | | | | X |
| 13 | Trimethoxymethane | | | | | X | X |
| 14 | 2-Methyl-1 propanol | X | | | X | X | |
| 15 | *N,N'*-Dimethylethylenediamine | | | | X | | |
| 16 | Isopropyl nitrate | | | | X | | |
| 17 | 3-Pentanone | | X | | | | |
| 18 | 2,4-Dimethyl-1,3-dioxane | X | X | X | | | X |
| 19 | 2-Methylpropanoic acid | | | | X | | |
| 20 | *tert*-Butoxyisopropoxymethane | | | | | | X |
| 21 | Butanoic acid | | | X | | | |
| 22 | *p*-Xylene or *o*-Xylene | | X | | | | |
| 23 | 2-Phenylpropane | | | X | | X | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 24 | Decane | | X | | | | |
| 25 | 2-Ethenyl-2-butenal | | | | X | | |
| 26 | 3-Furaldehyde or Furfural | | | | | X | |
| 27 | α-Methylstyrene | | | | | X | |
| 28 | Mysitylene or 1,2,4-trimethylbenzene | | | | | X | |
| 29 | Glycerol *tert*-butyl ether | | X | | | | X |
| 30 | 1-Methyl-3-isopropylbenzene | | X | | | | |
| 31 | Heptanal | | X | | | | |
| 32 | 1,3-Dioxolane-2-methanol | | | | X | | |
| 33 | 4-Hydroxy-4-methyl-2-pentanone | | X | | | | |
| 34 | 2,3,4-Trimethyldecane | | | X | | | |
| 35 | Undecane | | | | | X | |
| **No** | Compound Name | S1 RW | S1 FW $Cl_2$ | S2 RW | S2 FW $NH_2Cl$ | S3 RW | S3 FW $Cl_2$ |
| 36 | Indane | | | | | X | |
| 37 | Dodecane or 4-methylundecane | X | | | X | | X |
| 38 | 2,5-Dimethyl-2,5-dihydrofuran | X | | | | | |
| 39 | 2-Propylpyridine | X | | | | | |
| 40 | Cyclohexanone | | | | X | | |
| 41 | $N^1$-[4-(4-Methoxyphenyl)-6-(trichloromethyl)-2-pyrimidinyl]-$N^2$,$N^2$-dimethyl-1,2-propanediamine | X | | | | | |
| 42 | 2,2,7-Trimethyldecane or 2,2,5-Trimethyldecane | | | X | | | |
| 43 | 2,2-Dimethoxypropane | | | | X | | |
| 44 | 2-Methyl-2-nitro-1-propanol | | | | | | X |
| 45 | *tert*-Butyl hydroxycarbamate | | X | | | | |
| 46 | *N*,*N*-Dimethylacetamide | | | X | X | | X |
| 47 | 2-Butylpyridine | X | | | | X | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 48 | 6-Methyldodecane | X | | | | | |
| 49 | 1-Hexylcyclohexene | | | | | X | |
| 50 | Heptanonitrile | | | | X | | |
| 51 | 1-Butoxy-2-methylbutane | | | | | | X |
| 52 | Benzeneacetaldehyde | | X | | X | | X |
| 53 | 4-Cyanocyclohexene | | X | | | | |
| 54 | 2-*tert*-Butoxytetrahydrofuran | | | | | | X |
| 55 | Naphthalene | | X | X | X | | |
| 56 | Hexadecane | | X | | | | |
| 57 | Ethyl benzoate | | X | X | X | | |
| 58 | Benzothiazole | | X | X | X | X | X |
| 59 | 5-Ethyl-4-tridecanone | X | | | | | |
| 60 | 1,3-Butanediol diacetate | X | X | X | | | X |
| 61 | Phenylacetonitrile | | X | | | | |
| 62 | (2E)-3-Phenylacrylonitrile or Quinoline | | | X | X | X | |
| 63 | 5-Ethyldihydro-2(3H)-furanone | | X | | | | |
| 64 | 3-Methyl-2,4-pentanedione | | | X | X | | X |
| 65 | (4E)-6-Acetoxy-4-methyl-4-heptenoic acid | | X | | | | |
| 66 | 2-Ethylhexyl butyrate | X | | X | | | |
| 67 | 2,6,6-Trimethyl-2-cyclohexene-1,4-dione | | X | | | | |
| 68 | 2-Methyl-2-propanyl hexanoate | X | | | X | | X |
| 69 | Undecanal | X | | X | | | |
| 70 | 2-[1-(2-Pentanyloxy)ethoxy]pentane or 2-(2-Pentanyloxy)pentane | | | X | X | | X |
| 71 | 2-Ethyl-2-hexen-1-ol | X | | X | | | |
| **No** | Compound Name | S1 RW | S1 FW $Cl_2$ | S2 RW | S2 FW $NH_2Cl$ | S3 RW | S3 FW $Cl_2$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| 72 | 2-Methyl-3-phenyl-2-propenal | X | | X | | X |
| 73 | 4,7-Dimethyl-5-decyne-4,7-diol | X | | | | X |
| 74 | 2,4,7,9-Tetramethyl-5-decyn-4,7-diol | | X | | X | X |
| 75 | 4,5-Diethyl-3,5-octadiene or (2E,5E)-3,4,5,6-tetramethyl-2,5-octadiene | X | X | X | | X |
| 76 | Dodecanal | X | | X | X | |
| 77 | *N*-Nitrosodibutylamine | | X | | X | X |
| 78 | 4-Hydroxy-4-methyl-2,6-bis(2-methyl-2-propanyl) -2,5-cyclohexadien-1-one | | | X | | X |
| 79 | Dodecanoic acid methyl ester | X | X | | | |
| 80 | 4-Undecanylbenzene | | X | X | X | |
| 81 | Heptadecane | | X | | | |
| 82 | 4-Hydroxy-6-pentyltetrahydro-2H-pyran-2-one | | X | | | X |
| 83 | 3a,9-Dimethyldodecahydrocyclohepta[d]inden-3-one | X | | | | |
| 84 | 5-Dodecanylbenzene | | | X | | |
| 85 | 4-Dodecanylbenzene | | X | X | X | |
| 86 | Diphenylamine | X | | X | | |
| 87 | 3-Dodecanylbenzene | | | X | | |
| 88 | Dimethyl terephthalate | X | | X | X | |
| 89 | *o*-Cyanobenzoic acid | | | X | X | |
| 90 | 2,6-Diisopropylnaphthalene | | | | | X |
| 91 | Dodecyl acrylate | | | X | | X |

[1]Key: RW = Raw water; FW = Finished water.

Table 3.4. Non-target compounds tentatively identified with library match > 700 and mass accuracy < 600 ppm in duplicate[1]

| No | Compound Name | S1 RW | S1 FW $Cl_2$ | S2 RW | S2 FW $NH_2Cl$ | S3 RW | S3 FW $Cl_2$ |
|---|---|---|---|---|---|---|---|
| | ***Halogenated*** | | | | | | |
| 1 | Dibromochloromethane | | X | X | X | | X |
| 2 | Bromochloroacetaldehyde | | X | | X | | |
| 3 | Tribromomethane | | X | X | X | | X |
| 4 | 1-Bromo-1-chloropropan-2-one | | X | | X | | |
| 5 | Methanesulfonyl chloride | | | | | | X |
| 6 | 1-Bromooctane | | X | | X | | |
| 7 | 1,2,3-Tribromopropane | | | X | | | |
| | ***Non-halogenated*** | | | | | | |
| 8 | 3-Ethyl-2-methylpentane | | | | | | X |
| 9 | 3-Pentanone | | X | | | | |
| 10 | 2,4-Dimethyl-1,3-dioxane | X | X | X | | | X |
| 11 | 2-Methylpropanoic acid | | | | X | | |
| 12 | *tert*-Butoxyisopropoxymethane | | | | | | X |
| 13 | Butanoic acid | | | X | | | |
| 14 | *p*-Xylene or *o*-Xylene | | X | | | | |
| 15 | 2-Phenylpropane | | | X | | X | |
| 16 | Decane | | X | | | | |
| 17 | 2-Ethenyl-2-butenal | | | | X | | |
| 18 | 3-Furaldehyde or Furfural | | | | | X | |
| 19 | α-Methylstyrene | | | | | X | |
| 20 | Mysitylene or 1,2,4-trimethylbenzene | | | | | X | |
| 21 | Glycerol *tert*-butyl ether | | X | | | | X |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 22 | 1-Methyl-3-isopropylbenzene | | X | | | | |
| 23 | Heptanal | | X | | | | |
| 24 | Undecane | | | | | X | |
| 25 | Indane | | | | | X | |
| 26 | Dodecane or 4-methylundecane | X | | | X | | |
| 27 | 2,5-Dimethyl-2,5-dihydrofuran | X | | | | | |
| 28 | Cyclohexanone | | | | X | | |
| 29 | *N,N*-Dimethylacetamide | | | X | X | | X |
| 30 | 2-Butylpyridine | X | | | | | |
| 31 | 6-Methyldodecane | X | | | | | |
| 32 | 1-Hexylcyclohexene | | | | | X | |
| 33 | Benzeneacetaldehyde | | X | | X | | X |
| 34 | 4-Cyanocyclohexene | | X | | | | |
| 35 | Naphthalene or Azulene | | X | X | X | | |
| **No** | Compound Name | S1 RW | S1 FW $Cl_2$ | S2 RW | S2 FW $NH_2Cl$ | S3 RW | S3 FW $Cl_2$ |
| 36 | Ethyl benzoate | | X | X | X | | |
| 37 | Benzothiazole | | X | X | X | X | X |
| 38 | Phenylacetonitrile | | X | | | | |
| 39 | (2E)-3-Phenylacrylonitrile or Quinoline | | | X | X | X | |
| 40 | 5-Ethyldihydro-2(3H)-furanone | | X | | | | |
| 41 | 3-Methyl-2,4-pentanedione | | | X | X | | X |
| 42 | 2,6,6-Trimethyl-2-cyclohexene-1,4-dione | | X | | | | |
| 43 | 2-[1-(2-Pentanyloxy)ethoxy]pentane or 2-(2-Pentanyloxy)pentane | | | | X | | X |
| 44 | 2-Ethyl-2-hexen-1-ol | X | | X | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 45 | 2-Methyl-3-phenyl-2-propenal | X | | X | | X |
| 46 | 4,5-Diethyl-3,5-Octadiene or (2E,5E)-3,4,5,6-Tetramethyl-2,5-octadiene | X | X | X | | X |
| 47 | Dodecanal | X | | X | | |
| 48 | *N*-Nitrosodibutylamine | | X | | X | X |
| 49 | 4-Hydroxy-4-methyl-2,6-bis(2-methyl-2-propanyl) -2,5-cyclohexadien-1-one | | | X | | X |
| 50 | Dodecanoic acid methyl ester | X | X | | | |
| 51 | 4-Undecanylbenzene | | X | X | X | |
| 52 | Heptadecane | | X | | | |
| 53 | 3a,9-Dimethyldodecahydrocyclohepta[d]inden-3-one | X | | | | |
| 54 | 5-Dodecanylbenzene | | | X | | |
| 55 | 4-Dodecanylbenzene | | X | X | X | |
| 56 | Diphenylamine | X | | X | | |
| 57 | 3-Dodecanylbenzene | | | X | | |
| 58 | Dimethyl terephthalate | X | | X | X | |

[1]Key: RW = Raw water; FW = Finished water.

Table 3.5. Non-target compounds supported with high-resolution mass spectrometry data (retention time, similarity > 700, mass accuracy < 2ppm) [1].

| No. | Compound Name | S1 RW | S1 FW $Cl_2$ | S2 RW | S2 FW $NH_2Cl$ | S3 RW | S3 FW $Cl_2$ |
|---|---|---|---|---|---|---|---|
| | *Halogenated* | | | | | | |
| 1 | Dibromochloromethane | | X | X | X | | X |
| 2 | Tribromomethane | | X | | X | | X |
| | *Non-halogenated* | | | | | | |
| 3 | 3-Pentanone | | X | | | | |
| 4 | 3-Furaldehyde/Furfural | | | | | X | |
| 6 | *N,N*-Dimethylacetamide | | X | X | X | | X |
| 7 | Benzeneacetaldehyde | | X | | X | | X |
| 8 | Ethyl benzoate | | | X | | | |
| 9 | Benzothiazole | | X | X | X | X | X |
| 10 | Phenylacetonitrile | | X | | | | |
| 11 | Quinoline | | | X | X | X | |
| 12 | *N*-Nitrosodibutylamine | | X | | X | | X |

[1]Key: RW = Raw water; FW = Finished water.

Table 3.6. Non-target compounds detected with BT GC-TOF-MS and supported with high-resolution mass spectrometry (HRMS) for Site 1 finished waters (S1 – FW $Cl_2$) in duplicate

| | | HRMS | | | | | BT | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound Name** | Molecular Formula | $t_R$ (min) | Average Library match | Theoretical (m/z) | Observed (m/z) | Average Error (ppm) | Observed (m/z) | Average Error (ppm) | Identification Confidence Level |
| ***Halogenated*** | | | | | | | | | |
| **Dibromochloromethane** | $CHBr_2Cl$ | 3.94 | 921 | 205.8128 | 205.8127 | 0.5 | 205.79 | 88 | 1 |
| **Bromoform** | $CHBr_3$ | 6.15 | 861 | 249.7623 | 249.7620 | 1.2 | 249.74 | 101 | 1 |
| ***Non-halogenated*** | | | | | | | | | |
| **3-Pentanone** | $C_5H_{10}O$ | 4.96 | 730 | 86.0726 | 86.0727 | 1.2 | 86.07 | 37 | 2* |
| ***N,N*-Dimethylacetamide** | $C_4H_9NO$ | 12.19 | 922 | 87.0679 | 87.0678 | 1.1 | | | 1 |
| **Benzeneacetaldehyde** | $C_8H_8O$ | 13.26 | 817 | 120.0570 | 120.0569 | 0.8 | 120.05 | 36 | 2* |
| **Benzothiazole** | $C_7H_5NS$ | 15.02 | 878 | 135.0137 | 135.0137 | 0 | 135.01 | 61 | 1 |
| **Phenylacetonitrile** | $C_8H_7N$ | 15.66 | 797 | 117.0573 | 117.0574 | 0.9 | 117.05 | 43 | 2* |
| ***N*-Nitrosodibutylamine** | $C_8H_{18}N_2O$ | 17.95 | 796 | 158.1414 | 158.1416 | 1.3 | 158.13 | 53 | 1 |

* High resolution mass spectrometry data supported tentative structural assignments, but these were not confirmed with analytical standards.

Table 3.7. Non-target compounds detected with BT and supported with high resolution mass spectrometry (HRMS) data for Site 2 raw waters (S2 – RW) in duplicate

| | | HRMS | | | | | BT | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound Name** | Molecular Formula | $t_R$ (min) | Average Library match | Theoretical (m/z) | Observed (m/z) | Average Error (ppm) | Observed (m/z) | Average Error (ppm) | Identification Confidence Level |
| ***Halogenated*** | | | | | | | | | |
| **Dibromochloromethane** | $CHBr_2Cl$ | 3.94 | 941 | 205.8128 | 205.8128 | 0 | 205.80 | 79 | 1 |
| ***Non-halogenated*** | | | | | | | | | |
| ***N,N*-Dimethylacetamide** | $C_4H_9NO$ | 12.19 | 922 | 87.0679 | 87.0678 | 1.1 | 87.07 | 47 | 1 |
| **Ethyl benzoate** | $C_9H_{10}O_2$ | 14.65 | 838 | 150.0675 | 150.0676 | 0.7 | 150.06 | 57 | 2* |
| **Benzothiazole** | $C_7H_5NS$ | 15.02 | 831 | 135.0137 | 135.0137 | 0 | 135.01 | 51 | 2* |
| **Quinoline** | $C_9H_7N$ | 15.71 | 886 | 129.0573 | 129.0573 | 0 | 129.05 | 55 | 2* |

* High resolution mass spectrometry data supported tentative structural assignments, but these were not confirmed with analytical standards.

Table 3.8. Non-target compounds detected with BT and supported with high resolution mass spectrometry (HRMS) data for Site 2 finished waters (S2 – FW $NH_2Cl$) in duplicate

| | | HRMS | | | | | BT | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound Name** | Molecular Formula | $t_R$ (min) | Average Library match | Theoretical (m/z) | Observed (m/z) | Average Error (ppm) | Observed (m/z) | Average Error (ppm) | Identification Confidence Level |
| ***Halogenated*** | | | | | | | | | |
| **Dibromochloromethane** | $CHBr_2Cl$ | 3.94 | 908 | 205.8128 | 205.8130 | 1.0 | 205.79 | 101 | 1 |
| **Bromoform** | $CHBr_3$ | 6.15 | 902 | 249.7623 | 249.7619 | 1.6 | 249.74 | 112 | 1 |
| ***Non-halogenated*** | | | | | | | | | |
| ***N,N*-Dimethylacetamide** | $C_4H_9NO$ | 12.19 | 920 | 87.0679 | 87.0678 | 1.1 | 87.07 | 44 | 1 |
| **Benzeneacetaldehyde** | $C_8H_8O$ | 13.26 | 847 | 120.0570 | 120.0569 | 0.8 | 120.05 | 64 | 2* |
| **Benzothiazole** | $C_7H_5NS$ | 15.02 | 826 | 135.0137 | 135.0137 | 0 | 135.00 | 76 | 1 |
| **Quinoline** | $C_9H_7N$ | 15.71 | 886 | 129.0573 | 129.0573 | 0 | 129.05 | 75 | 2* |
| ***N*-Nitrosodibutylamine** | $C_8H_{18}N_2O$ | 17.95 | 796 | 158.1414 | 158.1416 | 1.3 | 158.13 | 71 | 1 |

* High resolution mass spectrometry data supported tentative structural assignments, but these were not confirmed with analytical standards.

Table 3.9. Non-target compounds detected with BT and supported with high resolution mass spectrometry (HRMS) data for Site 3 raw waters (S3 – RW) in duplicate

| | | HRMS | | | | | BT | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound Name** | Molecular Formula | $t_R$ (min) | Average Library match | Theoretical (m/z) | Observed (m/z) | Average Error (ppm) | Observed (m/z) | Average Error (ppm) | Identification Confidence Level |
| ***Non-halogenated*** | | | | | | | | | |
| **Furfural** | $C_5H_4O_2$ | 9.64 | 813 | 96.0206 | 96.0205 | 1.0 | 96.02 | 45 | 2* |
| **Benzothiazole** | $C_7H_5NS$ | 15.02 | 847 | 135.0137 | 135.0137 | 0 | 135.01 | 53 | 2* |
| **Quinoline** | $C_9H_7N$ | 15.71 | 874 | 129.0573 | 129.0573 | 0 | 129.05 | 58 | 2* |



* High resolution mass spectrometry data supported tentative structural assignments, but these were not confirmed with analytical standards.

Table 3.10. Non-target compounds detected with BT and supported with high resolution mass spectrometry (HRMS) data for Site 3 finished waters (S3 – FW $Cl_2$) in duplicate

| | | HRMS | | | | | BT | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound Name** | Molecular Formula | $t_R$ (min) | Average Library match | Theoretical (m/z) | Observed (m/z) | Average Error (ppm) | Observed (m/z) | Average Error (ppm) | Identification Confidence Level |
| ***Halogenated*** | | | | | | | | | |
| **Dibromochloromethane** | $CHBr_2Cl$ | 3.94 | 916 | 205.8128 | 205.8127 | 0.5 | 205.80 | 81 | 1 |
| **Bromoform** | $CHBr_3$ | 6.15 | 873 | 249.7623 | 249.7621 | 0.8 | 249.74 | 94 | 2* |
| ***Non-halogenated*** | | | | | | | | | |
| ***N,N*-Dimethylacetamide** | $C_4H_9NO$ | 12.19 | 920 | 87.0679 | 87.0678 | 1.1 | 87.07 | 19 | 1 |
| **Benzeneacetaldehyde** | $C_8H_8O$ | 13.26 | 829 | 120.0570 | 120.0567 | 2.5 | 120.05 | 42 | 2* |
| **Benzothiazole** | $C_7H_5NS$ | 15.02 | 865 | 135.0137 | 135.0136 | 0.7 | 135.01 | 56 | 1 |
| ***N*-Nitrosodibutylamine** | $C_8H_{18}N_2O$ | 17.95 | 861 | 158.1414 | 158.1417 | 1.9 | 158.13 | 45 | 1 |

* High resolution mass spectrometry data supported tentative structural assignments, but these were not confirmed with analytical standards.

Table 3.11. Software filters used to search for molecular ions that contain one, two, or three bromine atoms

| Parameter | Response | Syntax |
|---|---|---|
| Br | | |
| **Intensity threshold** | Intensity | m > 100 |
| **Bromine isotopes** | Relative abundance | m > 90% of m+2~0.05Da |
| | | m < 110% of m+2~0.05Da |
| | | m+4~0.05Da < 2% of m+2~0.05Da |
| **Exclude siloxanes** | Relative abundance | m+1 < m+2~0.05Da |
| **Molecular ion** | Intensity | m+Br~0.05Da < 20 |
| | | m+$Br_2$~0.05Da < 20 |
| | | m+Cl~0.05Da < 20 |
| | | m+$Cl_2$~0.05Da < 20 |
| | | m > 78 |
| $Br_2$ | | |
| **Intensity threshold** | Intensity | m > 100 |
| **Bromine isotopes** | Relative abundance | m > 44% of m+2~0.05Da |
| | | m < 58% of m+2~0.05Da |
| | | m+4~0.05Da >42% of m+2~0.05Da |
| | | m+4~0.05Da <56% of m+2~0.05Da |
| **Exclude siloxanes** | Relative abundance | m+1 < m+2~0.05Da |
| **Exclude $Br_1Cl_2$** | Relative abundance | m+5.99~0.05Da < 2% of m+2~0.05Da |
| **Molecular ion** | Intensity | m+Br~0.05Da < 20 |
| | | m+$Br_2$~0.05Da < 20 |
| | | m+Cl~0.05Da < 20 |
| | | m+$Cl_2$~0.05Da < 20 |
| | | m > 157 |
| $Br_3$ | | |
| **Intensity threshold** | Intensity | m > 100 |
| **Bromine isotopes** | Relative abundance | m > 29% of m+2~0.05Da |
| | | m < 41% of m+2~0.05Da |
| | | m+4~0.05Da > 85% of m+2~0.05Da |
| | | m+4~0.05Da < 100% of m+2~0.05Da |
| | | m+5.99~0.05Da > 27% of m+2~0.05Da |
| | | m+5.99~0.05Da < 37% of m+2~0.05Da |
| **Exclude siloxanes** | Relative abundance | m+1 < m+2~0.05Da |
| **Exclude $Br_2Cl_2$** | Relative abundance | m+7.99~0.05Da < 2% of m+2~0.05Da |
| **Molecular ion** | Intensity | m+Br~0.05Da < 20 |
| | | m+$Br_2$~0.05Da < 20 |
| | | m+Cl~0.05Da < 20 |
| | | m+$Cl_2$~0.05Da < 20 |
| | | m > 236 |

Table 3.12. Software filters used to search for molecular ions that contain one, two or three chlorine atoms

| Parameter | Response | Syntax |
|---|---|---|
| Cl | | |
| **Intensity threshold** | Intensity | m > 100 |
| **Chlorine isotopes** | Relative abundance | m > m+2~0.05Da |
| | | m+2~0.05Da > 27% of m |
| | | m+2~0.05Da < 39% of m |
| **Exclude siloxanes** | Relative abundance | m+1 < m+2~0.05Da |
| **Molecular ion** | Intensity | m+Br~0.05Da < 20 |
| | | m+$Br_2$~0.05Da < 20 |
| | | m+Cl~0.05Da < 20 |
| | | m+$Cl_2$~0.05Da < 20 |
| $Cl_2$ | | |
| **Intensity threshold** | Intensity | m > 100 |
| **Chlorine isotopes** | Relative abundance | m+2~0.05Da > 54% of m |
| | | m+2~0.05Da < 74% of m |
| | | m+4~0.05Da >5% of m~0.05Da |
| | | m+4~0.05Da <15% of m~0.05Da |
| **Exclude siloxanes** | Relative abundance | m+1 < m+2~0.05Da |
| **Molecular ion** | Intensity | m+Br~0.05Da < 20 |
| | | m+$Br_2$~0.05Da < 20 |
| | | m+Cl~0.05Da < 20 |
| | | m+$Cl_2$~0.05Da < 20 |
| $Cl_3$ | | |
| **Intensity threshold** | Intensity | m > 100 |
| **Chlorine isotopes** | Relative abundance | m+2~0.05Da > 82% of m |
| | | m+2~0.05Da < 110% of m |
| | | m+4~0.05Da > 26% of m~0.05Da |
| | | m+4~0.05Da < 36% of m~0.05Da |
| **Exclude siloxanes** | Relative abundance | m+1 < m+2~0.05Da |
| **Molecular ion** | Intensity | m+Br~0.05Da < 20 |
| | | m+$Br_2$~0.05Da < 20 |
| | | m+Cl~0.05Da < 20 |
| | | m+$Cl_2$~0.05Da < 20 |

# CHAPTER 4

# DOES GAC WITH CHLORINATION PRODUCE SAFER DRINKING WATER? FROM DBPS AND TOX TO CALCULATED TOXICITY[4]

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**Abstract**

Granular activated carbon (GAC) adsorption is well-established for controlling regulated disinfection by-products (DBPs), but its effectiveness for unregulated DBPs and DBP-associated toxicity is unclear. In this study, GAC treatment was evaluated at three full-scale chlorination drinking water treatment plants over different GAC service lives for controlling 61 unregulated DBPs, 9 regulated DBPs, and speciated total organic halogen (total organic chlorine, bromine, and iodine). Plants represented a range of impacts, including algal, agricultural, and industrial wastewater. This study represents the most extensive full-scale study of its kind and seeks to address the question of whether GAC can make drinking water safer from a DBP perspective. Overall, GAC was effective for removing DBP precursors and reducing DBP formation and total organic halogen, even after >22,000 bed volumes of treated water. GAC also effectively removed preformed DBPs at plants using pre-chlorination, including highly toxic iodoacetic acids and haloacetonitriles. However, 7 DBPs (mostly brominated and nitrogenous) increased in formation after GAC treatment. In one plant, an increase in tribromonitromethane had significant impacts on calculated cytotoxicity, which only had 7-17% reduction following GAC. While these DBPs are highly toxic, the total calculated cytotoxicity and genotoxicity for the GAC treated waters for the other two plants was reduced 32-79% (across young-middle-old GAC). Overall, calculated toxicity was reduced post-GAC, with pre-oxidation allowing further reductions.

## Introduction

Drinking water disinfection is vital for prevention of waterborne illness. Since its introduction in the U.S. in the early 1900s, disinfection is reported to have contributed significantly to an estimated 29-year increase in life expectancy.[1] An unintended consequence of disinfection is the formation of disinfection by-products (DBPs), which have been associated with adverse health effects, including bladder cancer, colon cancer, miscarriage, and birth defects.[2-10] In the U.S., regulations are enforced for four trihalomethanes (THMs), five haloacetic acids (HAAs), bromate, and chlorite under the Stage 2 Disinfectants and DBP Rule.[11] Several recent studies indicate that while THMs and HAAs are the dominant DBPs formed upon chlorination, they are not necessarily drivers of toxicity associated with DBP formation.[12-14]

DBPs are formed by the reaction of disinfectants with natural organic matter (NOM), bromide, and iodide.[6, 15] Many NOM fractions can react to form THMs and HAAs, while phenolic NOM structures have been shown to form haloacetaldehydes (Table 4.3, Supporting Information [SI]).[16] Free and combined amino acids, aldehydes, and aromatic NOM have been shown to form haloacetamides and haloacetonitriles.[17-19] The presence of inorganic nitrogen, such as ammonia, nitrite and nitrate, can play a role in the formation of nitrogenous DBPs (N-DBPs),[20] which are generally more toxic than DBPs without nitrogen,[21-24] yet no N-DBPs are currently regulated. Extensive studies have shown that iodo-DBPs, which are also not regulated, are typically more toxic than

brominated DBPs (Br-DBPs), which are much more toxic than chlorinated analogues.[25-28] Table 4.3 summarizes precursors associated with each DBP class measured in this study.

Use of GAC is well-established for controlling THMs and HAAs because it can effectively remove NOM fractions that serve as their precursors.[13, 29-31] Studies indicate that some N-DBP precursors are not as readily removed using GAC.[13, 32] GAC columns are biologically active, even if the influent contains a disinfectant, as GAC will reduce the disinfectant at the top of the GAC column, allowing biomass to grow in the rest of the bed.[33] Thus, in addition to adsorption of DBP precursors, biodegradation plays a role in GAC treatment.[33]

Many drinking water plants use pre-oxidation (e.g., pre-chlorination), which may result in the formation of DBPs within the treatment system. We refer to these as "preformed" DBPs; they can be removed by GAC, or with additional disinfection, can act as precursor material for the formation of other DBPs.[17, 18] One potential benefit of pre-oxidation is transformation of NOM into intermediate aromatic halogenated DBPs which may be more easily removed by GAC than larger precursor molecules.[34]

GAC preferentially removes dissolved organic carbon (DOC) over dissolved organic nitrogen (DON), and it does not remove bromide.[31, 35, 36] Therefore, the DON:DOC and $Br^-$:DOC ratios increase across GAC adsorbers, which may result in

increased formation of N- and Br-DBPs, due to increased competition of HOBr.[13, 31, 35, 36] Higher $Br^-$:DOC ratios cause a shift in halogen speciation to more brominated THMs, and some brominated THM concentrations can be higher after GAC treatment.[35, 36] A shift from dichloroacetonitrile to dibromoacetonitrile has also been reported in a bench-scale GAC study.[13] Shifts in halogen speciation to more cytotoxic and genotoxic brominated DBPs must be evaluated for possible adverse health implications.

Given concerns about potential increased formation of Br- and N-DBPs, it is important to ask: Do GAC treated waters have lower associated toxicity than waters not treated with GAC? One way to address this is the "TIC-Tox" approach, which multiplies molar concentrations of individual DBPs by their corresponding cytotoxicity and genotoxicity index values.[14] Other previously published studies have used this approach in modeling toxicity of DBP mixtures, [14, 37, 38] and have shown increases in calculated genotoxicity following GAC.[13] This approach can also be used to assess which DBPs are toxicity drivers, regulated or otherwise.

The goal of our study was to assess the effectiveness of full-scale GAC treatment at chlorination plants for controlling: (1) human exposure to a wide range of 70 regulated and unregulated DBPs, as well as speciated total organic halogen (TOCl, TOBr, TOI), and (2) the calculated toxicity associated with these DBPs. Both removal of preformed DBPs and control of DBPs under conditions that represent the utilities' distribution systems; i.e., simulated distribution system (SDS) conditions, were assessed. Using each

plant's SDS conditions allows for the study of more realistic DBP concentrations, and therefore, exposure to real populations within those systems. Water samples were collected at three full-scale chlorination plants in the U.S., with DBP control evaluated across GAC service life (e.g., youngest, middle-aged, and oldest GAC). At each plant, the same influent water was used across three different aged filters on the same type of carbon, allowing for the comparison of filter age with the same DBP composition. Because source water quality can impact the types of DBPs that form, plants were carefully chosen to represent a wide range of impacts, including algal, agricultural, and industrial wastewater impacts. This study was limited to three full-scale chlorine plants due to the extensive analysis required. Formation of 70 DBPs, including haloacetonitriles (HANs), haloacetamides (HAMs), halonitromethanes (HNMs), haloacetaldehydes (HALs), haloketones (HKs), iodinated acetic acids (IAAs), iodinated trihalomethanes (I-THMs), THMs, and HAAs, was studied during full-scale pre-chlorination (preformed DBPs) at two plants and in bench-scale SDS tests at all three plants. This is the most extensive list of DBPs studied across GAC filter lifetimes. TOCl, TOBr, and TOI were also measured and compared to the total measured DBP concentrations, with the balance representing *unknown* DBPs. This is the first study that evaluated TOX across GAC lifetimes in full-scale plants. Preformed DBPs were evaluated before and after GAC treatment, providing information on their adsorbability and/or biodegradability, which is unknown for many emerging DBPs. Cytotoxicity and genotoxicity were calculated using the TIC-Tox method across GAC lifetime.[14] Most importantly, this study seeks to address whether DBP concentrations correlate with calculated toxicity, and which DBPs are the driving forces of toxicity across GAC age.

## Materials and Methods

### *Sampling of drinking water treatment plants*

Three full-scale drinking water plants were sampled; for each plant, three GAC service lives were evaluated, which was quantified in terms of throughput in bed volumes (BV, i.e. the volume of water treated relative to the GAC bed volume) and was different for each plant to reflect early, middle, and late stages of GAC operation. Operating characteristics and source water quality parameters for each plant and water sample are summarized in Table 4.1. Total organic carbon (TOC), absorbance at 254 nm ($UV_{254}$), specific ultraviolet absorbance (SUVA), bromide, iodide, and total nitrogen (TN) were quantified as surrogates for DBP precursors (Table 4.1). All plants used one or more pre-oxidants prior to GAC treatment (chlorine dioxide and chlorine in Plant 1, $KMnO_4$ and chlorine in Plant 2, $KMnO_4$ in Plant 3) and relied on chlorine as the primary disinfectant post-GAC and as the secondary disinfectant throughout the distribution system. Information regarding sampling dates, flow rates, empty bed contact times (EBCT), GAC type, and DOC breakthrough is found in Table 4.4. Due to the real-world nature of this study, the activated carbons were slightly different at the different plants studied, but they were consistent within each plant, allowing the impact of carbon age and impact of GAC vs. no GAC to be evaluated at each plant. Schematic diagrams of each plant are found in Figures 4.7-4.10. All plants operated GAC contactors in a staged parallel mode and blended the effluents. GAC influents and effluents at different service times were collected. Plant 3 was sampled on two occasions: in the first event, samples were taken after treating 9,200 BV of water; the second event occurred after GAC was replaced in two contactors.

### *Chlorination of samples*

For each sample, water was analyzed for preformed DBPs, and SDS testing was carried out according to protocols set by each plant (Text S1). Chlorine residual concentrations and contact times were equivalent to each plant's longest water age in the distribution system (3-7 days). For example, Plant 1 was pH adjusted with borate buffer to 8.0 and spiked with 1.0 to 4.0 mg/L to achieve a chlorine residual of approximately 1.0 mg $Cl_2$/L after 24 h of reaction.

Samples were collected in duplicate in two 1-L bottles, one containing ascorbic acid and one ammonium chloride (quenching agents; chlorine to quencher molar ratio of 1:1.3 based on an assumed maximum potential residual chlorine concentration of 5 mg/L as $Cl_2$) and adjusted to pH 3.5-4 with 1 M $H_2SO_4$. Samples were shipped cold overnight and extracted the same day received or stored at 4°C and extracted within 2 days. Quantified DBPs were stable over this storage time.[39-41] Further details are provided in Text S2.

### *Chemicals and reagents*

Analytical standards for unregulated DBPs (Table 4.5) were purchased or custom-synthesized at the highest purity available (CanSyn Chem. Corp., Toronto, Ontario; Sigma-Aldrich, St. Louis, MO; Aldlab Chemicals, Boston, MA; TCI America, Boston, MA). Organic solvents were of the highest purity. Acetonitrile, methyl *tert*-butyl ether (MTBE), methanol, hexane, and pure water were purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA).

### *Analytical Methods*

***Background water quality and regulated DBPs.*** Water quality parameters (residual chlorine, DOC, $UV_{254}$, SUVA, TN, ammonia, nitrite, nitrate, bromide and iodide) were measured using methods described in Table 4.6. THMs and HAAs were measured using EPA Methods 551.1 and 552.3, respectively.[42, 43]

***Unregulated DBPs.*** Three extraction methods and two derivatization methods were required to analyze 57 unregulated DBPs.[41] For, HANs, HKs, I-THMs, HNMs, and tri-HALs, a single liquid-liquid extraction (LLE) with 100 mL of sample, 2 mL MTBE, and 30 g sodium sulfate was conducted for samples quenched with ascorbic acid (Text S3.1). For HAMs, IAAs, and a subset of compounds (bromodichloronitromethane, dibromochloronitromethane, tribromonitromethane, and tribromoacetonitrile), 100 mL were pH adjusted with $H_2SO_4$ to pH <2, followed by multiple LLEs (x3) were conducted with 5 mL MTBE and 30 g sodium sulfate (samples quenched with ammonium chloride), followed by concentrating under nitrogen (Text S3.2). Final extracts were spiked with 1,2-dibromopropane internal standard and analyzed using gas chromatography (GC)-mass spectrometry (MS) with electron ionization (EI) and selected ion monitoring (SIM) (7890 GC, 5977A mass spectrometer, Agilent Technologies, Santa Clara, CA) with a Rtx-200 column (30m x 0.25mm x 0.25 µm film thickness; Restek Corporation, Bellefonte, PA). A portion of the extract was removed for IAA analysis, which required diazomethane derivatization (Text S3.3),[41, 44] followed by GC-EI-MS/MS analysis (TRACE GC Ultra, Quantum GC™ MS/MS, Thermo Scientific, Waltham, MA). Mono-

and di-haloacetaldehydes were analyzed using O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA) derivatization followed by LLE and GC-EI-MS analysis[45] (Text S3.4). The summed mass concentration of all regulated and unregulated DBPS was termed as "DBP sum".

Minimum reporting limits (MRLs) for most compounds in this study were 0.10 µg/L, excluding IAAs, chloroacetamide (CAM), bromoacetamide (BAM), and iodoacetamide (IAM) which had reporting limits of 0.025, 0.75, 5.0, and 0.75 µg/L, respectively. CAM, BAM and IAM were not detected in this study.

***Total organic halogen (TOX).*** TOCl, TOBr, and TOI were determined using a TOX analyzer (Mitsubishi Chemical Analytech, Chigasaki, Japan; Cosa Xentaur, Yaphank, USA) [37, 46-48] (Text S4). Briefly, acidified samples (pH <2) were adsorbed on activated carbon, washed with nitric acid, and combusted at 1000°C in the presence of oxygen and argon as a carrier gas. Combusted gases were collected in a fresh aqueous solution containing 0.03% $H_2O_2$, which was analyzed for chloride using a Dionex 1600 ion chromatograph (Dionex, Sunnyvale, CA).[37, 46-48] An inductively coupled plasma (ICP)-mass spectrometer (Finnigan ELEMENT XR, Thermo Electron Corporation) was used for trace-level bromide and iodide analysis.[48, 49]

***Contributions of DBP classes to TOX and toxicity.*** The contribution of each DBP class to TOX was calculated by first multiplying the molar concentration of each compound pertaining to a specific DBP class by its corresponding number of halogens (i.e., 1-4 halogen atoms). Then, these values were added and divided by the sum molar concentration of TOCl, TOBr, and TOI. For example, the percent contribution of haloacetonitriles to TOCl (%$HAN_{TOCl}$) and the percent contribution of haloacetonitriles to TOX (%$HAN_{TOX}$) are defined as

$$\%HAN_{TOCl} = \frac{\sum b[CH_aCl_bBr_cI_dCN]}{[TOCl]} * 100\% \quad (1)$$

$$\%HAN_{TOX} = \frac{\sum (b+c+d)[CH_aCl_bBr_cI_dCN]}{[TOCl]+[TOBr]+[TOI]} * 100\% \quad (2)$$

where, a is the number of hydrogens, and b, c, and d are the number of chlorine, bromine, and iodine atoms for each individual HAN. Similar equations were used for other DBP classes.

Toxicity associated with DBPs in each sample was based on the "TIC-Tox" method.[14] In brief, molar concentrations of each DBP were mulitplied by their corresponding cyto- or genotoxicity index values for Chinese hamster ovary cells (CHO) and summed together.[14, 50] (Equations 3 and 4).

$$Total\ Calculated\ Water\ Cytotoxicity = \sum([DBP] * {LC_{50}}^{-1} * 10^6) \quad (3)$$

$$Total\ Calculated\ Water\ Genotoxicity = \sum([DBP] * 50\%\ TDNA^{-1} * 10^6) \quad (4)$$

where [DBP] is the molar concentration of each DBP, the cytotoxicity index is the inverse of the lethal concentration at 50 % ($LC_{50}$) in M, the genotoxicity index is the inverse of the 50% tail DNA (50%TDNA) measurement in M, and $10^6$ is a normalization factor.

***Results and Discussion***

***Overview.*** We evaluated the effectiveness of GAC for the removal of: (1) DBPs that formed via pre-chlorination and (2) DBP precursors. The former is important because many drinking water utilities add chlorine for iron and manganese control to filters that precede GAC adsorbers. Thus, it is common that these influents contain DBPs. Two of the three drinking water utilities evaluated used pre-chlorination, providing an opportunity to conduct a comprehensive evaluation of the effectiveness of GAC treatment for removal of preformed DBPs. Removal of DBP precursors is also critically important because many utilities use chlorine to meet disinfection requirements for GAC-treated water and to maintain a disinfectant residual in the distribution system. Chlorinated GAC influent water simulates DBP levels expected at the consumers' tap in the absence of GAC treatment, and chlorinated GAC effluent samples permit an evaluation of DBP precursor removal and effectiveness for controlling DBPs expected at the consumers' tap. This study provided an opportunity to assess realistic exposure

concentrations for each plant. The following sections are organized as follows: First, a general discussion of DBP formation at the three plants is presented; second, GAC effectiveness for the control of preformed DBPs is presented; third, DBP precursor control by GAC is discussed by presenting results from SDS tests; and fourth, GAC effectiveness for reducing cyto- and genotoxicity, as calculated from measured DBP levels, is discussed.

***DBP formation at the three full-scale plants***

As expected, Plants 1, 2, and 3, which have different impacts to their source waters (Table 4.1) exhibited different DBP formation (Table 4.8, 4.10, 4.11, and 4.12). Plant 2, which has agricultural and industrial impacts (indicated by discharge permits upstream of the plant), formed higher levels of N-DBPs, such as HAMs, HANs, and HNMs (Table 4.10). Water impacted by agriculture and industry often results in increased levels of inorganic and organic nitrogen in source waters, which can form increased N-DBP levels.[51-54] Plant 1, with algal impacts, formed moderate levels of trihalonitromethanes (Table 4.8), consistent with previous studies that have shown formation of trichloronitromethane from algae, while it is unknown whether a bloom was occurring during sampling.[55-59] Plant 3 has minimally impacted source water (and low concentrations of bromide and iodide) and produced mostly chlorinated DBPs. Forty-six DBPs (of 70 measured) were detected among all three plants, two of which were only preformed (1,1-dibromopropanone, 1,1-dichloropropanone).

***Behavior of preformed DBPs in GAC contactors***

As a result of pre-chlorination (and potentially pre-chlorine dioxide treatment), Plants 1 and 2 had measurable preformed DBPs, most of which were completely removed by GAC over the evaluated service times (Figure 4.1). Thirteen preformed DBPs, including HAMs, HANs, HKs, HNMs, IAAs, THMs, and HAAs (Table 4.7), were measured in Plant 1 GAC influent; eight were completely removed. The total molar concentration of measured preformed DBPs at Plant 1 accounted for approximately 1-10% of TOCl, 2-30% of TOBr, and 0-11% of TOI. At Plant 2, 37 preformed DBPs, including HALs, HAMs, HANs, HKs, HNMs, I-THMs, THMs, and HAAs (Table 4.9), were measured in the GAC influent; 30 were completely removed. The total molar concentration of measured preformed DBPs at Plant 2 accounted for 26-85% of TOCl, 25-78% of TOBr, and 0-100% of TOI. Both adsorption and biodegradation can contribute to DBP removal by GAC. Previous studies modeled adsorption breakthrough using the pH-dependent octanol-water partition coefficient (logD).[60] Lower logD values indicate higher hydrophilic character and, in general, lower adsorbability to GAC. DBPs measured in this study have logD values ranging from -3.70 to 3.66 (Table 4.5). Molecular weight, polarizability, and charged surface interactions are also important factors impacting GAC adsorption.[30] Because GAC columns are biologically active, correlations between GAC adsorption removal and logD values may under-predict removal of biodegradable DBPs in biologically active GAC. For example, IAAs have negative logD values, suggesting they are poorly adsorbable; but preformed IAAs were removed 100% at both Plants 1 and 2 (Figure 4.1), indicating that they may be biodegradable, as are many other haloacetic acids.[61] DBPs that were completely removed covered the entire range of logD values

studied, which suggests that both biodegradation and adsorption contributed to their removal. *The effectiveness of GAC treatment for the removal of most of the DBPs evaluated in this study has not been reported in the literature, including removal for the highly toxic IAAs, I-THMs, and iodinated HAMs.*

Five preformed DBPs were detected in Plant 1 GAC effluent: bromochloroacetamide, 1-bromo-1,1-dichloropropanone, bromodichloronitromethane, and two THMs (trichloromethane, bromodichloromethane) (Table 4.7). Seven preformed DBPs were detected in Plant 2 GAC effluent: two HAMs (bromochloroacetamide, bromoiodoacetamide), two THMs (trichloromethane, dibromochloromethane), and three HAAs (trichloroacetic acid, bromodichloroacetic acid, and dibromochloroacetic acid) (Table 4.9). In Plant 1, 1-bromo-1,1-dichloropropanone (logD 1.21) yielded complete breakthrough at early GAC service times (Table 4.7). At both plants, bromochloroacetamide (logD 0.35, 0.4 µg/L), and at Plant 1, bromodichloronitromethane (logD 2.63, 0.8 µg/L), broke through early (5600 BV), but were not present in the effluent at later times. This behavior is indicative of biological removal, as older GAC columns tend to have higher biological activity.[33] Trichloromethane (logD 1.91) at both plants, bromodichloromethane (logD 2.04) at Plant 1, and dibromochloromethane (logD 2.21) at Plant 2 displayed classic breakthrough behavior; i.e. adsorptive removal of THMs became less effective with increasing GAC service life, consistent with previous studies.[62, 63] Interestingly, in Plant 2, bromoiodoacetamide was not detected in the GAC influent, but was present in the GAC effluent at two of the three GAC service times (1.3 and 1.1 µg/L). This could be due to a surface-catalyzed reaction with chlorine (chlorine

was present in the GAC influent). Previous studies have shown that GAC can act as an effective catalyst in the oxidation and reduction of micropollutants where oxygen, nitrogen, and available functional groups on the surface of GAC play an important role.[64] These types of reactions may also cause the reductive dehalogenation of a trihaloamide and subsequent formation of bromoiodoacetamide. More research is needed to understand these possible reactions. Haloamides may also from form the hydrolysis of haloacetonitriles (Table 4.3).

The normalized breakthrough behavior of TOCl, TOBr, TOI, and total DBP sum is shown in Figure 4.10 and 4.11 for preformed DBPs at Plant 1 and Plant 2, respectively. GAC was effective in removing >50% of TOCl, TOBr, and total DBP sum over the studied throughput range of 22,000 bed volumes (>10 months of GAC service life at Plant 2).

***Impact of GAC on simulated distribution system (SDS) DBPs***

GAC effectively controlled SDS-DBPs at all three chlorination plants (Figure 4.2 and S6). The DBP sum decreased after treatment, with control ranging from 48 to 82% at Plant 1, 58 to 89% at Plant 2, and 6 to 67% at Plant 3, decreasing with service time. These combined results correlate with TOC removal (Figures 4.13-4.15), and similar correlations were obtained with $UV_{254}$ (Table 4.1). When accounting for molar concentrations, the total measured SDS Cl-DBPs accounted for 15-40% of the TOCl at Plant 1, 51-57% at Plant 2, and 53-100% at Plant 3. The total measured SDS Br-DBPs accounted for 45-52% of the TOBr at Plant 1, 73-100% at Plant 2, and 38-45% at Plant 3. Total measured I-DBPs only accounted for 3-10% of the TOI at Plant 1, 0-4% at Plant 2,

and <1% at Plant 3. TOCl, TOBr, and TOI were also lower after GAC treatment at all three plants (Figure 4.3, 4.16-4.19) for the duration of GAC service times. At Plants 1 and 2, TOI was the most effectively controlled TOX parameter, while TOBr was the most poorly controlled at all three plants. Bromide, iodide, TOBr, and TOI measurements were taken for the pre-chlorinated GAC influent water and not the raw water. TOBr and TOI exceeded bromide and iodide concentrations of GAC influent in some cases, which we believe is caused by pre-oxidation conversion to HOBr and HOI, which would not be detected by ion chromatography methods used to measure bromide and iodide. Another possible explanation is that brominated and iodinated contaminants, such as brominated flame retardants or iodinated X-ray contrast media may have been present in the raw source waters and contributed to the TOBr and TOI levels observed. [65, 66, 67] As expected, DBP control was most effective at the youngest GAC service life and decreased with increasing service life (Figure 4.2 and 4.12). However, GAC adsorbers at the longest service life still controlled overall SDS-DBP values (TOX and DBP sum concentrations) when compared to using no GAC.

***DBPs not well controlled by GAC***

While SDS concentrations of 28 of the 44 DBPs detected were effectively controlled by GAC, ten DBPs were unaffected, i.e, the concentrations were not significantly different between GAC influent and effluent (two-tailed t-test, 1 degree of freedom (d.f.), 95% confidence interval (CI)) (Table 4.2). In addition, the SDS concentrations of seven DBPs were *higher* in the GAC effluent (one-tailed t-test, 1 d.f.,

95% CI): tribromoacetonitrile, 1,1,1-trichloropropanone, tribromonitromethane, trichloronitromethane, bromodichloronitromethane, dibromochloromethane, and tribromomethane (Table 4.2). For example, tribromoacetonitrile increased in formation following GAC treatment at Plant 2 from below detection (MRL of 0.1 µg/L) to 0.7 µg/L (Table 4.10). Twelve of these 16 DBPs were brominated, and the lack of control or increase in Br-DBPs after GAC is consistent with earlier studies that showed increases in regulated brominated THMs,[35, 36, 68] as well as recent work showing an increase in dibromoacetonitrile.[32,13] Increased Br-DBP formation is partially explained by an increased $Br^-$:TOC ratio, as TOC is removed by GAC, while $Br^-$ is not, allowing increased competition of HOBr.[35, 36] TOBr:TOC ratios in SDS samples increased by 155-207% from 17.5 µg/L at Plant 2, and increased 144-205% from 0.9 and 1.9 µg/L at Plant 3, respectively as a result of GAC treatment (Table 4.14), but TOBr was still controlled overall by GAC, even after treating 22,000 BV of water (Plants 1 and 2, Figure 4.3). One compound was unchanged in one plant and higher in another plant (1,1,1-trichloropropanone).

N-DBP precursors were not well removed, as HANs, HNMs, and HAMs either increased in formation or remained unchanged. For example, tribromonitromethane increased in formation following GAC treatment at Plant 1 from below detection (MRL of 0.1 µg/L) to 1.9 µg/L (Figure 4.4). This significant increase, like bromoiodoacetamide, may be due to desorption or surface catalyzed reactions. A previous study showed formation of nitrosamines from GAC catalyzed oxidation of amines.[64] Another study showed that GAC can activate nitrite, which can nitrosate amines in turn forming

nitromethanes.[69] As GAC filters age, biofilms develop and can shed precursors for N-DBPs.[70] Future research is needed to understand the formation of other N-DBPs within GAC. Nine N-DBPs including bromochloroacetamide, dibromoacetamide, dichloroacetamide, tribromoacetonitrile, bromochloroacetonitrile, dibromoacetonitrile, trichloronitromethane, tribromonitromethane, and bromodichloronitromethane (Table 4.2, 4.7, and 4.8) were either poorly controlled or increased following GAC treatment, consistent with two recent studies that investigated HANs[13, 32] and one HNM.[32]

***Preformed vs. SDS-DBPs***

At Plant 1, 11 of the 23 SDS-DBPs formed in the GAC influent were also prefomed DBPs (formed by pre-chlorination and pre-chlorine dioxide), and at Plant 2, 29 of the preformed DBPs (formed by pre-chlorination) were among the 35 SDS-DBPs. Thus, only 12 and 6 DBPs, respectively, were in the SDS samples that were not in the preformed samples at Plants 1 and 2. At both plants, some of the unregulated DBPs (e.g. bromodichloronitromethane, bromodichloroacetaldehyde, dichloroiodomethane, and chloroiodoacetic acid) had similar concentrations in the SDS and preformed samples (Table 4.7-4.10), indicating that their formation was precursor-limited; i.e., additional chlorine had little effect on their formation. In contrast, concentrations of all of the THM4, HAA9 species, and some unregulated DBPs (e.g. trichloroacetaldehyde, dichloroacetonitrile, bromochloroacetonitrile, dichloroacetamide, dibromoacetamide, trichloroacetamide) increased after SDS chlorination (Tables 4.7-4.10), indicating chlorine-controlled DBP formation reactions; i.e., additional chlorine yielded additional

DBPs. At Plants 1 and 2, preformed TOCl represented about 50% found in the SDS sample for the GAC influent. However, for TOBr, preformed and SDS concentrations were similar for the GAC influent, indicating bromide was the limiting reagent in Br-DBP formation.

For GAC effluent at Plants 1 and 2, the contribution of preformed DBPs to the SDS-DBP concentrations was 6-26% for DBP sum and about 30-50% for TOCl (Tables 4.7-4.10). These percentages were similar to those for SDS-treated GAC influents. Thus, on average, GAC was equally removing preformed DBPs and DBP precursors within these measures. For TOBr, GAC removed more preformed Br-DBPs, as the preformed TOBr to SDS-TOBr ratio decreased after GAC treatment.

In Plant 1, GAC treatment removed preformed IAAs (iodo-, chloroiodo-, bromoiodo-, and diiodoacetic acid) to below detection (<25 ng/L), yet some of these compounds formed in the SDS test following GAC, indicating adsorbability and/or biodegradability of these compounds might be higher than the corresponding precursor material. Some studies have shown GAC removal of iodide, and unlike bromide, iodide sorption is not affected by competing anions.[71-73] As iodide reacts with chlorine in GAC influent, it converts to HOI, which reacts with NOM to form organic-iodine, which is more readily adsorbed by activated carbon than iodide. Iodide is also rapidly oxidized to iodate, which is less adsorbable but is a non-toxic inorganic sink for iodide.[74, 75] It has been proposed that iodide can be converted to iodine by dissolved oxygen, which can

then be easily removed by GAC, suggesting removal of iodide (and reduction in iodo-DBP formation) is dependent on dissolved oxygen concentration and disinfectant concentration.[71, 73, 76] Adsorption of IAAs to GAC has not been previously reported.

### *Calculated Water Toxicity*

***Overall results***. Because the use of GAC increased formation of some of the more toxic Br- and N-DBPs, it was not evident whether the effective control of overall DBP formation translated to safer drinking water. Therefore, cytotoxicity and genotoxicity associated with the 70 measured DBPs were calculated for GAC-treated samples across GAC service lives (Figure 4.2, 4.10, 4.20, and 4.21).[14, 50] Breakthrough of SDS cytotoxicity and genotoxicity is also shown in Figure 4.20. Calculated cytotoxicity and genotoxicity were substantially lower following GAC treatment at younger service lives, despite higher formation of some toxic Br- and N-DBPs. With increasing GAC run time, an increase in SDS-DBPs corresponded to increased calculated cytotoxicity and genotoxicity (Figure 4.2), but calculated toxicity values for all three plants remained below GAC influent values after pre-oxidation (without GAC treatment). Plant 1 had less significant reduction for calculated toxicity than its corresponding reduction in overall DBP formation following SDS. While overall DBP formation was reduced by 50-83%, cytotoxicity decreased only 7-17%, and genotoxicity by 29-34% following GAC (Figure 4.2). *This indicates that reductions in DBP concentrations do not necessarily reflect reductions in toxicity.* Plant 2 had highest overall DBP formation, along with the highest calculated cytotoxicity and genotoxicity (Tables 4.15-4.17); implementation of GAC treatment yielded 69, 65, and 34% reduction in calculated cytotoxicity and 50, 76, and

32% reduction in calculated genotoxicity for youngest, middle-aged, and oldest GAC, respectively, compared to not using GAC at all (Figure 4.2). Plant 3, which had the least impacted source water, formed lower calculated toxicity with 39-82% reduction in calculated cytotoxicity and 47-57% reduction in calculated genotoxicity following GAC (Figure 4.2).

***Preformed DBPs.*** A reduction in calculated cytotoxicity and genotoxicity for preformed DBPs was substantial and consistent for Plant 1 (74-100% and 83-100% for cytotoxicity and genotoxicity, respectively; Table 4.15), but more variable for Plant 2 (43-100% and 74-100%, respectively, Table 4.16), which was driven by inconsistent breakthrough of bromoiodoacetamide. This compound is significantly more cytotoxic than most regulated DBPs (except bromoacetic acid)[50]. At both plants, the preformed calculated toxicity in the GAC effluent was highest in the youngest GAC, but was lower in older GAC, indicative of biological acclimation.[29]

At Plant 1, calculated cytotoxicity for preformed DBPs in the GAC influent was 70% of the value after SDS, but dropped to <1-22% following GAC; calculated genotoxicity of the preformed DBPs in the GAC influent was 104% of the value after SDS, and dropped to 0-26% following GAC (Tables 4.15, 4.16). While preformed calculated toxicity was reduced by 74-100% in Plant 1, calculated cytotoxicity was only reduced by 7-17% following SDS procedures. Plant 2 preformed calculated cytotoxicity ranged from <1-61% of the value following SDS, and 0-33% of the calculated

genotoxicity was preformed. Calculated toxicity is a function of both DBP concentration and toxicity index values, and therefore does not always correlate with decreased DBP concentrations. This reduced efficiency of GAC following SDS procedures may indicate that individual DBPs may be more efficiently removed by GAC adsorption than corresponding precursors in some cases.

***Drivers of toxicity***. At all three drinking water plants studied, THMs and HAAs constituted a majority of the quantified DBPs (Figure 4.2 and 4.12). For example, at Plant 1, THMs were 18-25% and HAAs were up to 9% of the TOX (Figure 4.5), but THMs only contributed 2% to calculated cytotoxicity, and HAAs only contributed up to 3%. Cytotoxicity and genotoxicity drivers without GAC treatment were HNMs, IAAs, HALs, and HANs (Figure 4.5). Following GAC treatment, drivers of toxicity shifted to HNMs (70-76%), HANs (14-16%), and HAMs (9%), with little contribution of IAAs or HALs. The increase of tribromonitromethane after GAC treatment, contributed up to 41% of the cytotoxicity and 37% of the genotoxicity (Figure 4.2 and 4.5). Formation of tribromonitromethane, like bromoiodoacetamide, may be due to desorption or to surface catalyzed reactions. Reduction of cytotoxicity and genotoxicity increases to 41-51% and 53-59%, respectively, if tribromonitromethane is not considered, *highlighting that the increase in concentration of toxic unregulated DBPs can have a dramatic impact on toxicity*. Dibromochloronitromethane and bromodichloronitromethane also contributed significantly to he calculated cytotoxicity and genotoxicity (16-20% and 14-16%, respectively).

At Plant 2, THMs were 34-75% and HAAs were 14-25% of the TOX, respectively (Figure 4.20). However, THMs contributed only ~1% of the calculated cytotoxicity, while HAAs contributed 12-21%. Two groups contributing the most to cytotoxicity in the GAC influent of Plant 2 were HAMs and HANs, with 46% and 31%, respectively (Figure 4.20). Following GAC treatment, HAMs contributed to 21-32% and HANs to 43-50% of cytotoxicity. Major contributors to cytotoxicity were bromochloroacetamide (5-26%), dibromoacetamide (7-16%), bromochloroacetonitrile (14-20%), dibromoacetonitrile (11-31%), and bromoacetic acid (5-24%). Thus, while HAMs and HANs only contributed 4-8% and 1-3%, respectively, to TOX, they were the major drivers of cytotoxicity in the overall DBP mixture. *These results highlight that regulated DBP concentrations alone may not always provide an adequate basis for risk assessment*. THMs are not genotoxic in the Chinese hamster ovary (CHO) assay, and therefore, had no contribution to calculated genotoxicity. The drivers for genotoxicity at Plant 2 were HAAs (56 to 78%) and HANs (11 to 30%). Genotoxicity was largely driven by bromoacetic acid (40-81%) and dibromoacetonitrile (11-30%). For Plant 3 ($2^{nd}$ sampling) with and without GAC, HANs and HALs were drivers for cytotoxicity, while HNMs, HANs, and HALs were the drivers for genotoxicity (Figure 4.21). This study represents the most extensive evaluation of calculated toxicity over the life of GAC.

***Breakthrough relationships to TOC.*** For preformed concentrations in Plants 1 and 2, TOC breakthrough was a useful conservative indicator for TOCl, TOBr, and total DBP sum, but not for TOI, which was present in influent at two orders of magnitude lower than TOCl and one order of magnitude lower than TOBr (Figure 4.10 and 4.11).

This pattern was also seen at Plant 1, and correlations between TOC removal and speciated TOX removal for the combined data sets from both plants are shown in Figure 4.22. At both plants, TOC was also a useful conservative indicator for calculated toxicity breakthrough, except at 3,000 BV at Plant 2 for cytotoxicity (Figure 4.11). The relationships between TOC removal, removal of the SDS total DBP sum, and cytotoxicity and genotoxicity for all three plants are shown in Figure 4.6. The correlation coefficients ($R^2$ values) for TOC vs. SDS DBP sum, cytotoxicity, and genotoxicity are 0.72, 0.66, and 0.61 respectively. It should be noted that if cytotoxicity for Plant 1 is excluded, the $R^2$ is 0.98 indicating much stronger correlations to TOC removal at Plants 2 and 3. The relationships between TOC and SDS TOCl, TOBr, TOI, and DBP sum are shown in Figure 4.13, 4.14, 4.15. As with preformed DBP results (Figure 4.23), TOC is an acceptable surrogate indicator for TOCl, TOBr, and DBP sum (but not for TOI), though TOC is not always the best indicator for the formation of specific compounds, e.g., N-DBPs,[77] which have strong impacts on calculated toxicity. Given the differences in DBP speciation between plants, the effectiveness of GAC for DBP and toxicity control was location-specific and was likely a function of both precursor characteristics, halide concentration, and GAC type.

**Implications for Drinking Water Treatment and Risk Reduction**

While concentrations of some Br- and N-DBPs increased following GAC treatment, the overall performance of GAC in decreasing DBP concentrations, calculated cytotoxicity, and calculated genotoxicity in finished waters is promising. However, it is clear that calculated toxicity reductions are not as dramatic as reduction in DBP levels.

Moreover, decreased DBP concentrations do not necessarily reflect decreased toxicity, and small increases in some unregulated DBPs (such as tribromonitromethane) can have a dramatic impact on calculated toxicity. Thus, concentrations of regulated DBPs alone may not be adequate when conducting risk assessments.

The TIC-Tox method is a useful comparative tool, but it is based on *in vitro* models and excludes metabolic transformations. Future studies should also include real measurements of cytotoxicity and genotoxicity for GAC-treated waters to account for the total mixture toxicity, including unknown DBPs not measured in this study. In many cases, more than 50% of the halogenated material in chlorinated drinking water is not yet identified or quantified.[78] In this study, there was up to 79%, 45%, and 48% unknown TOX in Plant 1, 2 and 3 respectively (Figure 4.5, 4.18, and 4.19), indicating a need for further exploration of halogenated material in drinking waters, and in particular I-DBPs. Low levels of bromide and iodide limits the formation of Br- and I-DBPs at the plants in this study, so future studies should address the impact that bromide and iodide may have on GAC efficiency to reduce toxicity. Finally, results indicate that pre-chlorination before GAC treatment may be an effective strategy for further reducing DBP formation, but more research including measured toxicity is required.

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**Table 4.1. Water quality parameters for Plants 1-3**

| Plant | Impacts on Source Water | Pre-oxidant | GAC Operation | Bed Volumes Treated by GAC | TOC (mg/L as C) | $UV_{254}$ ($cm^{-1}$) | SUVA (L/mg-m) | TN (mg/L as N) | $Br^-$ (µg/L) | $I^-$ (µg/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | Algae | Chlorine dioxide, chlorine | Post-Filter Adsorber (seasonal partial treatment) | GAC Inf. | 1.5 | 0.018 | 1.2 | 0.13 | 9 | <5 |
| | | | | 5,600 | 0.5 | 0.003 | 0.64 | 0.07 | 13 | <5 |
| | | | | 12,600 | 0.8 | 0.007 | 0.8 | 0.09 | 14 | <5 |
| | | | | 22,400 | 1.1 | 0.01 | 0.9 | 0.11 | 15 | <5 |
| **2** | Industrial wastewater, agricultural | $KMnO_4$, chlorine | Post-Filter Adsorber | GAC Inf. | 2.1 | 0.037 | 1.8 | - | - | - |
| | | | | 3,000 | 0.3 | 0.007 | 1.0 | - | - | - |
| | | | | 8,700 | 0.6 | 0.006 | 1.0 | - | - | - |
| | | | | 22,000 | 1.2 | 0.020 | 1.7 | - | - | - |
| **3** (1st Sampling) | Minimal | $KMnO_4$ | Filter Adsorber | GAC Inf. | 2.0 | 0.037 | 1.8 | 0.19 | <5 | <5 |
| | | | | 9,200 | 1.4 | 0.022 | 1.6 | 0.14 | <5 | <5 |
| **3** (2nd Sampling) | | | | GAC Inf. | 2.0 | 0.046 | 2.3 | - | <5 | <5 |
| | | | | 3,400 | 0.7 | 0.015 | 2.0 | - | <5 | <5 |
| | | | | 3,800 | 0.8 | 0.017 | 2.0 | - | <5 | <5 |

**Table 4.2. DBPs that were not significantly reduced by GAC or increased after the use of GAC**

| Class | DBP | Change After GAC | Location |
|---|---|---|---|
| HAL | Bromodichloroacetaldehyde | No change | Plant 2 |
| HAM | Bromochloroacetamide | No change | Plant 1 |
| HAM | Dibromoacetamide | No change | Plant 2 |
| HAM | Dichloroacetamide | No change | Plant 1 |
| HAN | Tribromoacetonitrile | Increased | Plant 2 |
| HAN | Bromochloroacetonitrile | No change | Plant 1, Plant 3[b] |
| HAN | Dibromoacetonitrile | No change | Plant 1, Plant 2 |
| HK | 1,1,1-Trichloropropanone | Increased | Plant 2, Plant 3[a] |
| HK | 1,1,1-Trichloropropanone | No change | Plant 3[b] |
| HK | 1,1,3,3-Tetrachloropropanone | No change | Plant 3[b] |
| HNM | Tribromonitromethane | Increased | Plant 1 |
| HNM | Trichloronitromethane | Increased | Plant 3[a] |
| HNM | Bromodichloronitromethane | Increased | Plant 3[a] |
| THM | Dibromochloromethane | Increased | Plant 2 |
| THM | Tribromomethane | Increased | Plant 2 |
| THM | Bromodichloromethane | No change | Plant 3[a,b] |
| HAA | Bromoacetic acid | No change | Plant 2 |

[a] First sampling; [b] Second sampling

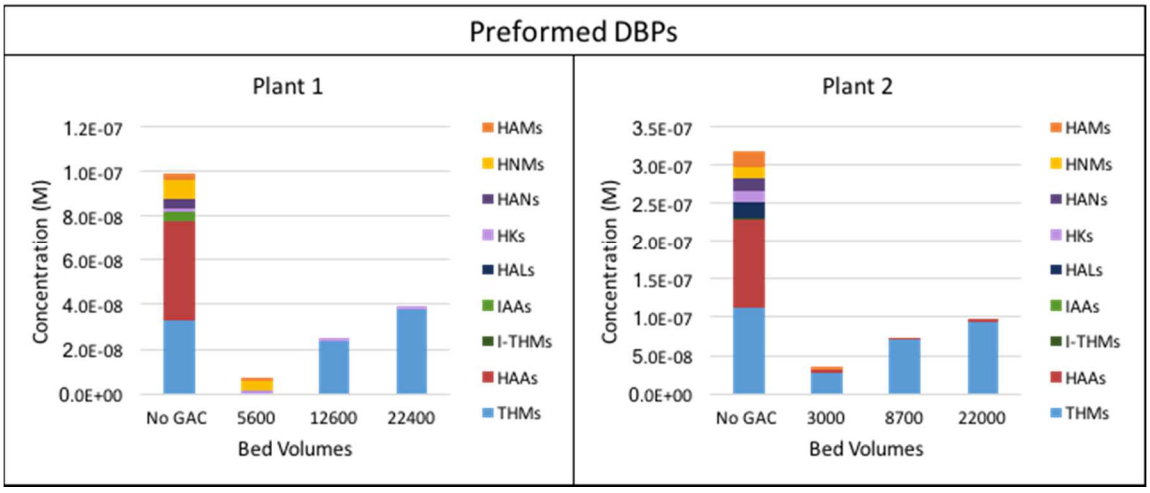


**Figure 4.1.** Effectiveness of GAC to control preformed DBPs formed during pre-oxidation using chlorine over a range of GAC service times in bed volumes (grouped by class). TCM, and BDCM are dominant breakthrough compounds at Plant 1. TCM and DBCM are the dominant breakthrough compounds at Plant 2.

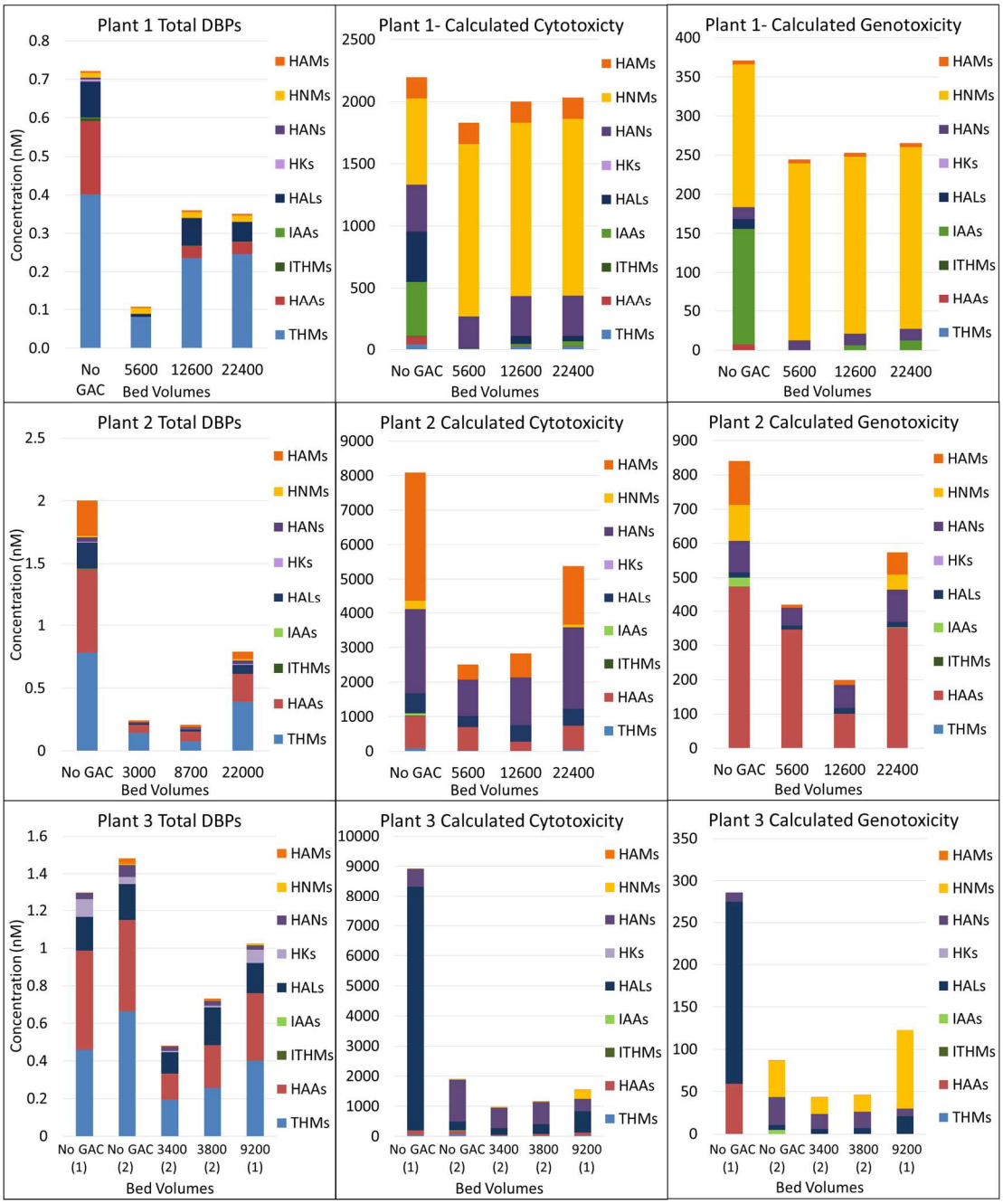


**Figure 4.2.** Effectiveness of GAC treatment for controlling regulated and unregulated DBPs (nM), calculated cytotoxicity and genotoxicity (unit-less) for Plant 1, 2 and 3. "No GAC" represents the level of DBPs that would be expected at the consumers' tap following simulated distribution system (SDS) chlorination in the absence of GAC treatment. Subsequent columns represent the effectiveness of GAC for DBP control following SDS chlorination over a range of GAC service times, indicated by the number of bed volumes (BV) treated.

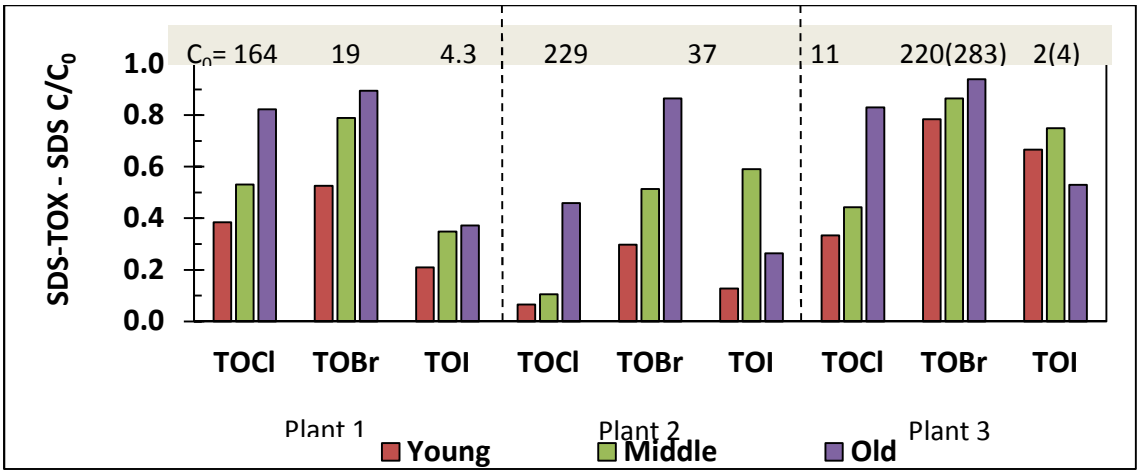


**Figure 4.3.** SDS total organic halogen (TOCl, TOBr, TOI) breakthrough for Plants 1, 2, and 3 with influent SDS. $C_0$ values (µg/L) from Plant 3 second sampling are shown in parentheses. Plant specific bed volumes are found in Table 1. $C_0$ is the GAC influent concentrations.

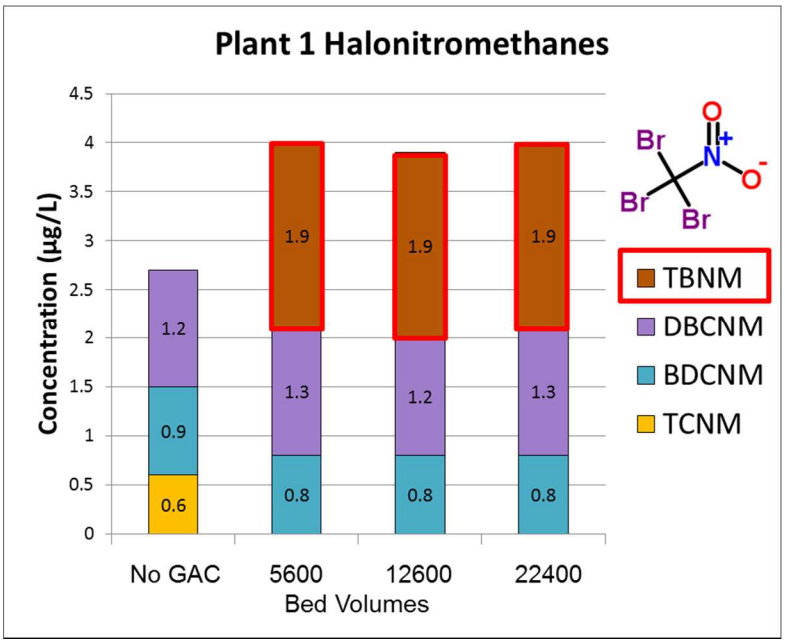


**Figure 4.4.** Plant 1 halonitromethanes before and after GAC following SDS procedures.

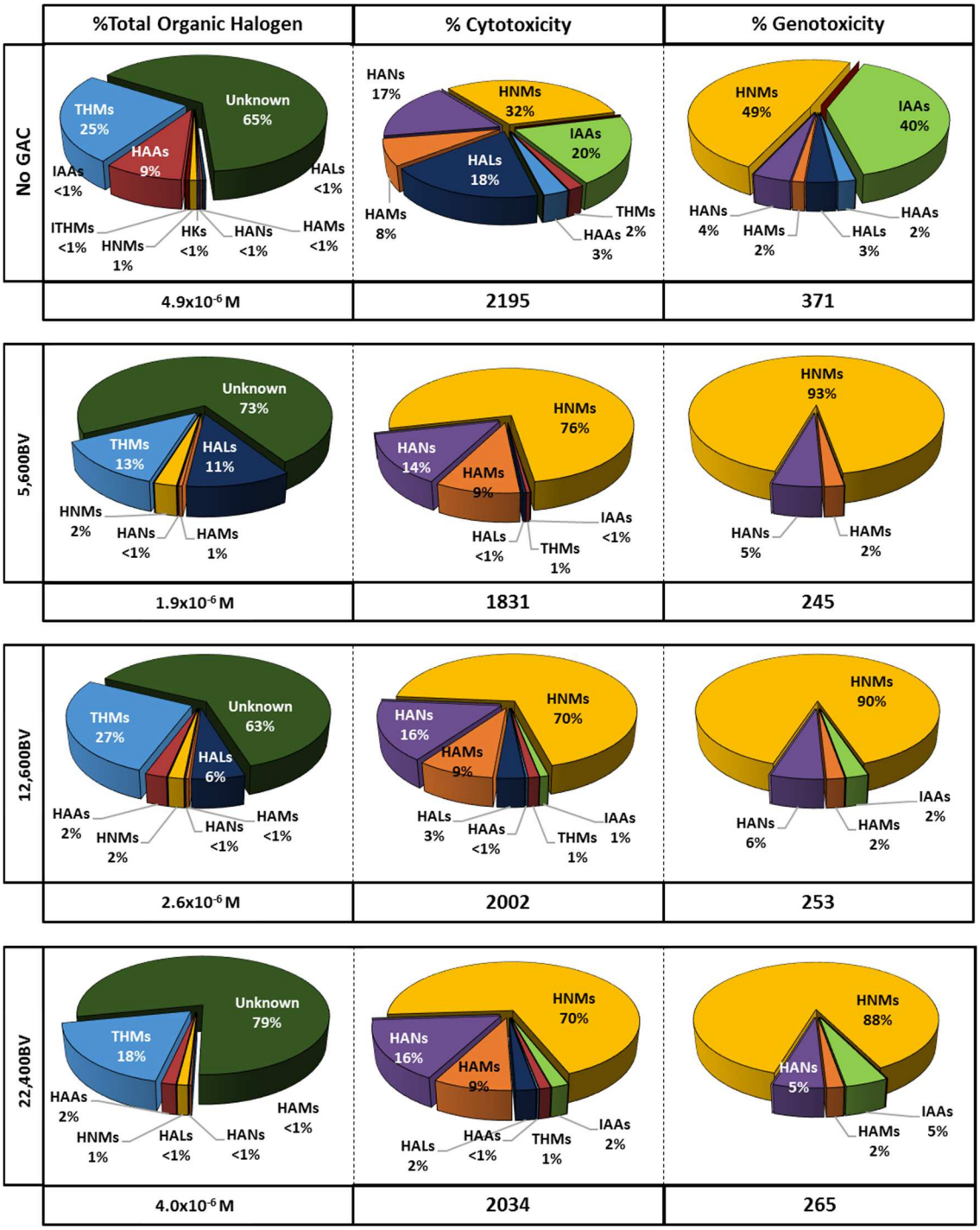


**Figure 4. 5.** Effectiveness of GAC treatment at Plant 1 for SDS-TOX, cytotoxicity and genotoxicity: % Total organic halogen (in molarity), % calculated cytotoxicity, and % calculated genotoxicity for each DBP class. Total toxicity = $\sum([DBP] \times (C_{1/2})^{-1} \times 10^{6})$. Note that there are no data on cytotoxicity or genotoxicity in the literature for haloketones or tribromoacetonitrile.

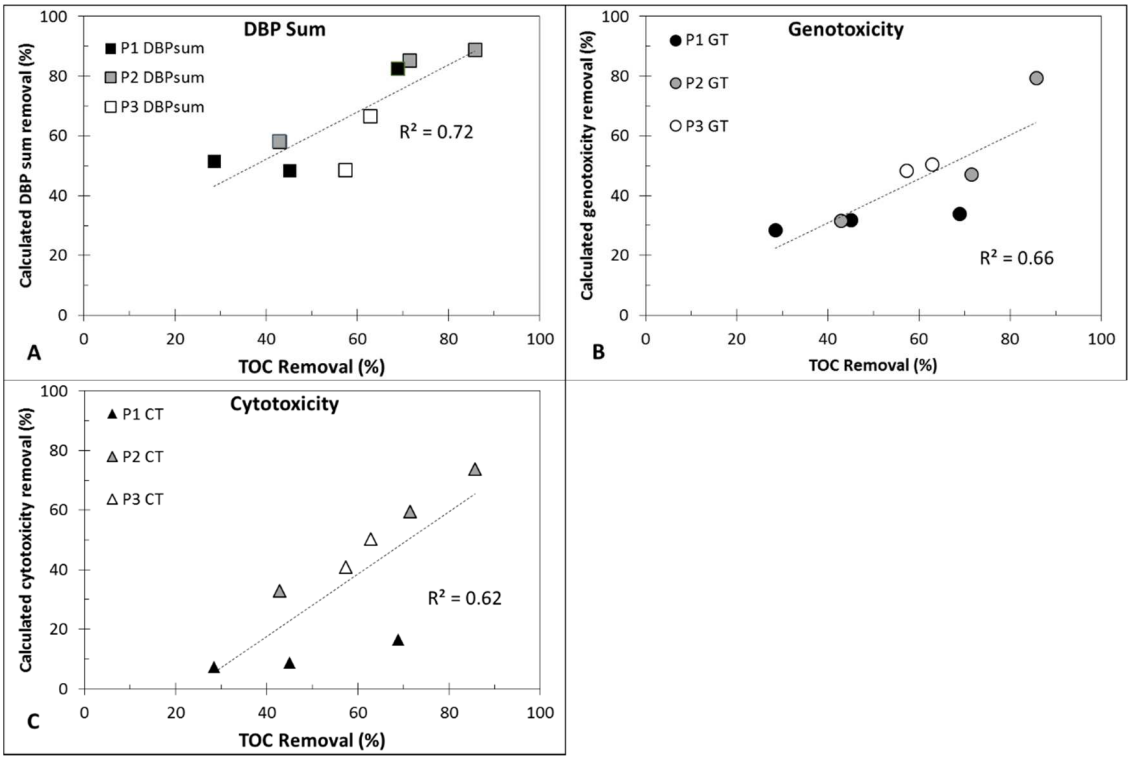


**Figure 4.6.** Relationship between TOC removal and removal of SDS-DBP sum (A), genotoxicity (GT) (B), and cytotoxicity (CT) (C).

# SUPPORTING INFORMATION FOR CHAPTER 4

**Text 4.1. Disinfection byproduct (DBP) formation tests**

Simulated distribution system (SDS) tests were employed to examine the effect of DBP precursors on subsequent chlorine applications. Water samples for SDS experiments were collected and analyzed depending on the conditions for each water treatment plant (WTP). SDS procedures were conducted on both settled influent waters and granular activated carbon (GAC) effluent waters.

*Plant 1:* Chlorine uniform formation conditions (UFC) tests were performed for Plant 1. The pH of the samples was adjusted to ~8.0 with borate buffer. Samples were spiked with 1.0 to 4.0 mg/L of chlorine to achieve a chlorine residual level of approximately 1.0 mg $Cl_2$/L after 24 hours of reaction time.

*Plant 2:* Chlorine SDS tests were performed to simulate Plant 2's distribution system. Samples were spiked with 1.0 to 4.0 mg/L of chlorine without any pH adjustments (ambient pH of ~7.5) to achieve a chlorine residual level of approximately 0.2 mg $Cl_2$/L after 168 hours of reaction time.

*Plant 3:* Chlorine SDS tests were performed to simulate the Plant 3's distribution system. Samples were spiked with 1.5 to 2.5 mg/L of chlorine without any pH adjustments (ambient pH of ~7.5) to achieve a chlorine residual level of approximately 1.0 mg $Cl_2$/L after 124 hours of reaction time.

**Text 4.2. Sample quenching and preservation procedure**

Priority DBPs that were quantified included haloacetamides (HAMs), halonitromethanes (HNMs), haloacetaldehydes (HALs), iodinated trihalomethanes (I-THMs), haloacetonitriles (HANs), haloketones (HKs), iodinated acetic acids (IAAs), and total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI). However, these priority DBPs have varying stability in the presence of different disinfectants, quenchers, pH, and temperature conditions. Namely, bromodichloronitromethane, dibromochloronitromethane, tribromonitromethane and tribromoacetonitrile (BDCNM, DBCNM, TBNM, TBAN) degrade in the presence of ascorbic acid. As a result, two separate quenching procedures were used.[1]

Previous stability studies showed that priority DBPs are stable within a few days with ascorbic acid and/or in the presence of chloramines at pH 3.5-4 at 4°C.[1] For this study, two sets of samples were collected in separate 1 L small-mouth amber bottles; one set was quenched with ascorbic acid for total organic halogen (TOX) and mono-/di-haloaldehydes analyses, while the other was quenched with ammonium chloride to analyze for trihalonitromethanes, haloacetamides, iodinated acetic acids, and tribromoacetonitrile. Samples were quenched in slight excess (chlorine to quenching agent molar ratio of 1:1.3) based on a maximum potential residual chlorine of 5 mg/L as $Cl_2$ and adjusted to pH 3.5-4 with 1 M $H_2SO_4$. All samples were collected headspace free in amber bottles and shipped overnight on ice. Samples were then stored at 4ºC until analyzed within 1-2 days.

**Text 4.3. Sample extraction methods and instrumentation for unregulated DBPs**

DBPs were analyzed using four analytical methods: 1) Liquid-liquid extraction (LLE) for tri-HALs, HANs, HKs, I-THMs, and HNMs; 2) Multiple LLE for HAMs, IAAs, and a subset of compounds (BDCNM, DBCNM, TBNM, and TBAN) with diazomethane derivatization procedure for IAAs; 3) PFBHA (*O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride) derivatization method for mono-, and di- HALs; 4) Total organic halogen (TOX).

**Text 4.3.1. Analytical Method 1: Single liquid-liquid extraction (LLE)**

A single LLE method was developed for volatile analytes that could be lost during sample extraction or concentration. Samples were brought to room temperature before extraction. Aliquots of 100 mL of sample were spiked with 30 g of sodium sulfate and 2 mL of MTBE in 125 mL amber bottles. Samples were shaken for 30 min on a mechanical shaker, followed by a 10 min wait to allow the organic phase to separate. The organic phase was then immediately removed into a separate container. Sodium sulfate was added to the extract to remove any excess water, and 250 μL was transferred with a syringe into a gas chromatography (GC) vial. Final extracts were spiked with the internal standard, 1,2-dibromopropane. Each sample extraction and analysis was processed in duplicate.

Analysis was performed by an Agilent 7890 GC coupled to an Agilent 5977A mass spectrometer (Agilent Technologies, Santa Clara, CA) with electron ionization (EI), which was carried out in selected ion monitoring (SIM) mode. The samples were injected using a multi-mode inlet (MMI) in pulsed splitless mode with the following program:

initial temperature of 35°C held for 0.1 min, ramped at a rate of 360°C/min to 220°C, held for 5 min, and then ramped at 720°C/min to 280°C for 20 min in order to clean the system of organic matter. Samples were injected onto a Restek Rtx-200 column (30 m x 0.25 mm ID x 0.25 μm film thickness; Restek Corporation, Bellefonte, PA). The GC program was as follows: an initial temperature of 35°C held for 5 min, then ramped at 9°C/min to 200°C, then ramped at 20°C/min to 280°C, and held for 20 min. The transfer line temperature was maintained at 250°C, the source temperature at 200°C with an electron energy of 70 eV, and the quadrupole at 150°C.

The retention times and the ions (m/z values) selected to monitor each compound can be found in Table 4.5. Quantification ions had a dwell time of 100 ms, and qualifier ions had a dwell time ranging from 50-75 ms. Quantification ions were selected based on relative abundance, generally selecting the most abundant ions.

**Text 4.3.2. Analytical Method 2: Multiple LLE and diazomethane derivatization**

A multiple LLE method was developed for semi-volatile DBPs, which improved detection limits for many compounds. Samples were brought to room temperature before extraction. Aliquots of 100 mL of sample (in duplicate) were spiked with 30 g of sodium sulfate and 5 mL of MTBE in 125 mL amber bottles and then shaken for 15 min on a mechanical shaker. This was followed by a 10 min wait to allow the organic phase to separate out, which was immediately removed into a conical vial. Samples were extracted two more times, for a total of three LLEs and a total of 15 mL of MTBE. The collected extract was passed through a sodium sulfate column, and concentrated under nitrogen to a

final volume of 200 µL. Final extracts were transferred to two GC vials containing 100 µL each. One 100 µL extract was spiked with the internal standard, 1,2-dibromopropane for analysis of trihalonitromethanes, haloacetamides, and tribromoacetonitrile by GC-MS. These compounds were analyzed using the same GC-EI-MS method in Analytical Method 1. The retention times and ions (m/z values) used for SIM analysis can be found in Table 4.5.

**Text 4.3.3. Diazomethane derivatization**.

To analyze for IAAs, the second 100 µL of extract underwent diazomethane derivatization. A 100 µL portion of the extract was spiked with 1,2-dibromopropane internal standard and derivatized using freshly-generated diazomethane. Diazomethane was generated following a U.S. Environmental Protection Agency Standard Operating Procedure.[2] An Aldrich® diazomethane-generator apparatus was used. Approximately 0.367 g of Diazald® and 1.0 mL of CARBITOL™ were added to the inner piece of the apparatus and 3.0 mL of MTBE was added to the outer portion. The apparatus was assembled, placed in ice. Potassium hydroxide (KOH, 1.5 mL of 37%) solution was injected dropwise through the septum into the inner tube. After reacting for 1 hour, 50 µL of the diazomethane (dissolved in MTBE in the outer tube) was added to each 100 µL sample. After 30 minutes of reaction, any excess diazomethane remaining in the samples was quenched with approximately 10 mg of silica gel. To remove solid silica, derivatized extracts were transferred to new vials.

Derivatized samples were analyzed by GC-tandem mass spectrometry (MS/MS) for four iodoacetic acids (IAAs), including iodoacetic acid (IAA), chloroiodoacetic acid (CIAA), bromoiodoacetic acid (BIAA), and diiodoacetic acid (DIAA), using a Quantum GC$^{TM}$ triple quadrupole mass spectrometer coupled to a TRACE GC Ultra gas chromatograph (Thermo Scientific, Waltham, MA). Sample volumes of 2.0 µL were injected at an inlet temperature of 250 °C with a splitless time of 0.80 min and split flow of 50 mL/min. GC separations were performed using an Rxi-5ms (30 m x 0.25 mm ID x 0.25 µm film thickness; Restek Corporation, Bellefonte, PA**)**, with the following oven temperature program: 35°C for 2 min, followed by a 9°C/min ramp to 280°C, and held for 20 min. The transfer line temperature was controlled at 280°C. An EI source was used at a temperature of 200°C, emission current of 50 µA, and electron energy of 70 eV. Multiple reaction monitoring (MRM) was used to quantify IAA, CIAA, BIAA, and DIAA. Two MS/MS transitions, one quantitative and one qualitative, were used for each of the IAAs, along with 1,2-dibromopropane internal standard as shown in Table 4.3.

**Text 4.3.4. Analytical Method 3: PFBHA (O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride) derivatization followed by LLE.**

Mono- and di-halogenated acetaldehydes were analyzed according to a published method by Jeong et al. (2014)[3] with minor alterations. Samples were brought to room temperature before extraction. A 100-mL sample was spiked with a surrogate standard, ammonium sulfate, potassium hydrogen phthalate/sodium hydroxide buffer, and PFBHA and reacted for two hours in a temperature controlled water bath at 35°C. Samples were cooled down to room temperature, and concentrated sulfuric acid and 1,2-

dibromopropane internal standard were added. Then, 10 mL of hexane was added to samples, shaken for 3 min with a mechanical shaker, transferred to separatory funnels, and held for 5 min for phases to separate. The organic extract was collected in 40 mL vials and the LLE was repeated two more times for a total of 30 mL of organic extract. The final extract was dried over a sodium sulfate column and concentrated with nitrogen to 0.5 mL for GC-MS analysis.

Analyte detection was performed by GC-EI-MS analysis, which was carried out in SIM mode. The injection port was run in splitless mode at 250°C at 15.5 psi. GC separations were performed using a Rxi-5ms (30 m x 0.25 mm ID x 0.25 µm film thickness; Restek Corporation, Bellefonte, PA**)**, with the following oven temperature program: 35°C for 2 min, followed by a 9°C/min ramp to 160°C, a 5°C/min ramp to 180°C, and a final ramp of 22°C/min to 280°C, which was held for 20 min. The transfer line temperature was controlled at 280°C, the source temperature at 200°C with an electron energy at 70 eV, and the quadrupole at 150°C.

The retention times and the ions (m/z values) selected to monitor each compound can be found in Table 4.5. Quantification ions had a dwell time of 100 ms, and qualifier ions had a dwell time ranging from 50-75 ms. Quantification ions were selected based on relative abundance.

**Text 4.4. Analytical Method for total organic halogen (TOX)**

Total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI) were determined using a previously published procedure (Smith et al., 2010; Echigo et al., 2000) with a few modifications.[4, 5] Samples were quenched with ascorbic acid in slight excess (chlorine to ascorbic acid molar ratio of 1:1.3) based on a residual chlorine of 5 mg/L as $Cl_2$ and adjusted to pH 3.5-4 with 1 M $H_2SO_4$. Samples were shipped overnight with icepacks and extracted the day they were received or stored at 4°C for 1-2 days. Samples were brought to room temperature before extraction and acidified to pH <2 with concentrated nitric acid. Samples were processed in duplicate with a sample adsorption and combustion unit (Mitsubishi, Chigasaki, Japan; Cosa Xentaur, Yaphank, NY). A 50 mL aliquot was passed through two granular activated carbons (GAC) that adsorbed halogenated organic compounds. Then, GAC columns were washed with nitric acid (5 mL, 5 mg $NO_3^-$ per mL) to remove inorganic halides and other inorganics. Each GAC column was loaded onto a ceramic boat and combusted at 1000°C in the presence of oxygen and argon as the carrier gas. Combusted gases, including hydrogen halides, were collected in centrifuge tubes that contained 5 mL of $H_2O_2$ solution (0.03%, prepared daily), and the gas line was washed with an additional 3 mL of $H_2O_2$ solution. Centrifuge tubes were weighed before and after collecting the off gases to determine exact volumes.

Recovered aqueous solutions were analyzed for chloride with a Dionex 1600 ion chromatograph (Dionex, Sunnyvale, CA) as described elsewhere.[6] A Finnigan ELEMENT XR double focusing magnetic sector field inductively coupled plasma (ICP)-

MS instrument (Thermo Electron Corporation) was used for bromide and iodide analysis.[7]

**Table 4.3. Precursor information by DBP class and adsorption potential (High > 80%, Mid 20-80%, Low <20%)**

| DBP Class | Precursor | Adsorption Potential[8] |
|---|---|---|
| THM | Many NOM fractions[9, 10] | High to mid |
| HAA | Many NOM fractions[10] | High to mid |
| HAL | phenolic NOM structures, proteinaceous materials [11,12] | Mid |
| HAM | phenolic NOM structures, proteinaceous materials, amino acids, haloacetonitrile, hydrophilic acid NOM fraction, aromatic NOM, aldehydes[13-17] | Low to mid |
| HAN | free amino acids, heterocyclic nitrogen in nucleic acids, proteinaceous materials, combined amino acid bound to humic structures, aldehydes[13-15] | Low |
| HK | Many NOM fractions[8] | High |
| HNM | hydrophilic natural organic matter, aromatic NOM, nitrophenols, nitromethane[14] | Low |
| I-THM | Many NOM fractions, higher $I^-$ content[18] | Not Studied |
| IAA | Many NOM fractions, higher $I^-$ content[18] | Not Studied |

**Table 4.4. Sampling information for Plants 1-3 including sampling collection date, flow and loading rate on each contactor, empty bed contact time (EBCT), pre-oxidant concentrations, GAC media type, bed volumes treated (BV) by GAC, and percent DOC breakthrough**

| WTP | Sample Collection Date | Source Water Type | Typical Flowrate per Contactor (MGD) | Typical Hydraulic Loading Rate (gpm/ft$^2$) | Typical EBCT$^{\blacklozenge}$ (min) | Chlorine Residual GAC Feed Water | GAC Installation Date | GAC Type | Bed Volume Treated by GAC (BV) | Percent DOC Break-through $^{\bigstar}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 10/6/15 | River Surface Water | 0.8 | 8.8 | 15 | 0.4-0.5 mg/L of chlorine | 7/31/15 | Virgin Calgon F300M | 5,600 | 31 |
| | | | | | | | 5/27/15 | Virgin Calgon F300M | 12,600 | 55 |
| | | | | | | | 8/21/13 | Virgin Calgon F300M | 22,400 | 71 |
| **2** | 1/11/16 | River Surface Water | 1.4 | 2.4 | 20 | 0.2-0.4 mg/L of chlorine | 9/23/15 | Regenerated Calgon F400 | 3,000 | 14 |
| | | | | | | | 5/13/15 | Regenerated Calgon F400 | 8,700 | 29 |
| | | | | | | | 6/2/14 | Regenerated Calgon F400 | 22,000 | 57 |
| **3 (1$^{st}$)** | 10/30/15 | Lake Reservoir | 4.5 | 2.4 | 15 | None | July 2015$^{*}$ | Virgin Norit Hydrodarco 820 | 9,200$^{\dagger}$ | 68 |
| **3 (2$^{nd}$)** | 9/6/16 | Lake Reservoir | 4.5 | 2.4 | 15 | None | 7/5/16 | Virgin Calgon Carbsorb 820 | 3,400 | 37 |
| | | | | | | | 7/26/16 | Virgin Calgon Carbsorb 820 | 3,800 | 43 |

MRL: Minimum reporting level

$^{*}$: One GAC contactor's media was replaced each week. All four GAC contactor's media were replaced in successive weeks of July 2015.

$^{\dagger}$: Average BV of all four GAC contactors is presented.

$^{\ddagger}$: Youngest GAC contactor is a single bed and the other selected contactors are dual bed. Single bed GAC contactors receive half the flow of the dual bed. Consequently, EBCT in all contactors are the same.

$^{\blacklozenge}$: EBCT values are calculated for the GAC media only. Underlying sand or anthracite media was not incorporated into EBCT calculations.

$^{\bigstar}$: Percent DOC breakthrough is calculated by dividing GAC influent total organic carbon (TOC) by GAC effluent TOC

**Table 4.5. GC-MS retention time (RT), vendor information, quantitative and qualifier ions, and method detection limits (MDLs) for priority DBPs quantified in this study [a]**

| DBP Class | DBP Name | Abbreviation | RT (min) | Quantitative Ion (m/z) | Qualifier Ion (m/z) | MDL (ng/L) | logD (pH 6-9) |
|---|---|---|---|---|---|---|---|
| ***Analytical Method 1*** | | | | | | | |
| HAL | Trichloroacetaldehyde[b] | TCAL | 3.82 | 82.0 | 110.9 | 3.0 | 1.80 |
| HAL | Bromodichloroacetaldehyde[c] | BDCAL | 5.17 | 83.0 | 111/163.8 | 3.0 | 1.88 |
| HAL | Dibromochloroacetaldehyde[c] | DBCAL | 7.17 | 128.9 | 127.9 | 3.0 | 1.80 |
| HAL | Tribromoacetaldehyde[b] | TBAL | 9.10 | 172.8 | 171.8 | 3.0 | 1.99 |
| HAN | Trichloroacetonitrile[b] | TCAN | 3.42 | 108.0 | 110.0 | 10 | 2.15 |
| HAN | Dichloroacetonitrile[b] | DCAN | 4.22 | 74.0 | 82.0 | 5.0 | 0.94 |
| HAN | Chloroacetonitrile[b] | CAN | 4.36 | 75.0 | 48.0 | 8.0 | 0.18 |
| HAN | Bromochloroacetonitrile[b] | BCAN | 6.00 | 74.0 | 155.0 | 5.0 | 1.22 |
| HAN | Bromoacetonitrile[b] | BAN | 6.16 | 118.90 | 120.9 | 48 | 0.51 |
| HAN | Dibromoacetonitrile[b] | DBAN | 8.17 | 117.9 | 199.0 | 38 | 1.57 |
| HAN | Iodoacetonitrile[b] | IAN | 8.85 | 167.0 | 126.9 | 31 | 0.79 |
| HK | 1,1-Dichloropropanone[b] | 11DCP | 4.63 | 43.0 | 83.0 | 26 | -0.28 |
| HK | Chloropropanone[b] | CP | 4.73 | 92.0 | 43.0 | 24 | -0.28 |
| HK | 1,1,1-Trichloropropanone[b] | 111TCP | 6.88 | 43.0 | 125.0 | 6.0 | 0.17 |
| HK | 1,1-Dibromopropanone[c] | 11DBP | 8.17 | 43.0 | 215.9 | 20 | 1.50 |
| HK | 1-Bromo-1,1-dichloropropanone[c] | 1B11DCP | 8.80 | 43.0 | 125.0 | 3.0 | 1.21 |
| HK | 1,3-Dichloropropanone[c] | 13DCP | 8.95 | 77.0 | 49.0 | 35 | 0.45 |
| HK | 1,1,3-Trichloropropanone[b] | 113TCP | 10.02 | 77.0 | 83.0 | 2.0 | -0.49 |
| HK | 1,1,3,3-Tetrachloropropanone[c] | 1133TeCP | 10.92 | 83.0 | 85.0 | 9.0 | -0.46 |
| HK | 1,1,3,3-Tetrabromopropanone[d] | 1133TeBP | 16.13 | 200.8 | 119.9 | 22 | 3.57 |
| HNM | Trichloronitromethane[b] | TCNM | 4.67 | 116.9 | 119.0 | 6.0 | 2.21 |
| HNM | Dichloronitromethane[c] | DCNM | 4.77 | 83.0 | 85.0 | 28 | -1.43 |
| HNM | Bromochloronitromethane[c] | BCNM | 6.64 | 129.0 | 127.0 | 24 | -0.97 |
| HNM | Dibromonitromethane[c] | DBNM | 8.51 | 172.8 | 171.0 | 4.0 | -0.45 |
| I-THM | Dichloroiodomethane[c] | DCIM | 4.34 | 83.0 | 126.9 | 12 | 1.90 |
| I-THM | Bromochloroiodomethane[c] | BCIM | 5.95 | 128.9 | 126.9 | 39 | 2.10 |
| I-THM | Dibromoiodomethane[c] | DBIM | 7.83 | 172.8 | 299.7 | 15 | 2.37 |
| I-THM | Chlorodiiodomethane[c] | CDIM | 8.33 | 174.9 | 126.9 | 39 | 2.30 |
| I-THM | Bromodiiodomethane[c] | BDIM | 10.09 | 218.8 | 220.8 | 90 | 2.59 |
| I-THM | Iodoform[b] | TIM | 12.06 | 393.7 | 266.8 | 47 | 3.12 |

| DBP Class | DBP Name | Abbreviation | RT (min) | Quantitative Ion (m/z) | Qualifier Ion (m/z) | MDL (ng/L) | logD (pH 6-9) |
|---|---|---|---|---|---|---|---|
| ***Analytical Method 2*** | | | | | | | |
| HAM | Bromoacetamide[b] | BAM | 11.73 | 137.0 | 44.0 | 100 | -0.52 |
| HAM | Dichloroacetamide[e] | DCAM | 12.03 | 44.0 | 127.0 | 16 | 0.07 |
| HAM | Bromochloroacetamide[c] | BCAM | 13.38 | 44.0 | 173.0 | 22 | 0.35 |
| HAM | Trichloroacetamide[b] | TCAM | 13.92 | 44.0 | 82.0 | 7.0 | 1.11 |
| HAM | Iodoacetamide[b] | IAM | 14.07 | 185.0 | 58.0 | 400 | -0.19 |
| HAM | Dibromoacetamide[c] | DBAM | 14.08 | 44.0 | 217.0 | 41 | 0.04 |
| HAM | Chloroiodoacetamide[c] | CIAM | 15.15 | 92.0 | 219.0 | 38 | 0.54 |
| HAM | Bromodichloroacetamide[c] | BDCAM | 15.21 | 44.0 | 128.0 | 30 | 1.34 |
| HAM | Bromoiodoacetamide[c] | BIAM | 16.28 | 136.0 | 138.0 | 50 | 0.75 |
| HAM | Dibromochloroacetamide[c] | DBCAM | 16.44 | 44.0 | 128.0 | 23 | 1.64 |
| HAM | Tribromoacetamide[c] | TBAM | 17.59 | 44.0 | 173.0 | 40 | 1.03 |
| HAM | Diiodoacetamide[c] | DIAM | 17.91 | 184.0 | 311.0 | 23 | 0.62 |
| HAN | Tribromoacetonitrile[c] | TBAN | 9.11 | 197.8 | 195.8 | 100 | 3.06 |
| HNM | Bromodichloronitromethane[c] | BDCNM | 6.71 | 163.0 | 161.0 | 100 | 2.63 |
| HNM | Dibromochloronitromethane[c] | DBCNM | 8.87 | 206.8 | 209.0 | 100 | 3.12 |
| HNM | Tribromonitromethane[c] | TBNM | 10.81 | 251.0 | 253.0 | 100 | 3.66 |
| IAA | Iodoacetic acid[b] | IAA | 7.53 | 200>73 | 169>141 | 10 | -1.99 to -2.96 |
| IAA | Chloroiodoacetic acid[c] | CIAA | 9.68 | 234>79 | 234>107 | 25 | -2.55 to -2.61 |
| IAA | Bromoiodoacetic acid[c] | BIAA | 11.08 | 278>123 | 278>151 | 25 | -2.00 to -2.10 |
| IAA | Diiodoacetic acid[c] | DIAA | 12.91 | 326 >171 | 326>199 | 25 | -1.42 to -1.72 |
| ***Analytical Method 3*** | | | | | | | |
| HAL | Chloroacetaldehyde[b] | CAL | 14.62 | 238 | 181/182 | 50 | 0.16 |
| HAL | Bromoacetaldehyde[d] | BAL | 15.87 | 287 | 238 | 25 | 0.30 |
| HAL | Iodoacetaldehyde[d] | IAL | 17.48 | 293 | 335 | 25 | 0.59 |
| HAL | Dichloroacetaldehyde[e] | DCAL | 15.49 | 272 | 181/182 | 100 | 0.66 |
| HAL | Bromochloroacetaldehyde[c] | BCAL | 16.80 | 272 | 238 | 50 | 0.76 |
| HAL | Dibromoacetaldehyde[c] | DBAL | 18.21 | 137 | 135 | 50 | 0.93 |

[a] DBPs are classified by their corresponding analytical method and DBP class. LogD values were calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2018 ACD/Labs). When ranges are shown for logD values, these represent the combination of charged states (anions) and small portion of neutral species present according to their individual $pK_a$s. [b] Sigma Aldrich. [c] CanSyn Chem. Corp. [d] Aldlab Chemicals. [e] TCI America. [f] THMs measured by EPA method 551.1. [g] HAAs measured by EPA method 552.3. [h] Regulated DBPs.

**Table 4.6. List of analytes and associated analytical methods**

| Analyte | Method |
|---|---|
| Residual chlorine | *N,N*-diethyl-*p*-phenylendiamine titrimetric method[19] |
| Dissolved organic carbon (DOC) | SM 5310 B |
| Free chlorine, high range (>10 mg-$Cl_2$/L) | Modified 4500-Cl B |
| Free chlorine, low range (<10 mg-$Cl_2$/L) | SM 4500-Cl G; using a Hach Pocket Colorimeter II, Chlorine kit |
| Ammonia | SM 4500-NH G |
| Nitrite | SM 4500-$NO_2^-$ |
| Nitrate | SM 4500-$NO_3^-$ |
| pH | SM 4500-H+ B |
| Total nitrogen (TN) | Shimadzu TOC-TN Analyzer |
| Total Chlorine | SM 4500-Cl G; using a Hach Pocket Colorimeter II, Chlorine kit |
| $UV_{254}$ | SM 5910 B |
| Bromide | Ion chromatography |
| Iodide | Ion chromatography |

**Table 4.7. Full scale Plant 1 results: Effect of GAC age on preformed DBP and TOX removal** [a]

| DBP Class | DBP | GAC Inf | 5,600 BV | 12,600 BV | 22,400 BV |
|---|---|---|---|---|---|
| HAM | **BCAM** | 0.4±0.04 | 0.3±0.04 | ND | ND |
| HAN | **TBAN** | 1.3±0.0001 | ND | ND | ND |
| HK | **1B11DCP** | 0.3 | 0.3±0.08 | 0.3±0.02 | 0.3±0.02 |
| HNM | **BDCNM** | 0.8±0.003 | 0.8±0.001 | ND | ND |
| HNM | **DBCNM** | 1.2±0.002 | ND | ND | ND |
| IAA | **IAA** | 0.4±0.1 | ND | ND | ND |
| IAA | **CIAA** | 0.30±0.04 | ND | ND | ND |
| IAA | **BIAA** | 0.06±0.01 | ND | ND | ND |
| IAA | **DIAA** | 0.10±0.002 | ND | ND | ND |
| THM | **TCM** | 2.5 | ND | 2.8 | 3.1 |
| THM | **BDCM** | 2.0 | ND | ND | 1.9 |
| HAA | **DCAA** | 3.7 | ND | ND | ND |
| HAA | **TCAA** | 2.6 | ND | ND | ND |
| TOC | **TOCl as Cl** | 86.6±2.2 | 31.2±4.7 | 35.6±1.1 | 38.1±0.4 |
| TOX | **TOBr as Br** | 22.1±0.5 | 1.9±0.5 | 5.6±0.1 | 16.7±0.02 |
| TOX | **TOI as I** | 4.9±0.02 | 3.2±0.2 | 9.6±0.2 | 3.7±0.1 |

[a] All concentrations in µg/L (± standard deviation); ND: Not detected.

**Table 4.8. Full scale Plant 1 simulated distribution system results: Effect of GAC age on DBP removal [a]**

| DBP Class | DBP | GAC Inf | 5,600 BV | 12,600 BV | 22,400 BV |
|---|---|---|---|---|---|
| HAL | **TCAL** | 12.2±5.0 | 0.9±0.03 | 10.4±0.8 | 7.5±5.7 |
| HAL | **DCAL** | 1.1±0.007 | ND | ND | ND |
| HAM | **BCAM** | 0.5±0.0003 | 0.5±0.0001 | 0.5±0.0004 | 0.5±0.0008 |
| HAM | **DCAM** | 0.2±0.001 | 0.1±0.0001 | 0.2±0.0002 | 0.2±0.002 |
| HAN | **DCAN** | 0.3±0.006 | ND | ND | ND |
| HAN | **BCAN** | 0.2±0.006 | 0.1±0.002 | 0.2±0.01 | 0.2±0.002 |
| HAN | **DBAN** | 0.1±0.007 | 0.1±0.02 | 0.1±0.001 | 0.1±0.003 |
| HK | **CP** | 0.6±0.07 | ND | ND | ND |
| HNM | **TCNM** | 0.6±0.02 | ND | ND | ND |
| HNM | **DBCNM** | 1.2±0.004 | 1.2±0.002 | 1.2±0.002 | 1.3±0.0004 |
| HNM | **BDCNM** | 0.9±0.01 | 0.8±0.0008 | 0.8±0.001 | 0.8±0.0006 |
| HNM | **TBNM** | ND | 1.9±0.0008 | 1.9±0.002 | 1.9±0.00007 |
| I-THM | **DCIM** | 1.2±0.09 | ND | ND | ND |
| I-THM | **BCIM** | 0.2±0.001 | ND | ND | ND |
| IAA | **IAA** | 0.24±0.09 | ND | 0.01±0.001 | 0.02±0.0008 |
| IAA | **CIAA** | 0.23±0.06 | 0.09±0.03 | 0.07 ± 0.003 | 0.08 ± 0.006 |
| IAA | **BIAA** | 0.08±0.005 | ND | ND | ND |
| IAA | **DIAA** | 0.12±0.005 | ND | ND | ND |
| THM | **TCM** | 40.1 | 6.1 | 21.3 | 22.5 |
| THM | **BDCM** | 10.8 | 4.9 | 9.5 | 9.5 |
| HAA | **DCAA** | 16.5 | ND | 4.0 | 4.0 |
| HAA | **TCAA** | 5.9 | ND | ND | ND |
| HAA | **BCAA** | 4.5 | ND | ND | ND |
| TOX | **TOCl as Cl** | 164±1.2 | 62.9±2.0 | 86.5±1.9 | 135±53.3 |
| TOX | **TOBr as Br** | 19.1±1.1 | 10.2 ± 0.04 | 15.2 ± 0.7 | 17.1±0.5 |
| TOX | **TOI as I** | 4.3±0.1 | 0.9 ± 0.04 | 1.5 ± 0.02 | 1.6±0.3 |

[a] All concentrations in µg/L (± standard deviation) ; ND: Not detected.

**Table 4.9. Plant 2: Effect of GAC age on preformed DBP and TOX removal a**

| DBP Class | DBP | GAC Inf | 3,000 BV | 8,700 BV | 22,000 BV |
|---|---|---|---|---|---|
| HAL | **TCAL** | 2.5±0.04 | ND | ND | ND |
| HAL | **BDCAL** | 0.5±0.0006 | ND | ND | ND |
| HAL | **DBCAL** | 0.5±0.007 | ND | ND | ND |
| HAM | **DCAM** | 1.8±0.2 | ND | ND | ND |
| HAM | **BCAM** | 0.4±0.07 | 0.7±0.05 | ND | ND |
| HAM | **TCAM** | 0.2±0.01 | ND | ND | ND |
| HAM | **DBAM** | 0.6±0.03 | ND | ND | ND |
| HAM | **BIAM** | ND | 1.3±0.02 | ND | 1.1±0.3 |
| HAN | **TCAN** | 0.04±0.0001 | ND | ND | ND |
| HAN | **DCAN** | 1.5±0.04 | ND | ND | ND |
| HAN | **BCAN** | 0.2±0.003 | ND | ND | ND |
| HAN | **DBAN** | 0.4±0.003 | ND | ND | ND |
| HK | **11DCP** | 0.2±0.0007 | ND | ND | ND |
| HK | **111TCP** | 1.1±0.02 | ND | ND | ND |
| HK | **11DBP** | 0.2±0.002 | ND | ND | ND |
| HK | **1B11DCP** | 0.6±0.02 | ND | ND | ND |
| HK | **1133TeCP** | 0.2±0.0005 | ND | ND | ND |
| HNM | **TCNM** | 0.6±0.004 | ND | ND | ND |
| HNM | **DCNM** | 0.3±0.001 | ND | ND | ND |
| HNM | **BCNM** | 0.6 ±0.003 | ND | ND | ND |
| HNM | **BDCNM** | 0.5±0.00007 | ND | ND | ND |
| HNM | **DBCNM** | 0.8±0.002 | ND | ND | ND |
| I-THM | **DCIM** | 0.3±0.009 | ND | ND | ND |
| I-THM | **BCIM** | 0.2±0.002 | ND | ND | ND |
| IAA | **CIAA** | 0.09±0.01 | ND | ND | ND |
| THM | **TCM** | 11.9 | 3.3 | 8.51 | 9.5 |
| THM | **BDCM** | 0.4 | ND | ND | ND |
| THM | **DBCM** | 2.1 | 0.1 | ND | 3 |
| HAA | **CAA** | 0.1 | ND | ND | ND |
| HAA | **BAA** | 0.1 | ND | ND | ND |
| HAA | **DCAA** | 6.5 | ND | ND | ND |
| HAA | **TCAA** | 4.3 | ND | ND | 0.2 |
| HAA | **BCAA** | 2.8 | ND | ND | ND |
| HAA | **BDCAA** | 2.9 | 0.3 | 0.3 | 0.4 |
| HAA | **DBAA** | 0.7 | ND | ND | ND |
| HAA | **DBCAA** | 1.1±0.02 | 0.4 | ND | 0.4 |
| TOX | **TOCl as Cl** | 101.2±8.5 | 7.5±1.2 | 9.1±1.4 | 30.1±4.3 |
| TOX | **TOBr as Br** | 32.7±0.2 | 1.5±0.04 | 2.1±0.06 | 12.3±0.3 |
| TOX | **TOI as I** | 1.6±0.06 | 0.6±0.01 | 1.6±0.1 | 1.1±0.02 |

[a] All concentrations in µg/L (± standard deviation); ND: Not detected.

**Table 4.10. Plant 2 simulated distribution system results: Effect of GAC age on DBP and TOX removal [a]**

| DBP Class | DBP | GAC Inf | 3,000 BV | 8,700 BV | 22,000 BV |
|---|---|---|---|---|---|
| HAL | **TCAL** | 29.6±0.2 | 1.1±0.004 | 2.2±0.2 | 9.9±0.8 |
| HAL | **BDCAL** | 0.6±0.03 | 0.3±0.01 | 0.5±0.04 | 0.7±0.06 |
| HAL | **DBCAL** | ND | 0.3±0.006 | 0.4±0.005 | 0.3±0.009 |
| HAL | **DCAL** | 0.8±0.007 | ND | ND | ND |
| HAM | **DCAM** | 28.4±0.8 | 0.8±0.06 | 1.1±0.1 | 4.1±0.2 |
| HAM | **BCAM** | 6.1±0.4 | 0.4±0.2 | 0.7±0.2 | 1.7±0.2 |
| HAM | **TCAM** | 2.1±0.1 | 0.4±0.04 | 0.3±0.04 | 0.8±0.04 |
| HAM | **DBAM** | 1.5±0.03 | 0.8±0.08 | 1.2±0.1 | 1.2±0.1 |
| HAM | **BDCAM** | 1.7±0.1 | ND | ND | 1.2±0.005 |
| HAN | **DCAN** | 2.6±0.02 | 0.2±0.0007 | 0.3±0.03 | 1.5±0.1 |
| HAN | **BCAN** | 1.5±0.07 | 0.4±0.01 | 0.6±0.01 | 1.4±0.1 |
| HAN | **DBAN** | 0.5±0.01 | 0.4±0.006 | 0.5±0.001 | 0.6±0.04 |
| HAN | **TBAN** | ND | 0.7±0.002 | 0.7±0.002 | 0.7±0.0005 |
| HK | **111TCP** | 0.7±0.03 | 0.09±0.002 | 0.2±0.02 | 1.13±0.07 |
| HNM | **TCNM** | 0.7±0.01 | ND | ND | 0.2±0.0 |
| HNM | **DCNM** | 0.2±0.03 | ND | ND | 1.1±0.006 |
| HNM | **BCNM** | 0.3±0.004 | ND | ND | 0.3±0.0005 |
| HNM | **BDCNM** | 0.6±0.004 | ND | ND | ND |
| I-THM | **DCIM** | 0.30±0.009 | ND | ND | ND |
| I-THM | **BCIM** | 0.30±0.005 | ND | ND | ND |
| IAA | **IAA** | 0.04±0.02 | ND | ND | ND |
| IAA | **CIAA** | 0.2±0.02 | ND | ND | ND |
| IAA | **BIAA** | 0.07±0.006 | ND | ND | 0.1±0.04 |
| THM | **TCM** | 76.0 | 10.2 | 4.4 | 30.2 |
| THM | **BDCM** | 20.5 | 5.9 | 3.8 | 16.7 |
| THM | **DBCM** | 4.7 | 4.0 | 2.9 | 7.3 |
| THM | **TBM** | 0.0 | 0.5 | 0.5 | 0.4 |
| HAA | **CAA** | 4.3 | ND | ND | 1.7 |
| HAA | **BAA** | 0.8 | 0.8 | 0.2 | 0.7 |
| HAA | **DCAA** | 44.4 | 2.4 | 1.4 | 12.0 |
| HAA | **TCAA** | 28.8 | 1.0 | 0.3 | 7.3 |
| HAA | **BCAA** | 11.7 | 2.1 | 7.5 | 4.7 |
| HAA | **BDCAA** | 1.6 | 1.6 | 1.3 | 3.3 |
| HAA | **DBAA** | 2.3 | 1.3 | 1.2 | 1.4 |
| HAA | **DBCAA** | 2.7 | 2.0 | 2.2 | 2.6 |
| TOX | **TOCl as Cl** | 229.0±17.6 | 14.9±0.4 | 23.8±0.9 | 105.5±0.3 |
| TOX | **TOBr as Br** | 36.8±1.0 | 10.9±0.2 | 19.0±0.8 | 32.6±0.8 |
| TOX | **TOI as I** | 11.2±0.3 | 1.4±0.1 | 6.5±0.07 | 2.9±0.2 |

[a] All concentrations in μg/L (± standard deviation); ND: Not detected.

**Table 4.11. Plant 3 (first sampling) preformed DBP and simulated distribution system (SDS) results: Impact of GAC on removal of DBPs, TOCl, TOBr, and TOI a**

| DBP Class | DBP | Preformed GAC Inf | Preformed 9,200 BV | SDS GAC Inf | SDS 9,200 BV |
|---|---|---|---|---|---|
| HAL | **TCAL** | ND | ND | 16.6±5.8 | 21.0±3.5 |
| HAL | **CAL** | ND | ND | 1.8±0.2 | ND |
| HAL | **DCAL** | ND | ND | 4.9±0.2 | 1.9±0.01 |
| HAM | **DCAM** | ND | ND | 0.2±0.0006 | 0.1±0.0001 |
| HAN | **DCAN** | ND | ND | 3.7±0.1 | 2.6±0.1 |
| HK | **111TCP** | ND | ND | 5.4±0.2 | 6.9±0.1 |
| HK | **CP** | ND | ND | 6.1±1.7 | 2.7±0.4 |
| HNM | **TCNM** | ND | ND | ND | 0.5±0.01 |
| HNM | **BDCNM** | ND | ND | ND | 0.8±0.0002 |
| THM | **BDCM** | ND | ND | 1.4 | 1.4 |
| THM | **TCM** | ND | ND | 54 | 47 |
| HAA | **DCAA** | ND | ND | 37 | 28 |
| HAA | **CAA** | ND | ND | 2.3 | 0 |
| HAA | **TCAA** | ND | ND | 35 | 23 |
| TOX | **TOCl as Cl** | 49.6±1.6 | 50.3±7.0 | 220.0±3.5 | 182.0±0.3 |
| TOX | **TOBr as Br** | 1.8±0.2 | 0.9±0.1 | 1.8±0.2 | 1.7±0.1 |
| TOX | **TOI as I** | 0.7±0.01 | 0.6±0.03 | 17.9±0.9 | 9.5±0.2 |

[a] All concentrations in µg/L (± standard deviation); ND: Not detected.

**Table 4.12. Plant 3 (second sampling): Effect of GAC age on preformed TOCl, TOBr, and TOI removal a, b**

| Class | DBPs | GAC Inf | 3,400 BV | 3,800 BV |
|---|---|---|---|---|
| TOX | **TOCl as Cl** | 5.0±0.4 | 5.1±0.6 | 6.4±1.9 |
| TOX | **TOBr as Br** | 4.4±0.2 | 3.7±0.2 | 3.5±0.1 |
| TOX | **TOI as I** | 1.6±0.3 | 1.3±0.6 | 1.2±0.6 |

[a] All concentrations in µg/L (± standard deviation); ND: Not detected.

[b] No DBPs detected for native GAC with no disinfectant added

**Table 4.13. Plant 3 (second sampling) simulated distribution system results: Effect of GAC age on DBP and TOX removal a**

| Class | DBPs | GAC Inf | 3,400 BV | 3,800 BV |
|---|---|---|---|---|
| HAL | **TCAL** | 28.4±0.6 | 16.0±1.06 | 29.4±0.2 |
| HAL | **BDCAL** | 0.5±0.006 | 0.5±0.005 | 0.6±0.007 |
| HAM | **DCAM** | 3.6±0.3 | 0.3±0.04 | 0.7±0.1 |
| HAM | **TCAM** | 0.5±0.05 | 0.4±0.08 | 0.5±0.1 |
| HAN | **TCAN** | 0.4±0.001 | 0.3±0.0002 | 0.3±0.002 |
| HAN | **DCAN** | 6.2±0.1 | 1.8±0.08 | 2.2±0.2 |
| HAN | **BCAN** | 0.5±0.005 | 0.5±0.007 | 0.5±0.004 |
| HK | **11DCP** | 4.2±0.01 | 0.4±0.003 | 0.5±0.03 |
| HK | **111TCP** | 0.5±0.005 | 0.5±0.007 | 0.5±0.004 |
| HK | **1133TeCP** | 0.6±0.004 | 0.5±0.02 | 0.5±0.005 |
| HNM | **TCNM** | 0.6±0.005 | 0.3±0.006 | 0.3±0.009 |
| HNM | **DCNM** | 0.2±0.002 | ND | ND |
| THM | **TCM** | 77.9 | 21.9 | 29.5 |
| THM | **BDCM** | 1.9 | 1.7 | 1.8 |
| HAA | **DCAA** | 37.6 | 11.3 | 18.8 |
| HAA | **TCAA** | 31.7 | 8.6 | 12.9 |
| TOX | **TOCl as Cl** | 282.2±1.7 | 93.7±0.5 | 124.9±2.5 |
| TOX | **TOBr as Br** | 3.7±0.2 | 2.9±0.2 | 3.2±0.1 |
| TOX | **TOI as I** | 1.2±0.1 | 0.8±0.07 | 0.9±0.007 |

[a] All concentrations in µg/L (± standard deviation); ND: Not detected.

**Table 4.14. Plants 1, 2, and 3: % TOC removal, TOBr to TOC ratios, and TOI to TOC ratios**

| Plant | BV treated by GAC | TOC (mg/L) | % TOC removal | TOBr/TOC $*10^3$ | TOI/TOC $*10^3$ |
|---|---|---|---|---|---|
| **1** | GAC Inf. | 1.51 | NA | 14.6 | 3.2 |
| | 5600 | 0.47 | 68.9 | 4 | 6.8 |
| | 12600 | 0.83 | 45.1 | 6.7 | 11.6 |
| | 22400 | 1.08 | 28.5 | 15.5 | 3.4 |
| **2** | GAC Inf. | 2.1 | NA | 17.5 | 5.3 |
| | 3000 | 0.3 | 85.7 | 36.3 | 4.7 |
| | 8700 | 0.6 | 71.4 | 31.7 | 10.8 |
| | 22000 | 1.2 | 42.9 | 27.2 | 2.4 |
| **3 (1st Sampling)** | GAC Inf. | 2 | NA | 0.9 | 9 |
| | 9200 | 1.36 | 32 | 1.3 | 7 |
| **3 (2nd Sampling)** | GAC Inf. | 1.99 | NA | 1.9 | 0.6 |
| | 3400 | 0.74 | 62.8 | 3.9 | 1.1 |
| | 3800 | 0.85 | 57.3 | 3.8 | 1.1 |

**Table 4.15. Plant 1 calculated cytotoxicity and genotoxicity for preformed DBPs and after SDS chlorination**

| | Cytotoxicity | | Genotoxicity | |
|---|---|---|---|---|
| **Bed Volumes** | **Preformed** | **SDS** | **Preformed** | **SDS** |
| **No GAC** | 1534 | 2195 | 387 | 371 |
| **5,600** | 399 | 1831 | 65 | 245 |
| **12,600** | 2 | 2002 | 0 | 253 |
| **22,400** | 4 | 2034 | 0 | 265 |

**Table 4.16. Plant 2 calculated cytotoxicity and genotoxicity for preformed DBPs and after SDS chlorination**

| | Cytotoxicity | | Genotoxicity | |
|---|---|---|---|---|
| **Bed Volumes** | **Preformed** | **SDS** | **Preformed** | **SDS** |
| **No GAC** | 2718 | 8093 | 285 | 840 |
| **3,000** | 1543 | 2513 | 75 | 419 |
| **8,700** | 10 | 2833 | 0 | 199 |
| **22,000** | 1116 | 5378 | 58 | 574 |

**Table 4.17. Plant 3 calculated cytotoxicity and genotoxicity after SDS chlorination**

| | Cytotoxicity | | Genotoxicity | |
|---|---|---|---|---|
| **Bed Volumes** | **Preformed** | **SDS** | **Preformed** | **SDS** |
| **No GAC (1)** | NA | 8910 | NA | 286 |
| **No GAC (2)** | | 1910 | | 87 |
| **3400 (2)** | | 963 | | 43 |
| **3800 (2)** | | 1158 | | 46 |
| **9200 (1)** | | 1538 | | 123 |

NA: Not applicable, as no preformed DBPs were detected.

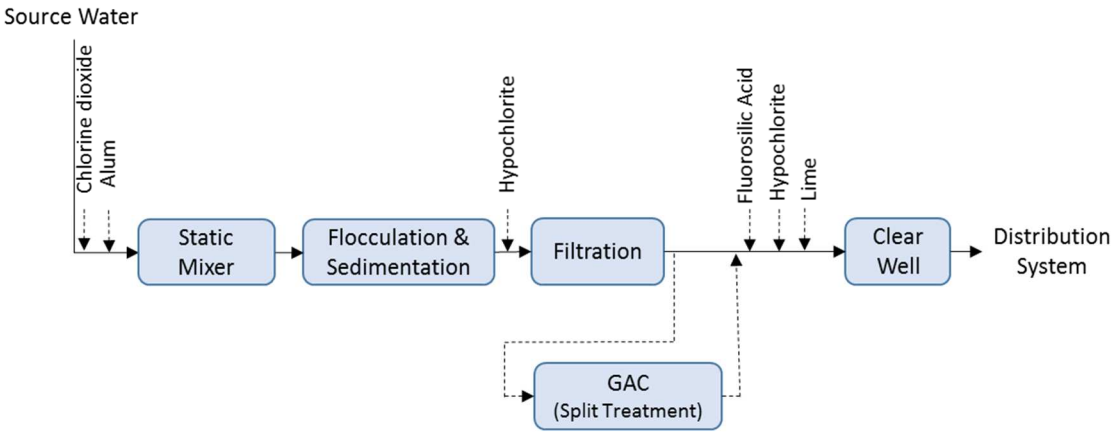


**Figure 4.7. Process flow diagram of Plant 1.**

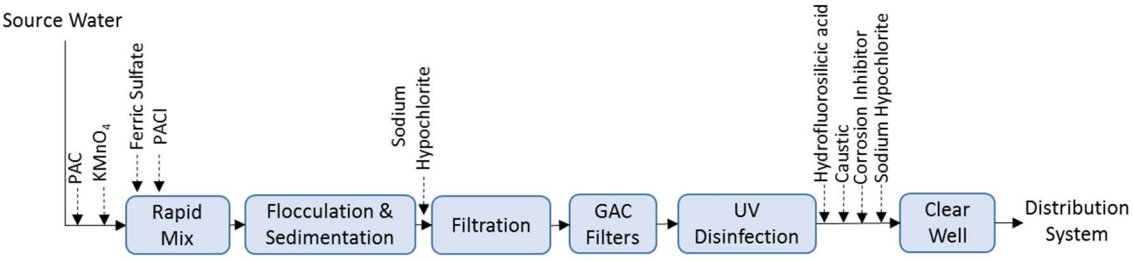


**Figure 4.8. Process flow diagram of Plant 2.**

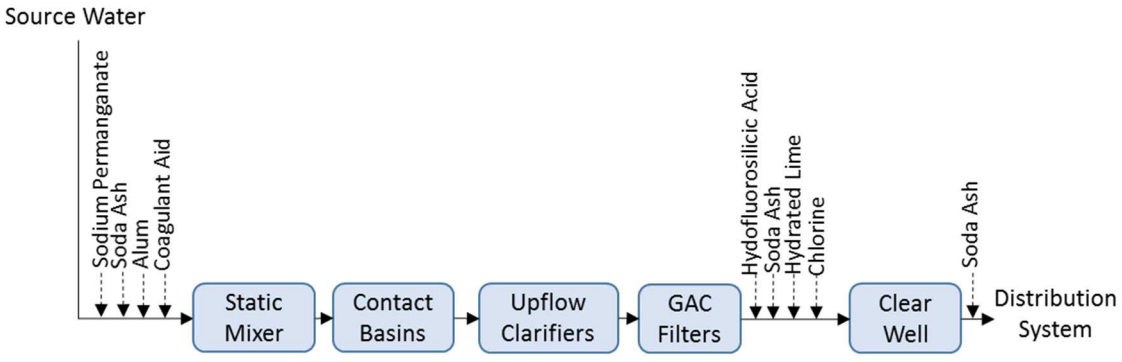


**Figure 4.9. Process flow diagram of Plant 3.**

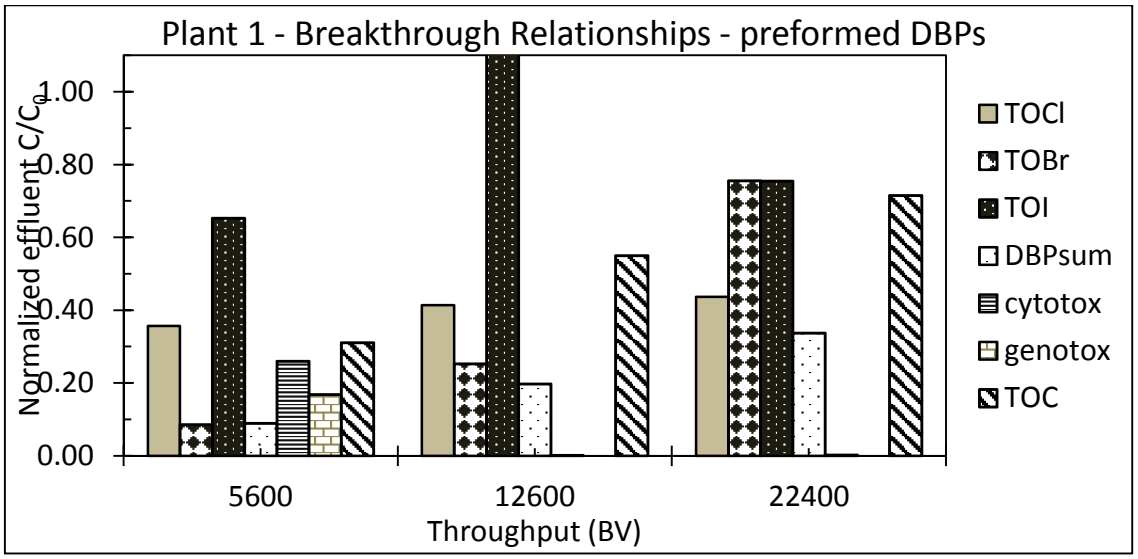


**Figure 4.10.** Breakthrough of background TOC, preformed DBPs as measured by total organic halogen (TOCl, TOBr, TOI), the mass sum of DBPs, and calculated cyto- and genotoxicity before final disinfection at Plant 1**.**

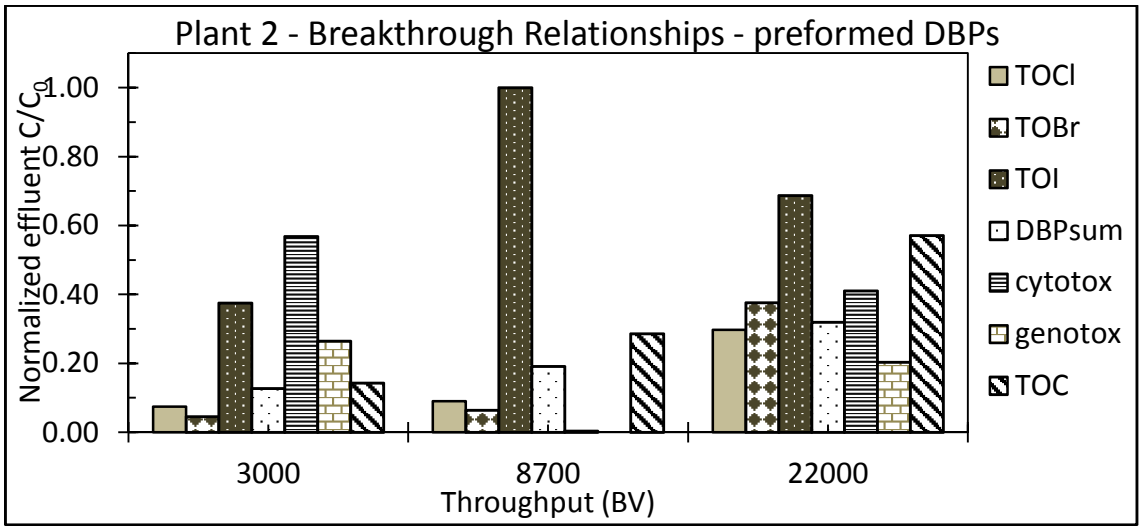


**Figure 4.11**. Breakthrough of background TOC, preformed DBPs as measured by total organic halogen (TOCl, TOBr, TOI), the mass sum of DBPs, and calculated cyto- and genotoxicity before final disinfection at Plant 2.

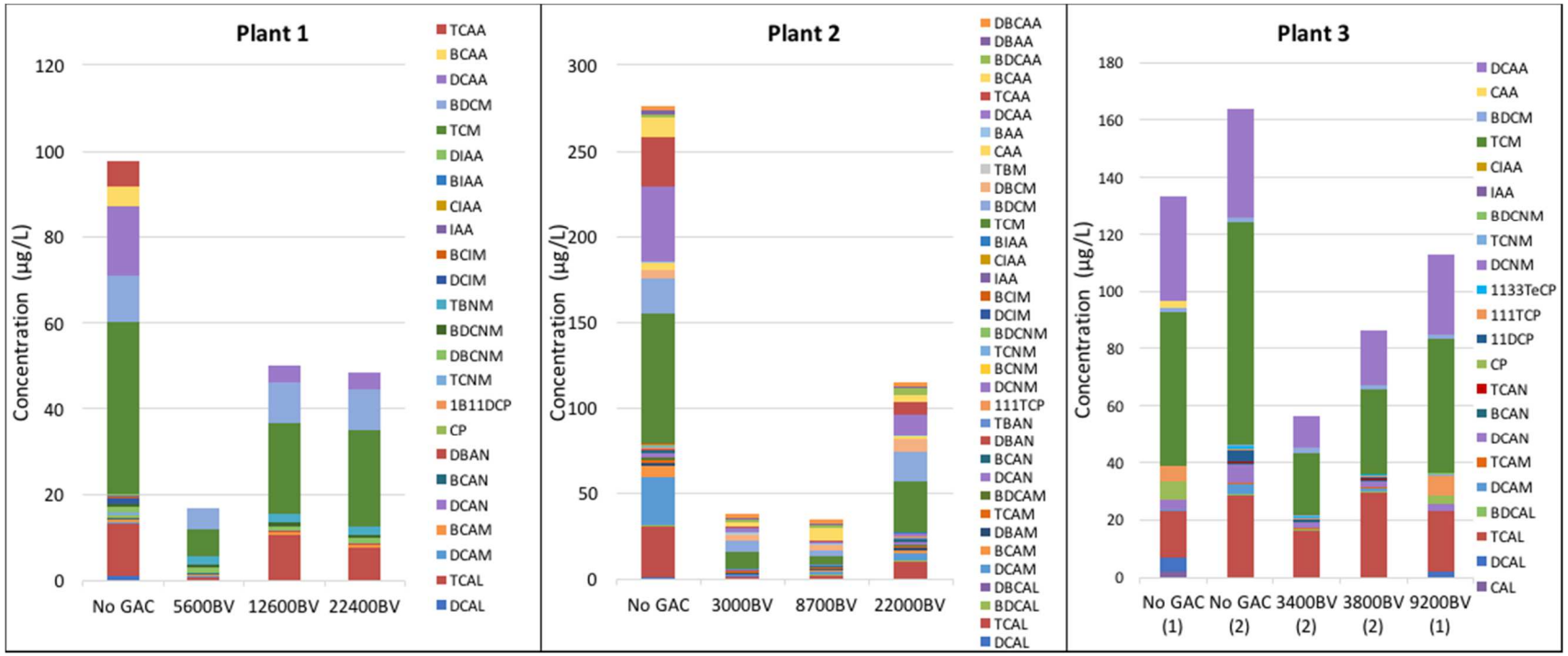


**Figure 4.12. Effectiveness of GAC treatment for controlling regulated and unregulated DBPs for Plants 1, 2, and 3.[a]**

[a] "No GAC" represents the level of DBPs that would be expected at the consumers' tap following simulated distribution system (SDS) chlorination, in the absence of GAC treatment. Subsequent columns represent the effectiveness of GAC for DBP control following SDS chlorination over a range of GAC service times, indicated by the number of bed volumes (BV) treated. For Plants 1 and 2, where pre-chlorination was practiced, the GAC effectiveness reflects removal of both preformed DBPs and DBP precursors. For Plant 3, where no chlorine was used before GAC treatment, results reflect DBP control by precursor removal only.

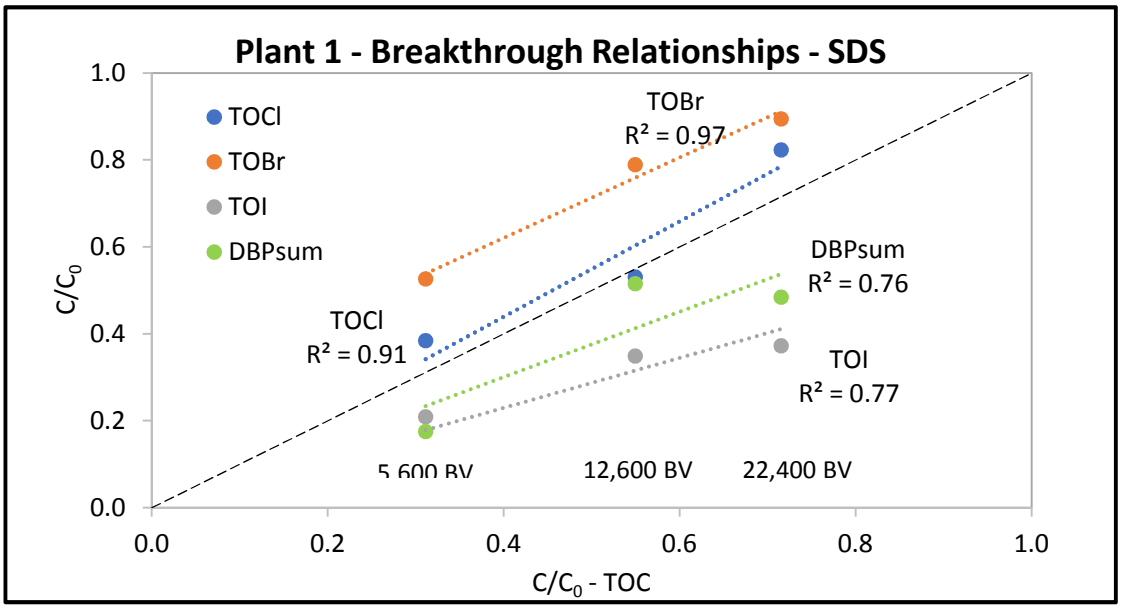


**Figure 4.13**. Plant 1 Breakthrough relationships to TOC for SDS total organic halogen (TOCl, TOBr, TOI), and total sum of DBPs.

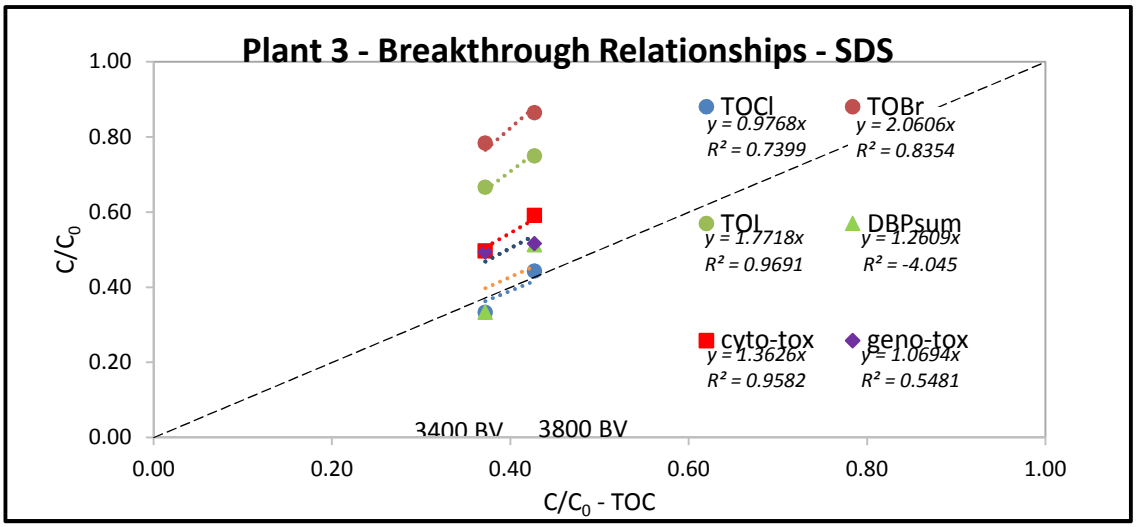


**Figure 4.14**. Plant 3 (2nd sampling) breakthrough relationships to TOC for SDS total organic halogen (TOCl, TOBr, TOI), total sum of DBPs, and calculated cytotoxicity and genotoxicity.

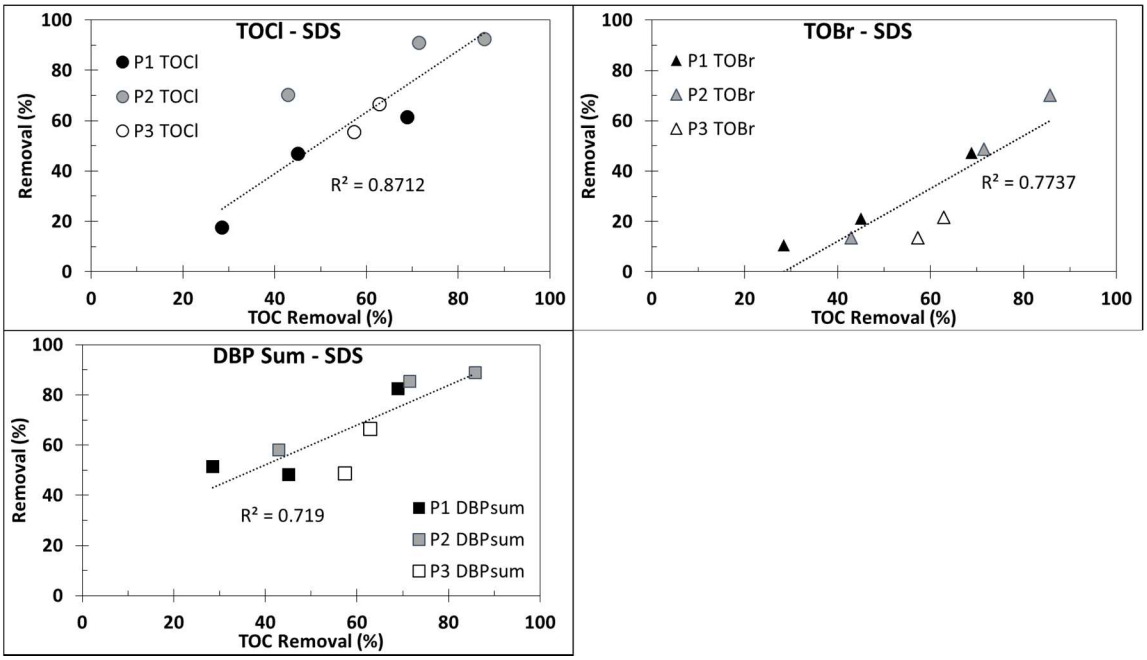


**Figure 4.15**. Relationship between TOC removal and SDS TOCl, TOBr, and DBP sum removal for Plants 1, 2, and 3 (P1, P2, P3).

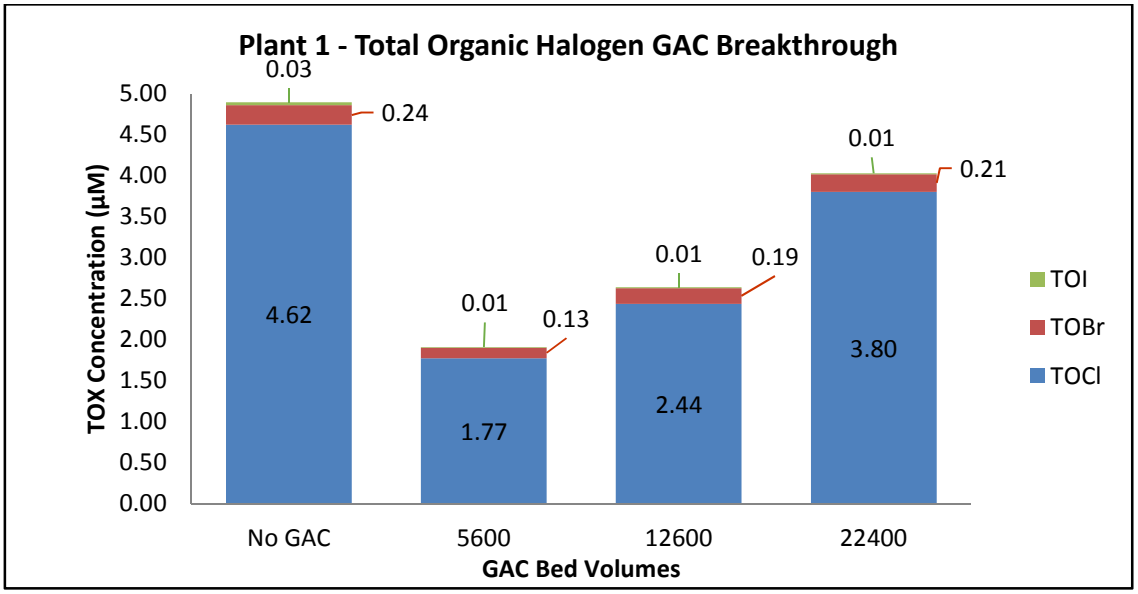


**Figure 4.16.** Plant 1 simulated distribution system: Effect of GAC age on TOCl, TOBr, and TOI removal in molarity.

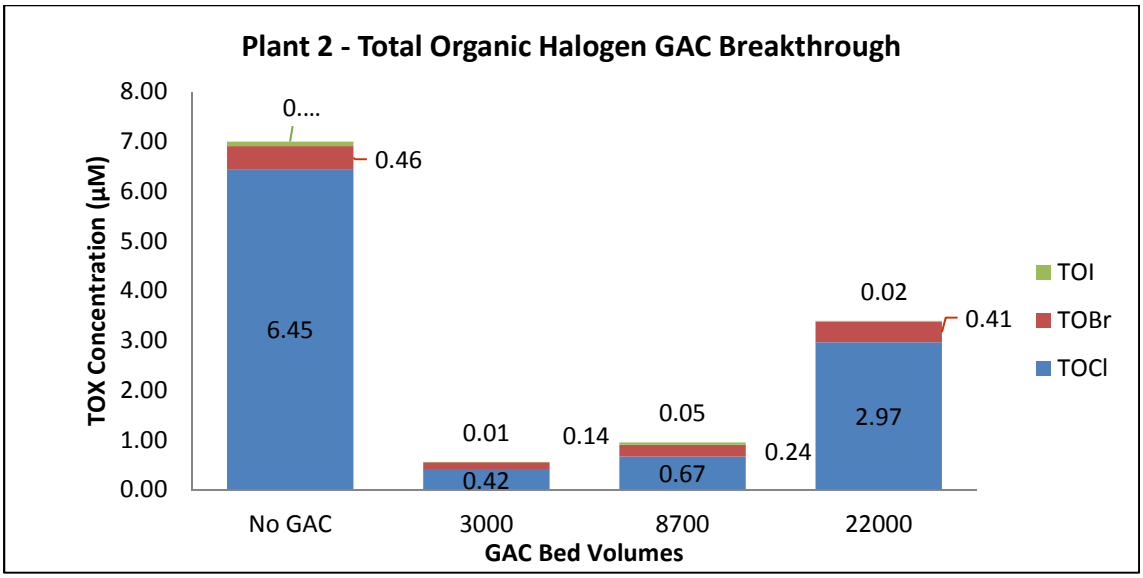


**Figure 4.17**. Plant 2: Effect of GAC age on TOCl, TOBr, and TOI removal in molarity.

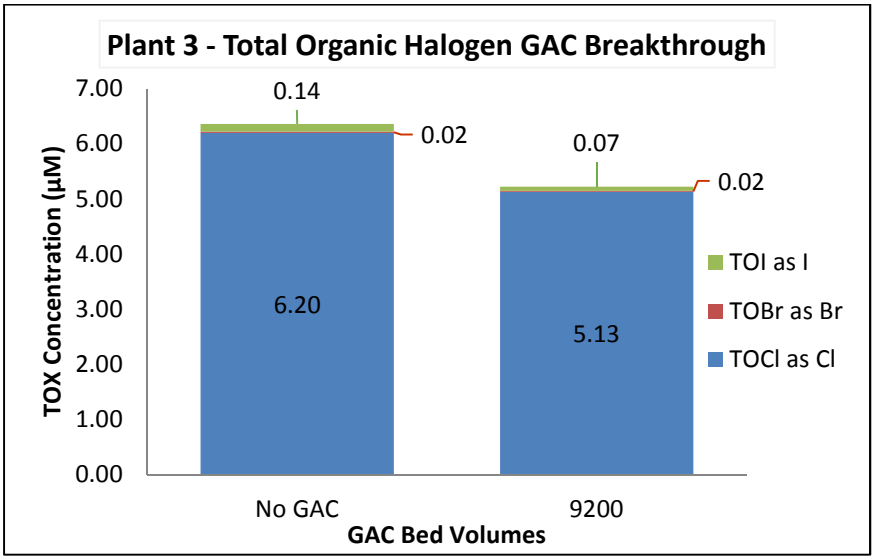


**Figure 4.18**. Plant 3 (first sampling): Effect of GAC on TOCl, TOBr, and TOI removal in molarity.

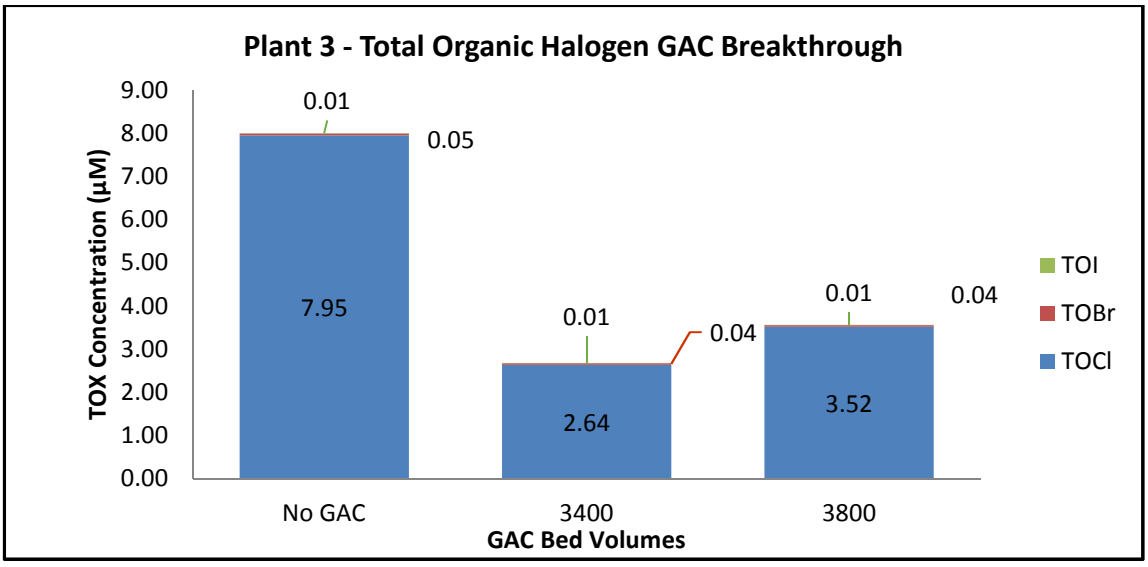


**Figure 4.19.** Plant 3 (second sampling): Effect of GAC age on TOCl, TOBr, and TOI removal in molarity.

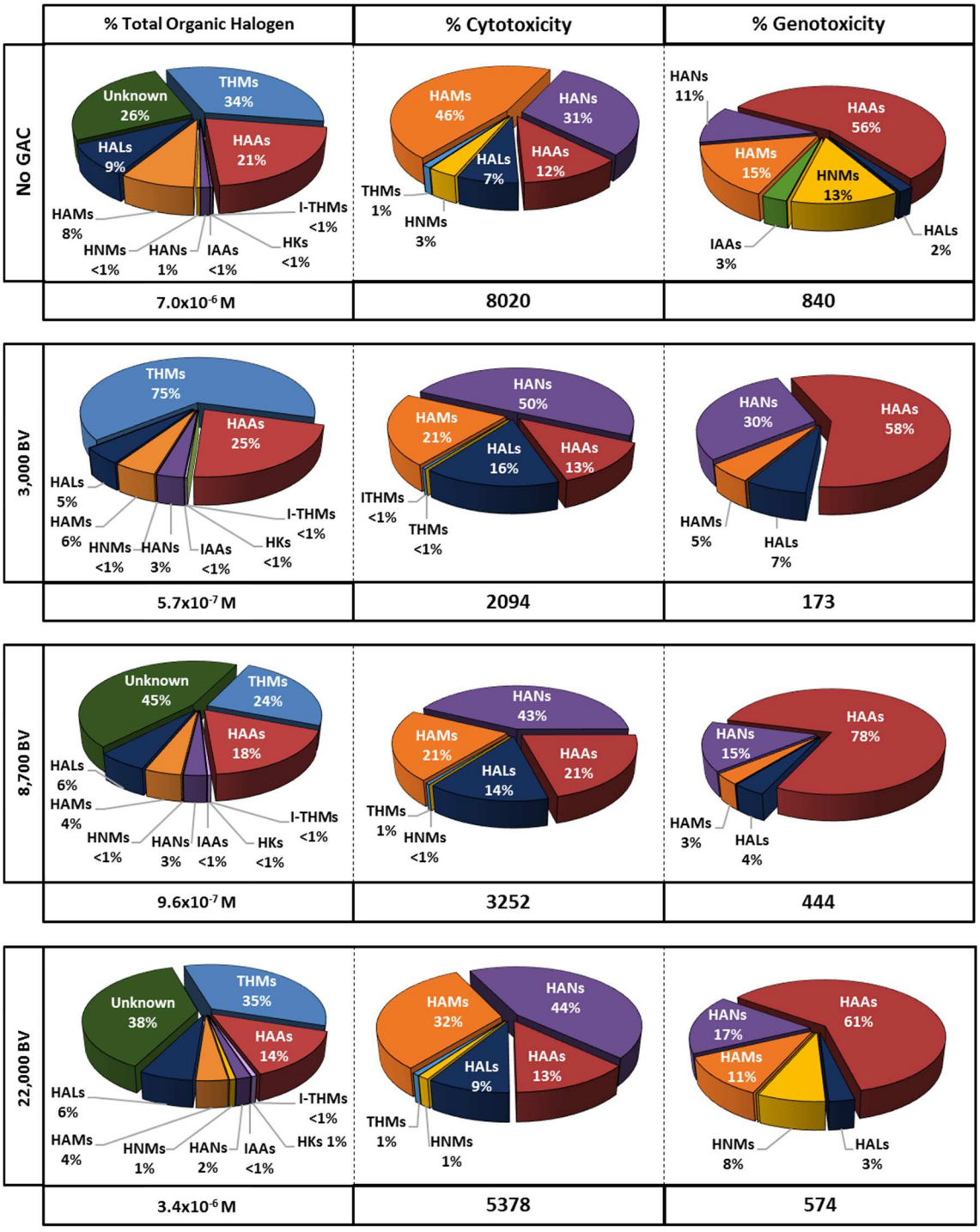


**Figure 4.20.** Plant 2: % Total organic halogen (in molarity, normalized by the number of halogens), % calculated cytotoxicity, and % calculated genotoxicity for each DBP class.[a,b]

[a] Note that there are no data on cytotoxicity or genotoxicity index values in literature for haloketones or tribromoacetonitrile.

[b] Total toxicity = $\sum([DBP] \times (C_{1/2})^{-1} \times 10^{6})$

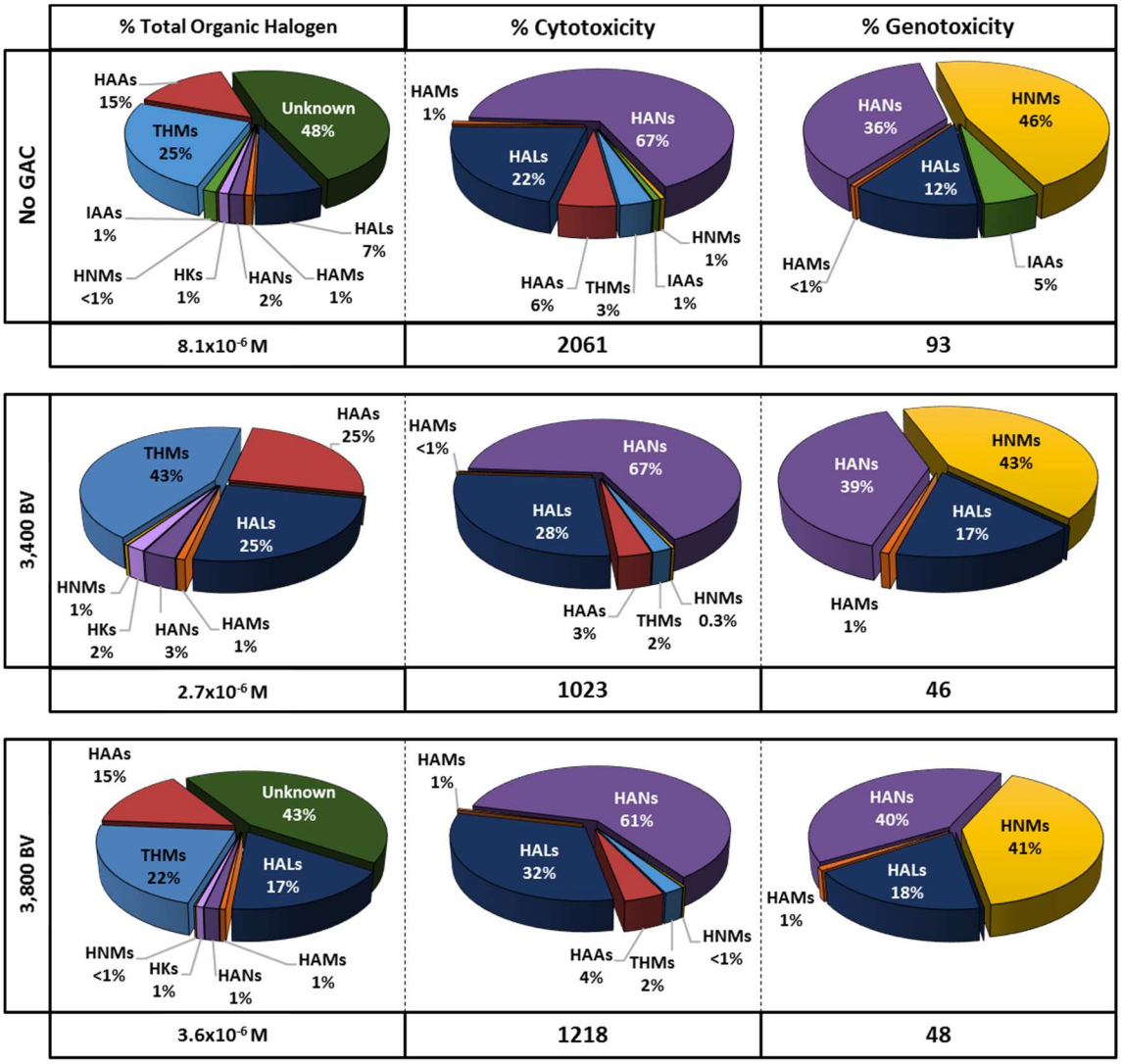


**Figure 4.21.** Plant 3 (second sampling): % Total organic halogen (in molarity), % calculated cytotoxicity, and % calculated genotoxicity for each DBP class.[a, b, c]

[a] Note that there is no data on cytotoxicity or genotoxicity index values in literature for haloketones or tribromoacetonitrile.

[b] All the DBPs on the youngest aged GAC were quantified; hence, no unknown fraction was included.

[c] Total toxicity = $\sum([DBP] \times (C_{1/2})^{-1} \times 10^{6})$

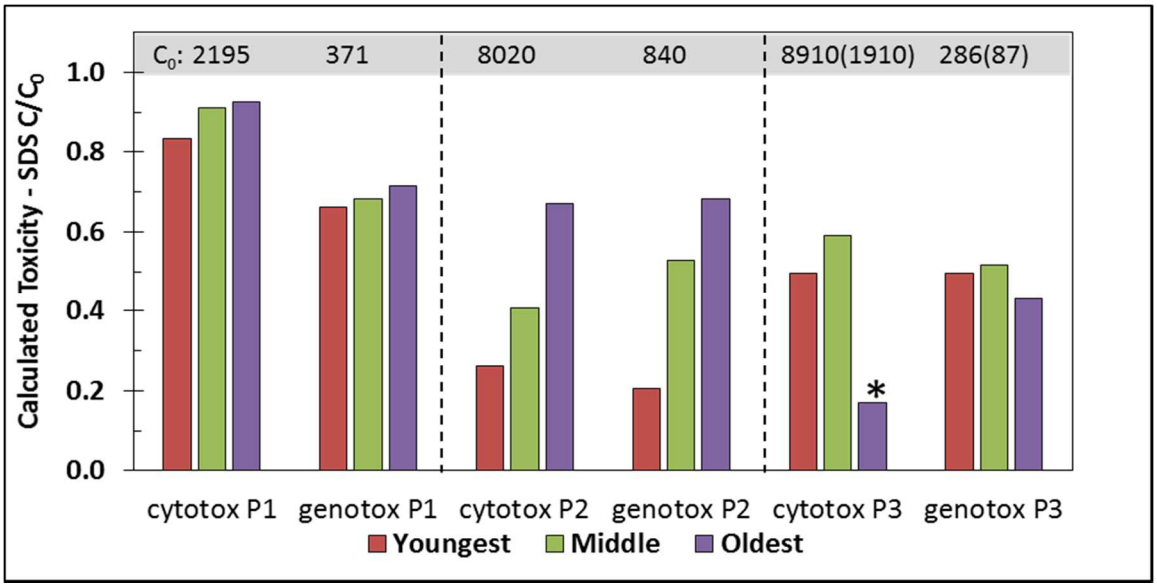


**Figure 4.22.** Calculated cytotoxicity (cytotox) and genotoxicity (genotox) for Plants 1, 2, and 3 (P1, P2, and P3)-SDS breakthrough. C0 values for P3 second sampling are in parentheses. Plant specific bed volumes are found in Table 4.1. *Influenced by substantially higher value for paired influent sample.

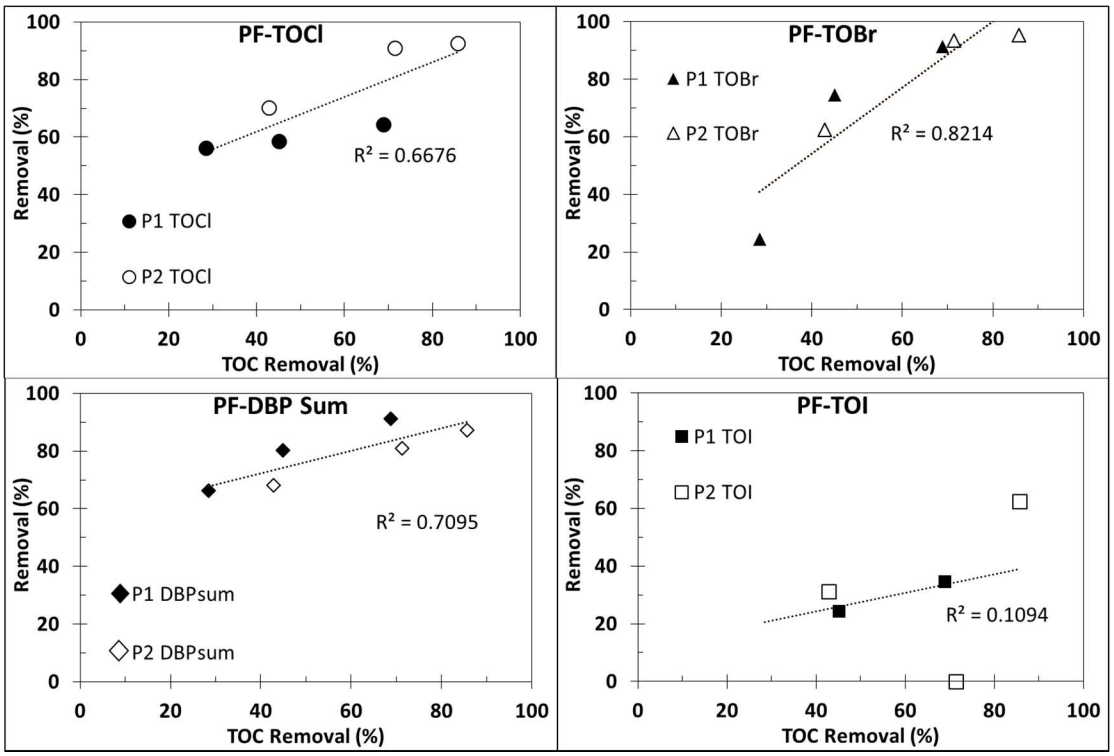


**Figure 4.23.** Relationship between TOC removal and preformed TOCl, TOBr, TOI, and DBP sum removal for Plants 1 and 2 (P1, P2).[a] One TOI value was not included in regression (P1 at 12,600 BV) due to increased concentrations.

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# CHAPTER 5

# GAC TO BAC: DOES IT MAKE CHLORAMINATED DRINKING WATER SAFER?[5]

[5] Amy A. Cuthbertson, Susana Y. Kimura, Hannah K. Liberatore, Detlef R. U. Knappe, Benjamin Stanford, R. Scott Summers, Eric Dickenson, Clark Maness, Riley E. Mulhern, Caitlin Glover, Meric Selbes, and Susan D. Richardson. To be submitted to *Water Research.*

Abstract

Biological activated carbon (BAC) is widely used as a clean-up step at full-scale drinking water plants to remove taste and odor compounds like 2-methylisoborneol (MIB) and geosmin. BAC has been previously studied for the reduction of regulated disinfection by-products (DBPs), particularly for with pre-ozonation in pilot-plants. However, most previous studies only include regulated or a handful of unregulated DBPs in pilot-scale conditions. This study explored two full-scale drinking water plants that used pre-chloramination followed by BAC with chloramine used as a final disinfectant. While chloramine generally produces lower concentrations of regulated DBPs, it may form increased levels of unregulated nitrogenous and iodinated DBPs. We evaluated 71 DBPs from eight DBP classes including haloacetonitriles, haloacetamides, halonitromethanes, haloacetaldehydes, haloketones, iodinated acetic acids, iodinated trihalomethanes, nitrosamines, trihalomethanes, and haloacetic acids, along with speciated total organic halogen (total organic chlorine, bromine and iodine) across 6 different BAC filters of increasing age. Most preformed DBPs were well removed by BAC. However, some DBPs were poorly removed, or increased following treatment with BAC including chloroacetaldehyde, dichloronitromethane, bromodichloronitromethane, N-nitrosodimethylamine, dibromochloromethane, tribromomethane, dibromochloroacetic acid, and tribromoacetic acid. Some compounds, including dibromoacetaldehyde, bromochloroacetamide, and dibromoacetamide, were formed only *after* treatment with BAC. While calculated genotoxicity decreased in all filters, decreases in overall formation of DBPs did not correlate with calculated cytotoxicity. In three of the six filters, calculated toxicity *increased* by 4-27%.

Keywords (6)

Biological activated carbon, drinking water, DBPs, disinfection by-products, total organic halogen, calculated cytotoxicity, genotoxicity

## 1. Introduction

Chemical disinfection is vital worldwide in providing safe drinking water free of waterborne illness, an unintended consequence is disinfection by-products (DBPs) that are formed by the reaction of disinfectants with organic matter, bromide, and iodide present in water (Richardson 2011). Several adverse health effects have been associated with DBPs, including bladder cancer, colon cancer, miscarriage, and birth defects (Richardson et al., 2007; Cantor et al., 2010; Nieuwenhuijsen et al., 2000; Savitz et al., 2005; Villanueva et al., 2004; Waller et al., 1998; Costet et al., 2011; Rahman et al., 2010; Villanueva et al., 2007). The main chemical disinfectant used for drinking water worldwide is chlorine, but to comply with DBP regulations in the U.S. many drinking water treatment plants have switched from chlorine to alternative disinfectants like chloramines (Zhang et al., 2000). Chloramines are less reactive with dissolved organic matter (DOM) compared to chlorine, and therefore, lower the formation of regulated DBPs such as THMs and HAAs (Seidel et al., 2005; Hua et al., 2007; Allard et al., 2015). However chloramines can form increased levels of more toxic unregulated iodinated, and nitrogenous DBPs (I-DBPs, and N-DBPs, respectively) (Plewa et al., 2004; Krasner et al., 2006; Richardson et al., 2008; Plewa et al., 2008; Chu et al., 2013; Huang et al., 2012). Current research shows that regulated THMs and HAAs account for only a small percentage of the toxicological effects seen in drinking water, and nitrogenous DBPs (N-

DBPs) appear to be the major forcing agents of toxicity (Plewa et al., 2017; Krasner et al., 2016; Cuthbertson et al., 2019). These include *N*-nitrosodimethylamine (NDMA), haloacetonitriles (HANs), halonitromethanes (HNMs), haloamides (HAMs), and cyanogen halides (CX). Currently, no N-DBPs are regulated.

Typically, there are two ways in which chloramination is conducted: either a free chlorine contact time is allowed before ammonia is added to form chloramines (most common), or chloramines are preformed before addition to water. Depending on the source water quality and which treatment process a drinking plant employs will result in varying concentrations of DBPs. For example, one study showed that waters with low aromaticity formed higher levels of iodinated trihalomethanes (I-THMs) when employing preformed $NH_2Cl$, while waters with higher aromaticity formed higher levels of I-THMs when a pre-chlorine contact time was allowed (Allard et al., 2015; Jones et al., 2011). While chloramination generally results in lower levels of total organic halogen (TOX) than chlorination (Hua et al., 2007), often increased levels of total organic iodine levels are formed (Kristiana et al., 2009). In several studies, less than 70% of TOX in chloraminated drinking water was accounted for by measured individual DBP species (Krasner et al. 2006; Hua et al., 2007; Kristiana et al., 2009; Tian et al., 2013). Some studies have shown that known, quantified DBPs rarely account the overall for toxicity effects of disinfected water in cells, and therefore, it is widely suspected that unknown or unquantified TOX contains toxicologically relevant DBPs (Hua et al., 2007; Bull et al., 2011).

The use of granular activated carbon (GAC) embodies both adsorption and biodegradation of DOM. The DOM adsorption process is a non-steady-state process in which the effluent concentrations of DOM increases with time until all the sorption sites are occupied. For the biodegradable DOM fractions, a bio-acclamation process is required, which might last from a few weeks to several months (Zearley et al., 2012). After acclamation has been achieved, biodegradation is a steady-state process when the influent conditions are constant (e.g., temperature) (Servais et al., 1994). After most of the adsorption capacity is exhausted, biodegradation is the dominant removal mechanism and the GAC process is often termed biological activated carbon (BAC) (Shukairy et al., 1992; Miltner et al., 1992). Operation in the BAC mode has the advantage of not requiring GAC change-out and can be effectively used to control biodegradable compounds, including taste and odor compounds, 2-methylisoborneol (MIB) and geosmin, and a wide range of other organic compounds of concern (Ho et al., 2007; Suffet et al., 1995). It is well established that a fraction of organic THM and HAA precursors can be removed by biofilters, including BAC columns (Shukairy et al., 1992; Miltner et al., 1992). However, in two studies, BAC yielded an increased dissolved organic nitrogen to dissolved organic carbon (DON:DOC) ratio, resulting in higher formation of N-DBPs (Shukairy et al., 1992; Chu et al., 2012). There is some information on the effect of BAC on the formation of unregulated DBPs (Table 5.1), however, many unregulated DBPs have not been studied.

Previous studies have shown that BAC is efficient at removing low molecular weight (MW) NOM, including some DBP precursors, algal toxins, ammonia and trace

level organics, but it is less effective for removing higher MW hydrophilic fractions. One potential drawback of BAC is that effluents can contain microbes, soluble microbial products (SMPs), and extracellular polymeric substances (EPS) excreted by microbes and released from the biofilm. Dead cells, SMPs, and EPS may serve as DBP precursors, in particular N-DBPs (Krasner et al., 2015). Important parameters to consider for effective BAC treatment include substrate concentration, pH, dissolved oxygen (DO), biofilm thickness, empty-bed contact time (EBCT), shear stress, and backwashing intensity and frequency. Some studies have shown that pre-disinfecting with chlorine, chloramines, chlorine dioxide, or ozone can potentially decrease the removal efficiency of organic matter, and may disturb or destroy the biofilm on the AC at higher concentrations. Furthermore, EPS may be produced by the biofilm as a protective response to chemical oxidants. One study reported the critical residual chlorine concentration that does not have an adverse effect on biological activity was approximately 0.2 mg/L. (Korotta-Gamage et al., 2017)

Given the concerns for the formation of more toxic Br-, I-, and N-DBPs with chloramines, along with increased DON:DOC ratios found in waters treated with BAC, it is important to assess whether water treated in this manner is in fact safer. To address this question, we used an approach termed “TIC-Tox” which multiplies the concentration of each individual DBP by their corresponding cytotoxicity and genotoxicity index values (Plewa et al., 2017). This method has been used in several published studies to assess potential toxicity of treated waters where toxicity bioassay tests were not available or

possible (Plewa et al., 2017; Smith et al., 2010; Allard et al., 2015; Krasner et al., 2016; Cuthbertson et al., 2019).

While there are many studies on the use of pre-ozonation with BAC, only seven full-scale studies evaluated the use of BAC on the formation of DBPs without the use of pre-ozonation (Liu et al., 2017; Lyon et al. 2014). Four of these studies evaluated a few unregulated DBPs, including nitrosamines (Gerrity et al., 2015), NDMA (Bukhari et al., 2016; Page et al., 2016), haloketones, haloacetaldehydes, halonitromethanes, haloacetonitriles, and haloacetamides (Zeng et al., 2016a; Liu et al., 2017). No plants in previous studies carried a chloramine residual prior to treatment with BAC, as is the case with the plants evaluated in this study. Our current study provided an opportunity to study the biodegradation capacity of BAC with chloramine to degrade many unregulated Br-, I-, and N-DBPs, including many DBPs that have not been previously investigated. To the best of our knowledge, no full-scale studies have evaluated regulated and unregulated DBP formation, along with TOX in plants that carry a chloramine residual prior to BAC without the use of pre-ozonation, as is the case with the full-scale drinking plants evaluated in this study.

The goals of our study were to assess the effectiveness of full-scale BAC treatment for controlling: (1) DBPs formed during pre-oxidation, and biodegradation; (2) formation of speciated TOX, including total organic chlorine, bromine, and iodine (TOCl, TOBr, TOI); (3) formation of DBPs following final disinfection after treatment

with BAC; and (4) calculated toxicity associated with quantified DBPs. Water samples were collected at two full-scale plants in the United States that use chloramine disinfection, and DBP precursor control was evaluated across a range of BAC service lives at each plant (Table 5.2). Since source waters impact DBP formation, these two plants were chosen because both have source waters impacted by industry, wastewater, and agriculture. Both plants also use amine-based polymers during coagulation, which can impact the formation of N-DBPs. Formation of 71 regulated and unregulated DBPs were studied in bench-scale simulated distribution system (SDS) tests for DBPs from eight DBP classes: haloacetonitriles (HANs), haloacetamides (HAMs), halonitromethanes (HNMs), haloacetaldehydes (HALs), haloketones (HKs), iodinated acetic acids (IAAs), iodinated trihalomethanes (I-THMs), NDMA, THMs, and HAAs. This is the first time that many of these DBPs have been investigated for the removal of BAC in a full-scale study. TOCl, TOBr, and TOI were also measured and compared to the total concentration of quantified DBPs, with the balance representing a quantitative measure of *unknown* or unquantified DBPs (Smith et al., 2010; Echigo et al., 2000; Daiber et al., 2016). Based on the 71 DBPs quantified, cytotoxicity and genotoxicity were calculated using the TIC-Tox method (Plewa et al., 2017). This study seeks to address the question of whether BAC is effective in controlling toxic unregulated DBPs and whether it impacts calculated cytotoxicity and genotoxicity from a DBP perspective.

## 2. Materials and methods

### 2.1 Sampling of drinking water treatment plants

Two full-scale drinking water plants (Plants A and B) were sampled, and characteristics such as impacts on source water quality, pre-oxidant type, use of coagulant aids, and BAC operation were evaluated. Both plants used chloramine as a pre-oxidant and as the secondary disinfectant throughout the distribution system, and both plants carried a chloramine residual throughout the plant, including into the BAC adsorber (Table 5.2). Samples were taken of the BAC influent and the BAC effluent and analyzed for preformed DBPs. Simulated distribution system (SDS) tests were carried out according to protocols set by each plant, and chloramine contact times were equivalent to each drinking water plant's longest water age in the distribution system with a target chloramine residual of 1 to 2.5 mg/L of $NH_2Cl$ as $Cl_2$.

#### 2.1.1 Operational conditions

For both Plants A and B, GAC media had not been replaced for the past several years (Table 5.2) prior to sampling. At Plant A, BAC filters typically received a flow with a loading rate of 1.5 gpm/ft$^2$ to achieve an EBCT of 10-15 min. In general, 1.5 to 2.0 mg/L of chloramines are present in the BAC feed water. Plant B operated under similar conditions; typically BAC filters received a flow with a loading rate of 2.1 gpm/ft$^2$ to achieve an EBCT of 5 min. Since the GAC media depth was similar to underlying sand media, the EBCT of the BAC was approximately 2.5 min., with 2.0 to 4.0 mg/L of chloramines present in the feed water. Process flow diagrams for Plant A and B can be found in Figures 5.6 and 5.7 in Supporting Information (SI).

### 2.1.2 Simulated distribution system (SDS) conditions

In Plant A, samples were spiked with 4.0 to 4.5 mg/L of preformed chloramines without pH adjustment (pH in the range of 7 to 8) to achieve a chloramine residual of 1.0 to 2.0 mg $Cl_2$/L chloramine residual after 63 h residence time. In Plant B, samples were spiked with 3.5 to 5.5 mg/L of preformed chloramines without any pH adjustment (pH in the range of 7 to 8) to achieve a chloramine residual of 1.0 to 2.0 mg $Cl_2$/L after 124 h residence time. Water quality parameters for Plants A and B including bed volumes treated (BVs), total organic carbon (TOC), UV254, specific ultraviolet absorbance (SUVA), total nitrogen (TN), DON, ammonia, nitrite, nitrate, bromide, and iodide, can be found in Table 5.3. Information regarding sampling dates, flow rates, empty bed contact times (EBCT), pre-oxidant concentration levels, GAC type, throughput, and percent DOC breakthrough can be found in Table 5.6 in SI.

Samples from all plants and SDS testing were collected in two 1 L bottles, each containing ascorbic acid in excess (chloramine to quencher molar ratio of 1:1.3) based on a maximum residual chloramine of 5 mg/L and adjusted to pH 3.5-4 with 1 M $H_2SO_4$. Samples were shipped overnight with icepacks and extracted the day they were received or stored at 4°C and extracted within 2 days. Samples were analyzed in duplicate. Quantified DBPs were found to be stable under these conditions (Allen et al., 2017; Kristiana et al., 2014; Gong et al, 2016).

## 2.2 Chemicals and reagents

Analytical standards for priority DBPs were purchased or custom synthesized at the highest purity available from CanSyn Chem. Corp. (Toronto, Ontario), Sigma-Aldrich (St. Louis, MO), Aldlab Chemicals (Boston, MA), and TCI America (Boston, MA), as

shown in Table 5.5 (SI). Acetonitrile, methyl *tert*-butyl ether (MTBE), methanol, and pure water were purchased from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA) at the highest purity available.

## 2.3 Analytical methods

### 2.3.1 Water quality and regulated DBPs

Water quality parameters [residual chlorine, DOC, UV254, SUVA, total nitrogen, dissolved organic nitrogen, ammonia, nitrite, nitrate, bromide, and iodide] were measured using methods described in Table 5.6 in SI. THMs and HAAs were measured using Environmental Protection Agency (EPA) Methods 551.1 and 552.2, respectively (US EPA 1995; US EPA 2003).

### 2.3.2 Unregulated DBPs.

NDMA was measured using a modified version of EPA Method 521 (Holady et al., 2012). Briefly, samples were extracted via automated solid phase extraction (SPE) on activated charcoal SPE cartridges. Quantification was accomplished using isotope dilution with isotopically-labeled NDMA-$d_6$ and gas chromatography tandem mass spectrometry (GC-MS/MS) in positive chemical ionization mode. A Varian 4000 Ion Trap GC-MS/MS system was used with a DB-624 column (30 m x 0.25 mm x 1.4 µm, Agilent, Palo Alto, CA).

Extraction and analysis of the remaining 57 DBPs (Table 5.5, SI) were conducted using previously published procedures (Allen et al., 2017; Kimura et al., 2018). HALs, HANs, HKs, HNMs, I-THMs, and HAMs were analyzed using GC-MS with electron ionization (EI) and selected ion monitoring (SIM) on an Agilent 7890 GC coupled to an Agilent 5977A mass spectrometer (Agilent Technologies, Santa Clara, CA) with a Rtxi-200 column (30 m x 0.25 mm x 1.4 um µm film thickness; Restek Corporation, Bellefonte, PA). IAA analysis required diazomethane derivatization (Richardson 2009), and these compounds were analyzed using GC-EI-MS/MS with a Thermo Quantum GC$^{TM}$ triple quadrupole mass spectrometer coupled to a TRACE GC Ultra gas chromatograph (Thermo Scientific, Waltham, MA). Mono- and di-haloaldehydes were analyzed according to an *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) derivatization followed by liquid-liquid extraction (LLE) with hexane and GC-EI-MS analysis (Jeong et al., 2015; Allen et al., 2017). Minimum reporting limits (MRLs) ranged from 0.1 to 5.0 µg/L.

### 2.3.3 Total organic halogen (TOX)

TOCl, TOBr, and TOI were determined using a Mitsubishi TOX analyzer (Mitsubishi Chemical Analytech, Chigasaki, Japan; Cosa Xentaur, Yaphank, USA), according to previously published procedures (Smith et al., 2010; Echigo et al., 2000; Daiber et al., 2016; Kimura et al. 2017) with a few modifications (Allen et al., 2017). Briefly, acidified samples (pH <2) were adsorbed on activated carbon, washed with 5% potassium nitrate solution and combusted at 1000°C in the presence of oxygen and argon as a carrier gas. Combusted gases were collected in a fresh aqueous solution (0.03% $H_2O_2$). Recovered

aqueous solutions were analyzed for chloride, bromide, iodide with a Dionex 1600 ion chromatograph (Dionex, Sunnyvale, CA) as described elsewhere. A Finnigan ELEMENT XR double focusing magnetic sector field inductively coupled plasma (ICP)-mass spectrometer (Thermo Electron Corporation, Waltham, MA) was used for trace-level iodide and bromide analysis (Yang et al., 2014; Kimura et al., 2017).

### 2.3.4 Water toxicity and percent TOX calculations

The contribution of each DBP class to TOX was calculated by first multiplying the molar concentration of each compound that pertained to a specific DBP class by its corresponding number of halogens (i.e., 1-4 halogen atoms). These values were then added and this sum was divided by the sum molar concentration of TOCl, TOBr, and TOI.

The water toxicity of each sample was based on the "TIC-tox" method published by Plewa et al. (2017). In brief, the molar concentration of each DBP was mulitplied by its corresponding cyto- or genotoxicity index value in chinese hamster ovary (CHO) cells available in the literature (Plewa et al., 2017; Wagner et al., 2017). The total cyto- and genotoxicity value for each water sample was the sum of all the measured DBPs toxicity values. All comparisons of compound concentrations were subjected to a two-tailed t-test with 1 degree of freedom (d.f.) at the 95% confidence interval (CI) to show statistical differences.

## 3. Results and discussion

Chloraminated influent samples simulate the concentration of DBPs expected at a consumers' tap without the use of BAC, while chloraminated effluent samples simulate DBP concentrations with the use of BAC. Since disinfection procedures were based on the plants dosing and distribution times, this study provided an opportunity to assess realistic exposure concentrations. The former is important because it provided an opportunity to study the adsorption and biodegradation of unregulated DBPs directly using BAC in real-world conditions. Many of these compounds have never been studied in full-scale BAC systems including IAAs, iodinated-HAMs, and iodo-THMs. To assess precursor removal, chloramination SDS procedures were conducted on a settled influent sample and three different aged BAC contactor effluents within each plant for a total of 6 full-scale BAC contactors (Table 5.2).

### 3.1 Biodegradation of preformed DBPs

Plant A, 27 DBPs were preformed during preoxidation with chloramine (Table 5.7), while 24 were formed at Plant B (Table 5.9). These included HALs, HAMs, HANs, HKs, HNMs, IAAs, I-THMs, NDMA, THMs, and HAAs (Figures 5.8). Concentrations ranged from 8 ng/L to 11.9 µg/L. When accounting for molar concentrations, these preformed DBPs account for approximately 14-32% of the TOCl, 37-97% of the TOBr, and 0-2% of the TOI measured. TOX removal showed mixed results (Figure 5.1, Figure 5.8). BAC treatment reduced TOCl by 16-19% in the middle aged and oldest BAC (93500 and 140200 BVs), but increased TOCl in the youngest BAC contactor (46700 BV). TOBr

was reduced by 7-21%, and TOI was reduced by 37-57%. Classes of compounds which showed promising removal include HALs (71-88% reduction), HAMs (64-95% removal), IAAs (66-100%), and HAAs (75-81%) (Figure 5.1). Preformed NDMA was well removed in Plant A (100%) and Plant B (83-87%). Classes that had mixed results included HNMs, with 83% removal in the first two filters (youngest and middle aged), but increases in the oldest filer (140200 BV) (Figure 5.1). DCNM was not removed at all, and increased from 200 ng/L to 300 ng/L in the oldest filter. It is possible that reductive de-halogenation of TCNM, which was fully biodegradable, resulted in the increased formation of DCNM (Yin et al., 2018). BDCNM also increased from 800 ng/L to 1000 ng/L in oldest filter. THM removal in Plant A was limited at 2-5%, with 0-3% removal of TCM, and no removal of BDCM. This is consistent with previous studies indicating poor biodegradation of some THMs (Zainudin et al., 2018).

Preformed DBPs at Plant B (Table 5.9) accounted for 36-65% of the TOCl, 56-66% of the TOBr, and <1% of the TOI. BAC showed limited removal of preformed TOX, with 3-28% TOCl, 8-26% TOBr, and 0-37% TOI reduction (Figure 5.8). Many of these compounds were well removed (70-100%) via biodegradation through all BAC contactor ages and include TCAL, BDCAL, BCAM, 11DCP, TCNM, CIAA, DCIM, NDMA, BAA, DCAA, BCAA, and TBAA. Others were less readily removed (30-70%), including DCAM, 111TCP, 1133TeCP, BCM, TCAA and BDCM. Many nitrogenous and iodinated DBPs were highly biodegradable, with 100% removal in all three contactors (BCAM, TCNM, CIAA, DCIM, and NDMA). Compounds that were reduced less than 10% included DCNM, BDCAA, TCM, and BDCM.

While many preformed DBPs were well removed by BAC, some compounds *increased* following BAC. For Plant A, these included DCNM which increased by 50% (from 0.2 µg/L), BDCNM which increased by 25% (from 0.8 µg/L), and CAA which increased slightly by 5% in the oldest contactor (from 2.0 µg/L). In Plant B, DBCM doubled in concentration in the middle and oldest contactors (from 0.2 µg/L), and DBCAA increased 63% in the youngest contactor (from 0.8 µg/L). Increases in unregulated DBPs poses possible health consequences, as many

The presence of nitrifying bacteria in both plants, indicated by the oxidation of ammonia to nitrate (Table 5.3) may possibly account for the increased formation of some N-DBPs following BAC, including N-nitrosamines, HANs, and HAMs (Zeng et al., 2016b). Another pilot study showed that increasing halogenation on HAAs decreased biodegradation rates in the presence of suspended bacteria (Zhou and Xie 2002). For example, CAA had the highest biodegradation rates while TCAA had the slowest rates. Our study supports this, and shows that the unregulated HAAs, BDCAA and DBCAA, may have poor biodegradation capacity in real-world full-scale plant conditions.

For many of these compounds, this is the first time that their removal is reported using BAC, and includes mono- and di- HALs, HAMs, I-THMs, IAAs, and trihalonitromethanes including bromodichloronitromethane (BDCNM) and dibromochloronitromethane (DBCNM).

### 3.2 Preformed vs. SDS DBP formation

Many compounds formed similar concentrations during preoxidation and following final disinfection. For example, Plant A had 50.3 µg/L total preformed DBPs in the BAC influent, while there was 56.0 µg/L total DBP formation following SDS treatment. Comparing the biodegradation of preformed DBPs vs. their formation following SDS allows the determination of whether a DBP or a corresponding precursor is more biodegradable. Many compounds formed at similar concentrations in the BAC influent, but had different % reductions following BAC and BAC SDS treatment. For example, at Plant A, preformed DCNM had the same concentration in the influent as the youngest and middle aged filter effluents, and then increased in the oldest BAC filter effluent, indicating that this compound is not biodegradable. Following SDS treatment, DCNM decreased by 50% indicating that the precursors for this compound were either biodegradable, or that transformation of this compound occurred with increased chloramination treatment.

Compounds formed *only* during SDS treatment include TBM, CAL, DBAL, BCAN, and DBCNM (Plant A), and BAA, CAL, DCAL, BCAN, BCAM, DBAM, and DCNM (Plant B). It should be noted that many of these compounds are brominated and/or nitrogenous, providing further evidence of the increased formation of N-DBPs following BAC filtration and final chloramination. Several compounds were only observed after BAC and final disinfection including DBAL (1.5 to 2 µg/L), BCAM (0.2 to 0.4 µg/L), and DBAM (0.2 to 0.6 µg/L). At Plant A, total preformed TOX constituted approximately 83-123% of SDS TOX, and was actually preformed at high concentration

in the youngest contact from 82.4 to 110.3 ug/L. At Plant B, total preformed TOX constituted 37-95% of the TOX formed following SDS treatment.

### 3.3 Impact of BAC on DBP formation following SDS treatment

Plants A and B achieved reductions in total DBP formation of 37-46% and 12-32% total reductions in DBP formation (Figure 5.3). Coincidentally, TOC removal for these plants was relatively low, with 8-11% and 4-5% for Plants A and B, respectively (Table 5.4). Most likely, the adsorption capacity of GAC for DBP precursors was exhausted since these plants were operating in BAC mode. Total summed Cl-DBPs were reduced 39-49% at Plant A, and 30-52% at Plant B. Total summed N-DBPs had 37-55% reduction at Plant A, and 32-62% at Plant B. Total summed Br-DBPs reduction was 25-30% at Plant A, and 48-67% at Plant B. Interestingly Total I-DBPs had significant removal at Plant A (91-100%), indicating the high biodegradation capacity for these compounds and their precurors. These results complement a pilot study (Fu et al., 2017) which showed signifcant reduction via biodegration of a subset of similar compounds including HKs, HANs, NDMA, THMs, and HAAs, but did not include I-DBPs.

TOCl, TOBr, and TOI *increased* following BAC filtration, and patterns did not match with reductions in quantified DBPs (Figure 5.2, and 5.9). For example, Plant A had reductions of TOCl, TOBr, and TOI of 19-31%, 15-26%, and 22-28%, respectively, in the youngest (46700 BV) and middle aged BAC (93500 BV), but TOI *increased* slightly by 10% in the oldest GAC (140200 BVs). There was not a significant increase in the

quantified iodinated-DBPs, suggesting the formation of unknown iodinated DBPs. On the other hand, Plant B saw consistent *increases* of TOCl and TOBr (by 15-143% and 1-17%, respectively, following BAC), with a reduction TOI of 19-31% (Figure 5.9 and 5.11). The variation in the results for these two plants highlights how dynamic and difficult to predict BAC systems can be. However, it should be noted that neither facilities used BAC as a DBP compliance strategy, but rather a taste and odor control strategy.

While the formation of many DBPs were effectively controlled by BAC, some DBPs were unaffected (concentrations were not significantly different between BAC influent and effluent). In Plant A, these precursors included dichloroacetamide (DCAM), bromochloroacetonitrile (BCAN), and dichloroiodomethane (DCIM). Some DBPs *increased* following the use BAC and chloramine, including chloroacetic acid (CAA), dibromoacetaldehyde (DBAL), chloroacetaldehyde (CAL), bromochloroacetamide (BCAM), dibromoacetamide (DBAM), bromochloroacetonitrile (BCAN), and NDMA. The increases of Br- and N-DBPs are consistent with earlier studies that showed increases in regulated brominated THMs, as well as more recent work by Chiu et al. (2012) and Krasner et al. (2016), who showed an increase in DBAN as a result of GAC treatment. At Plant A, there was a shift in speciation to more toxic Br-DBPs following GAC, as DCAL levels decreased and DBAL formed with the use of GAC. While NDMA was reduced significantly following Plant B SDS (36-84%), NDMA increased after BAC following SDS treatment at Plant A by 76% in the youngest filter (46700 BV) and by 10% in the oldest filter (140200 BV), from 50 ng/L to 88 ng/L. Plant A had significantly

more DON than Plant B (0.74 to 0.86 mg/L DON as N for Plant A, compared to <MRL for Plant B). DON precursors, such as dimethylamine, tertiary amines, and dimethylamides, can form significant amounts of NDMA upon chloramination (Mitch et al., 2004). These types of precursors may also be released by the biofilms into effluent waters. Previous studies have also shown increases in nitrosamines through nitrification by the presence of nitrifying bacteria. (Teng et al., 2016).

In both plants, there was a dramatic decrease in ammonia, with an accompanying increase in nitrite and nitrate after the use of BAC (Table 5.3). A possible reason that ammonia promotes the growth of nitrifying bacteria on the surface of GAC, which in turn converts ammonia to nitrite and nitrate (Vahala et al., 1999; Carrico et al., 2008). Nitrite and nitrate have been shown to be important precursors in the formation of many DBPs including halonitromethanes, trihalomethanes, and cyanogen chloride (Choi et al., 2004; Lyon et al., 2012). This may partially explain increases in N-DBPs (bromochloroacetamide, dibromoacetamide, bromochloroacetonitrile, and NDMA) following BAC in these plants. Bacterial strains most commonly associated with oxidizing ammonia to nitrite are in the nitrosomonas genus, while nitrosococcus and nitrosospira may also play a role (Feng et al., 2012; Maestre et al., 2013; Tatari et al., 2017). The bacteria belonging to the nitrobacter genus along with ammonia oxidizing archaea are known to oxidize nitrite to nitrate (Feng et al., 2012). Future research is needed to understand how to maintain or manipulate bacteria populations in full-scale BAC filters to maximize contaminant biodegradation, and thus promoting healthier drinking water.

## 3.4 Calculated water toxicity

The total calculated water toxicity and percent contribution by DBP class for both plants analyzed in this study are shown in Figures 5.3, 5.4 and 5.5. Because the use of BAC increased the formation of some of the more toxic Br- and N-DBPs, it is not evident that the effective control of overall DBP formation translates to safer drinking water. Therefore, cytotoxicity and genotoxicity were calculated for the BAC-treated samples using the TIC-Tox approach (Plewa et al., 2017) and the $\%C_{1/2}$ values published for >100 DBPs (Wagner et al., 2017).

### 3.4.1. Calculated toxicity for preformed DBPs

Preformed calculated cytotoxicity at Plant A was reduced by 45-87%, while calculated genotoxicity was reduced 37-94% over the lifetime of the BAC filters (Figure 5.3). At Plant B, preformed calculated cytotoxicity was reduced by 77-80% across BAC filter lifetime, and calculated genotoxicity was reduced 60-85%. Preformed calculated toxicity was driven by HNMs, and HAAs at Plant A, and HAMs, HNMS, and HAAs in Plant B (Figure 5.3). At Plant A, preformed calculated cytotoxicity accounted for 39% of the SDS cytotoxicity, but only 4-21% of the calculated cytotoxicity following treatment with BAC. At Plant B, preformed calculated cytotoxicity only accounted for 10% of the influent water, and 2-3% of the BAC effluents.

### 3.4.2 Calculated toxicity for SDS DBPs

Calculated cytotoxicity at both BAC plants following SDS treatement showed mixed results, with these plants showing both an increase and decrease in calculated cytotoxicity following BAC across different filters with no apparent correlation to age (Figure 5.3). At Plant A, calculated cytotoxicity increased in the youngest (46,700 BV) by 27%, decreased by 37% in the middle-aged filter (93,500 BV), and remained virtually unchanged in the oldest filter (140,200 BV) with a slight increase of 4%. *These increases of calculated toxicity at Plant A occurred despite a total reduction in quantified DBP levels of 30-35%*, indicating shifts to more toxic DBPs following treatment with BAC. Plant B had calculated cytotoxicity reductions of 1-22% in the youngest (157,400 BV) and middle aged filter (333,200 BV), but a slight increase of 9% in the oldest contactor (555,300 BVs). The variations in the results for these two plants indicate these types of BAC systems should be studied further. The calculated genotoxicity decreased at both plants in all 6 filters, and regardless of BAC age. Plant A saw a decrease of calculated genotoxicity by 38-65%, and Plant B genotoxicity decreased by 82-87%.

### 3.4.3 Drivers of toxicity.

At both plants, THMs and HAAs constituted a majority of the quantified DBPs, contributing 22-25% of the TOX at Plant A, and 10-43% of the TOX at Plant B across BAC filter age, while 64-75% at Plant A and 32-83% at Plant B remains unknown (Figures 5.4 and 5.5). Classes of DBPs that contributed the most to calculated cytotoxicity at Plan A were HALs (60-86%) and HANs (8-16%). Plant B calculated

cytotoxicity following treatment with BAC was driven by HALs (68-79%), HAMs (8-13%), HANs (5-8%) and HNMs (6-11%). Chloroacetaldehyde (CAL) and dibromoacetaldehyde (DBAL) were the drivers of increased cytotoxicity at Plant A, and respectively constituted 22-55% and 49-53% of the calculated cytotoxicity following BAC. Chloroacetaldehyde (CAL), bromochloroacetonitrile (BCAN), and dichloronitromethane (DCNM) were the drivers of increased cytotoxicity in the oldest contactor of Plant B. CAL constituted 68-78% of the calculated cytotoxicity following BAC at Plant A, and played the most significant role in increased calculated cytotoxicity and genotoxicity. While NDMA continues to be a compound of concern for potential regulation, the formation of this compound was low in this study and therefore played only a small role in the calculated toxicity compared to other unregulated, toxic DBPs that formed in higher concentrations (such as chloroacetaldehyde).

While calculated genotoxicity decreased significantly following treatment with BAC at both plants, drivers of genotoxicity at Plant A included HALs (35-55%), HNMs (8-20%), and HAAs (19-31%). At Plant B, calculated genotoxicity in the BAC influent was driven largely by BAA (87%), which was removed 100% following treatment with BAC. Calculated genotoxicity was then shifted to HALs, HAMs, HANs, and HNMs following a similar profile to calculated cytotoxicity. These results are supported by previous studies indicating that unregulated DBPs including Br- and N-DBPs, are predicted toxicity drivers in these types of systems, particularly HANs (Krasner et al., 2016; Zhang et al., 2017).

While most of the DBPs that increased in formation following BAC likely formed by the reaction of chloramine with NOM and bromide, some DBPs may have formed from other DBPs on the BAC surface. For example, an increase in chloroacetaldehyde (CAL) formation was observed following BAC adsorption for Plant A. CAL was a driver of calculated cytotoxicity and calculated genotoxicity for this drinking water following BAC and chloramination. A corresponding increase in TOCl was also observed. It is possible that trihaloaldehydes were reduced and dehalogenated on the BAC surface, forming increased levels of CAL. This may be signified by the decrease of trichloroacetaldehyde (TCAL) following BAC. Aldehydes may also form from higher molecular weight phenolic compounds that are not readily removed by BAC filtration (Chuang et al., 2017). Dibromoacetaldehyde also increased following BAC in Plant A, and contributed signficantly to both calculated cytotoxicity (53% in the youngest filter and 49% in the oldest) and genotoxicity (46% in the youngest filter, and 38% in the oldest), though it's formation was not consistent. These results highlight that the *DBP concentration alone* may not always provide an adequate basis for risk assessment, and that the water equivalent from a regulatory perspective is not necessarily equivalent to a risk perspective.

### 3.5 Implications for drinking water treatment and risk reduction

While BAC showed promising results for reducing the formation of many DBPs, in some cases certain DBPs increased which also increased calculated toxicity. It should be noted that these results were highly dependent on a number of factors, such as source water quality, type of organic matter, concentration of organic matter, dose of

disinfectant, and type of disinfectant prior to filtration. While some studies have shown that BAC removes MIB and geosmin, compounds that produce taste and odor problems (Ho et al. 2007; Suffet et al., 1995), this study shows that BAC provides a mixed performance in the overall reduction of regulated and unregulated DBPs (with pre-chloramination). In addition, the use of BAC with chloramination should be studied further to better understand the relationship between oxidation strategy, age of filter, water quality, and treatment operation on the potential increase or decrease in formation of cytotoxic DBPs. Special attention should be paid to DON and nitrification of ammonia, which may play a significant role in the formation of N-DBPs, some of which were drivers of calculated toxicity in this study. Certain parameters that can be adjusted to improve performance of BAC includes lowering pre-oxidant doses to reduce impacts on the biofilm, or adding a reducing agent prior to BAC. Studies indicate that BAC removes lower MW compounds, therefore coagulation after BAC may offer some additional benefits. Previous work showed increased performance for BAC when pre-ozonation was employed as opposed to pre-chloramination, particularly for reduction of NDMA (Liu et al., 2017; Vatankhah et al., 2019). Finally, these results demonstrate a holistic method for evaluating not only removal of DBP concentrations, but also relating that to potential reductions in calculated genotoxicity and cytotoxicity.

4. Conclusions

- Unregulated DBPs showed high biodegradation capacity
- BAC reduced total regulated and unregulated DBP concentrations
- Some DBPs increased following BAC (chloroacetaldehyde, dichloronitromethane, bromodichloronitromethane, NDMA,

dibromochloromethane, tribromomethane, dibromochloroacetic acid, and tribromoacetic acid)

- Calculated cytotoxicity in BAC effluents did not correlate with reduced DBP concentrations
- BAC showed promising reductions in calculated genotoxicity across 6 different BAC filter ages, while calculated cytotoxicity was more variable
- A large portion of total organic halogen remains unknown in chloraminated waters pre- and post-BAC

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**Supporting Information Available**

Additional details of materials and methods including extraction methods, and sampling information. Additional tables and figures including bulk water parameters, and raw data with accompanying graphs.

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**Figures and Tables**

**Table 5.1. Precursor and biodegradation potential for each DBP class**

| DBP Class | Precursors | Biodegradation Potential[a,b] |
|---|---|---|
| **THMs** | Many natural organic matter (NOM) fractions | Low-Mid |
| **HAAs** | Many NOM fractions | Low-Mid |
| **HALs** | Phenolic NOM structures | High |
| **HAMs** | Phenolic NOM structures, amino acids, haloacetonitriles, hydrophilic acid NOM fractions, aromatic NOM | Mid |
| **HANs** | Amino acids, nucleic acids, proteinaceous materials, combined amino acids bound to humic structures | Low-Mid |
| **HKs** | Many NOM fractions | Mid to high |
| **HNMs** | Hydrophilic NOM, aromatic NOM, nitromethanes | Low |
| **I-THMs** | Many NOM fractions | Not Studied |
| **IAAs** | Many NOM fractions | Not Studied |
| **NDMA** | Secondary and tertiary amines, amine based polymers, ammonia, nitrite | Low-Mid |

[a]High:> 75%, middle: 25-75%, low: <25%

[b](Liu et al., 2017; Zainudin et al., 2018; Hua et al., 2007; Chuang et al., 2015; Fang et al., 2019; Kimura et al., 2013; Shah et al., 2012; Bond et al., 2012; Chu et al., 2010; Zhang et al., 2016)

**Table 5.2. Sampling information for Plants A and B including collection date, flow, and loading rate on each contactor, empty bed contact time (EBCT), pre-oxidant concentrations, GAC media type, bed volumes treated (BV) by BAC, and percent DOC breakthrough**

| Plant | Sample Collection Date | Typical Flowrate per Contactor (MGD) | Typical GAC Loading Rate ($gpm/ft^2$) | Typical EBCT[b] (min) | Oxidant in the GAC Feed Water | Sample Location | GAC Installation Date | GAC Media | Bed Volume Treated by GAC (BV) | Percent DOC Break-through[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 3/28/2016 | 1.0-2.0 | 1.5 | 15 | 1.5-2.0 mg/L of chloramine | Youngest BAC Eff. | 5/5/2015 | Virgin Calgon F300M | 46,700 | 88.8 |
| | | | | | | Middle Aged BAC Eff. | 5/13/2014 | Virgin Calgon F300M | 93,500 | 92.3 |
| | | | | | | Oldest BAC Eff. | 4/9/2013 | Virgin Calgon F300M | 140,200 | 92.5 |
| B | 5/10/2016 | 1.0-1.5 or 2.0-3.0[a] | 2.1 | 2.5 | 2.0-4.0 mg/L of chloramine | Youngest BAC Eff. | Sept. 2015 | Virgin Calgon F300M | 157,400 | 94.6 |
| | | | | | | Middle Aged BAC Eff. | May 2013 | Virgin Calgon F300M | 333,200 | 95.7 |
| | | | | | | Oldest BAC Eff. | May 2011 | Virgin Calgon F300M | 555,300 | 96.6 |

[a]: Youngest GAC contactor is a single bed and the other selected contactors are dual bed. Single bed GAC contactors receive half the flow of the dual bed contactor. Consequently, EBCT in all contactors are the same.
[b]: EBCT values are calculated for the GAC media only. Underlying sand or anthracite media was not incorporated into EBCT calculations.
[c]: Percent DOC breakthrough is calculated by dividing GAC influent total organic carbon (TOC) by GAC effluent TOC

**Table 5.3. Water quality parameters for Plants A and B.**

| Plant | Bed Volume Treated by GAC (BV) | TOC (mg/L) as C | UV254 (abs. /cm) | SUVA (L/mg-m) | TN (mg/L) as N | DON (mg/L) as N | $NH_3$ (mg/L) as N | $NO_2^-$ (mg/L) as N | $NO_3^-$ (mg/L) as N | $Br^-$ (µg/L) | $I^-$ (µg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | GAC Inf. | 3.76 | 0.080 | 2.13 | 5.10 | 0.74 | 0.44 | 0.006 | 5.1 | 41 | <5 |
| | 46,700 | 3.34 | 0.066 | 1.98 | 5.50 | 0.75 | 0.02 | 0.013 | 5.5 | 42 | <5 |
| | 93,500 | 3.47 | 0.068 | 1.96 | 5.40 | 0.86 | 0.03 | 0.051 | 5.6 | 42 | <5 |
| | 140,200 | 3.48 | 0.067 | 1.93 | 5.40 | 0.84 | 0.03 | 0.050 | 5.4 | 42 | <5 |
| B | GAC Inf. | 3.49 | 0.077 | 2.21 | 5.03 | <MRL | 0.95 | 0.004 | 5.2 | 33 | <5 |
| | 157,400 | 3.30 | 0.063 | 1.91 | 5.23 | <MRL | <MRL | 0.591 | 5.4 | 36 | <5 |
| | 333,200 | 3.34 | 0.070 | 2.10 | 5.12 | <MRL | <MRL | 0.389 | 5.6 | 36 | <5 |
| | 555,300 | 3.37 | 0.068 | 2.02 | 4.94 | <MRL | 0.35 | 0.172 | 5.4 | 34 | <5 |

MRL: Minimum reporting limit

**Table 5.4. Plant A and B BAC removal for total organic carbon, total organic halogen, and calculated cytotoxicity and genotoxicity**

| Parameter | Removal (%) Plant A | | | Removal (%) Plant B | | | Overall Removal (%) |
|---|---|---|---|---|---|---|---|
| | Influent | **avg.** | std dev. | Influent | **avg.** | std dev. | |
| | | n=3 | | | n=3 | | |
| **TOC** | 3.76 | **9** | 1.7 | 3.49 | **4** | 0.8 | 7 |
| **Background (µg/L)** | | | | | | | |
| **TOCl as Cl** | 84 | **5** | 17.7 | 68 | **14** | 11.3 | 9 |
| **TOBr as Br** | 9 | **13** | 5.9 | 8 | **18** | 7.7 | 15 |
| **TOI as I** | 5.1 | **50** | 8.8 | 1.9 | **19** | 15.1 | 34 |
| DBPsum | 40 | **53** | 1.2 | 60 | **39** | 8.2 | 46 |
| Calc cytotox | 21751 | **61** | 11.1 | 1027 | **79** | 1.1 | 70 |
| Calc genotox | 867 | **53** | 5.1 | 679 | **73** | 10.1 | 63 |
| **SDS (µg/L)** | | | | | | | |
| **TOCl as Cl** | 92 | **27** | 5.2 | 82 | **-55 (-12)** | 61.3 | -14 (8) |
| **TOBr as Br** | 8.6 | **21** | 4.3 | 8.2 | **4** | 8.9 | 13 |
| **TOI as I** | 1.8 | **13** | 17.2 | 1.6 | **25** | 5.1 | 19 |
| sum DBPs | 57 | **43** | 4.4 | 102 | **48** | 7.8 | 46 |
| Calc cytotox | 3119 | **2** | 27.0 | 9843 | **5** | 13.0 | 4 |
| Calc genotox | 308 | **49** | 13.1 | 1451 | **84** | 2.2 | 66 |

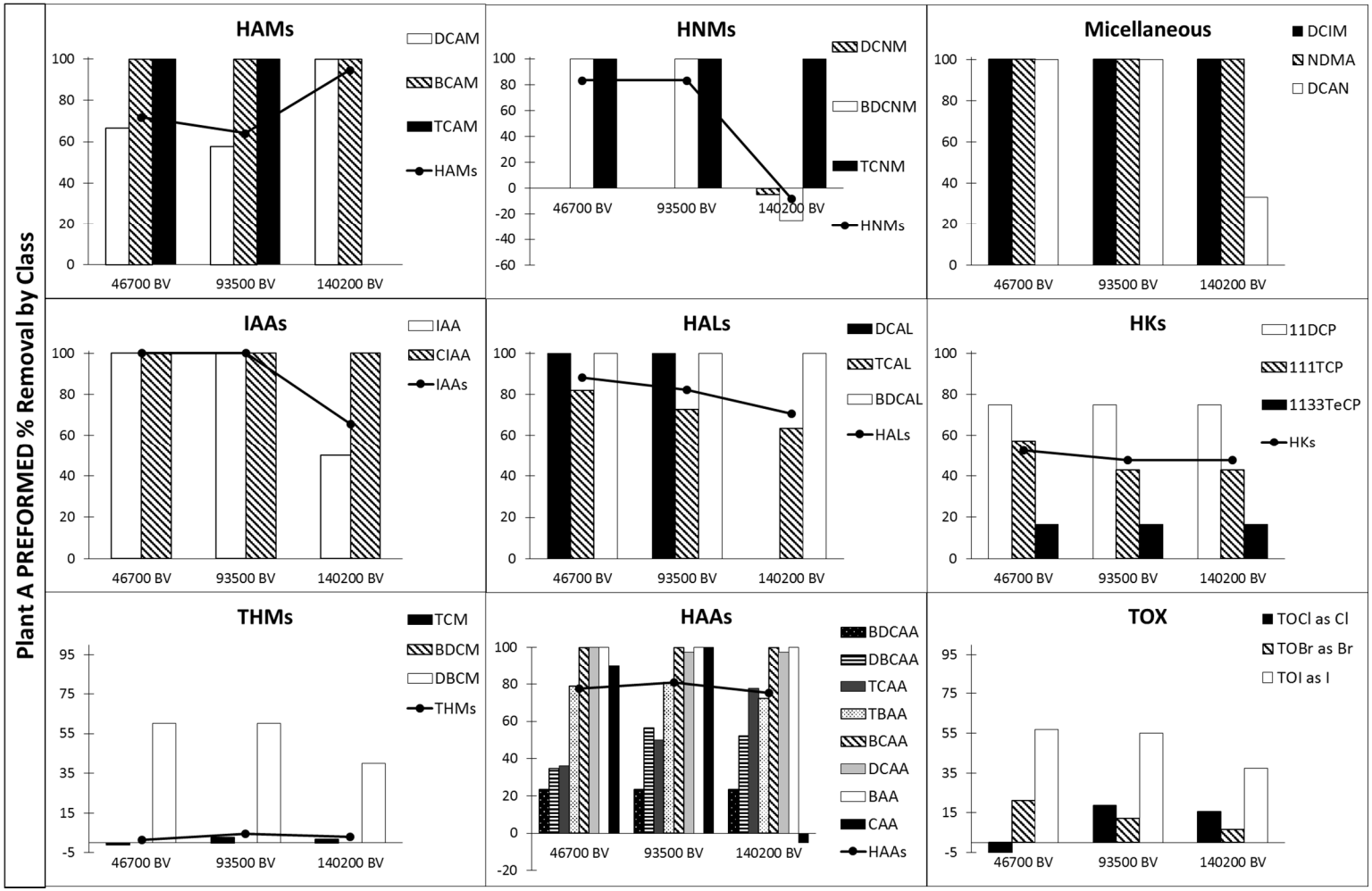


**Figure 5.1.** Plant A: % Removal of preformed DBPs by BAC grouped by class and total organic halogen (TOX). Effect of BAC age in bed volumes (BV) Class removals are based on summed concentrations, not average removal. Negative % removal indicates increased concentrations following treatment with BAC

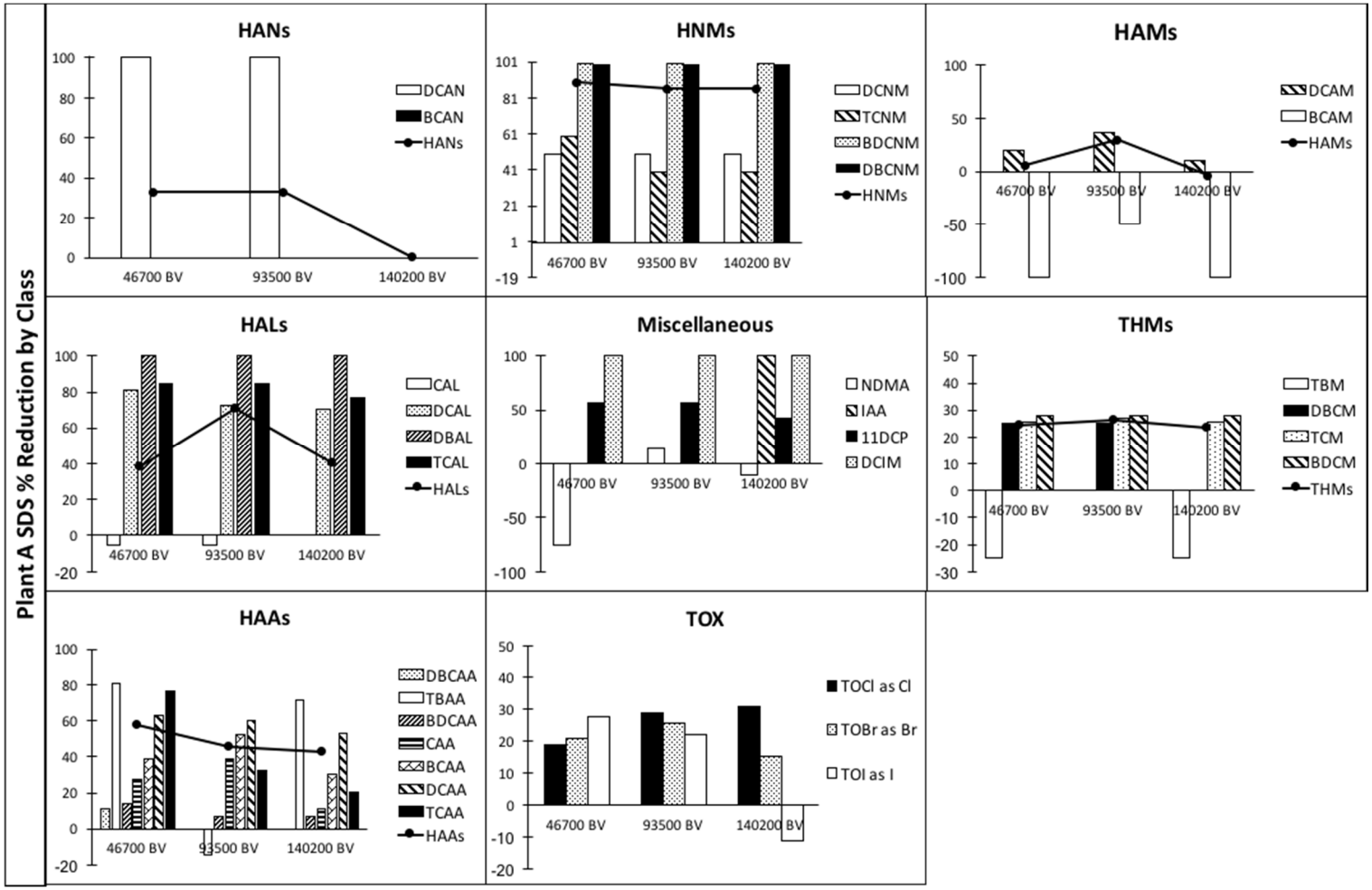


**Figure 5.2.** Plant A SDS: Effect of BAC age by bed volumes (BV) on DBP removal and breakthrough of quantified DBPs by class and total organic halogen (TOX). Class removals are based on summed concentrations, not average removal. Negative % removal indicates increased concentrations following treatment with BAC.

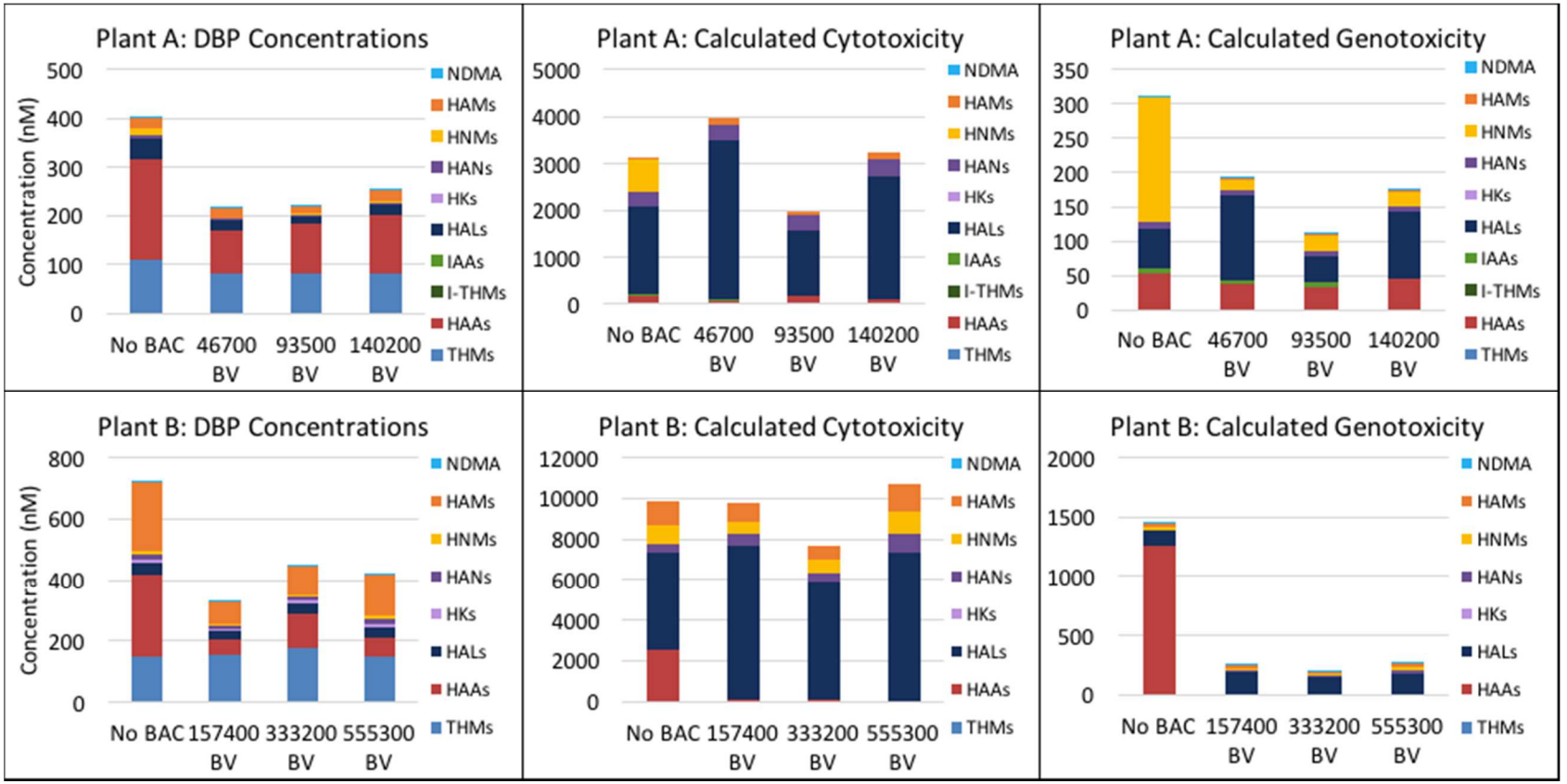


**Figure 5.3.** Plant A and B SDS: Total DBP concentrations (nM), calculated cytotoxicity and genotoxicity following treatment with BAC by bed volumes (BV).

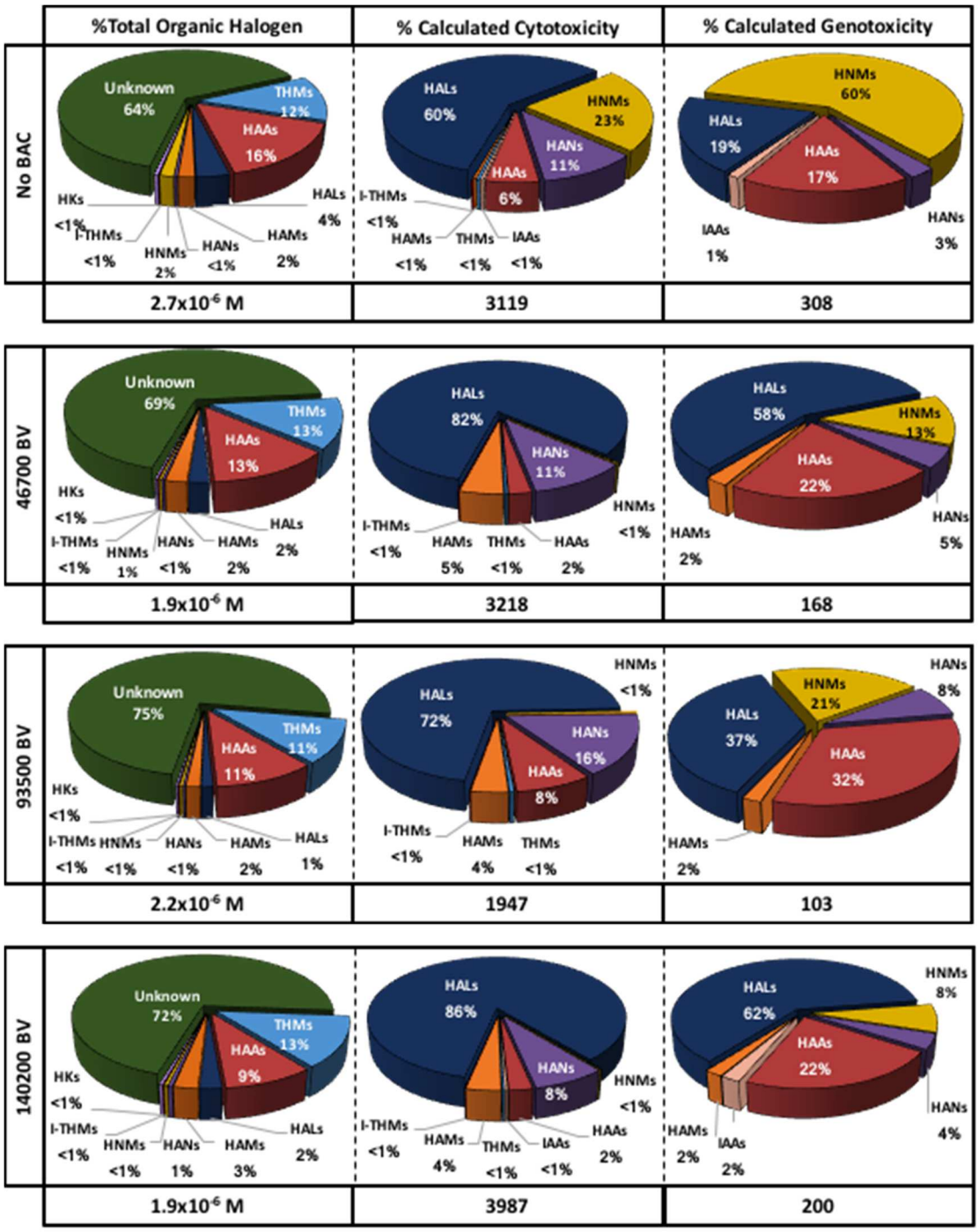


**Figure 5.4.** Plant A SDS: % Total organic halogen (%TOX) in molarity, % calculated cytotoxicity, and % calculated genotoxicity for each class of DBPs. Total calculated toxicity = $\sum([DBP] \times (C_{1/2})^{-1} \times 10^6)$. Note that there is no data on the cytotoxicity or genotoxicity in the literature for haloketones or tribromoacetonitrile.

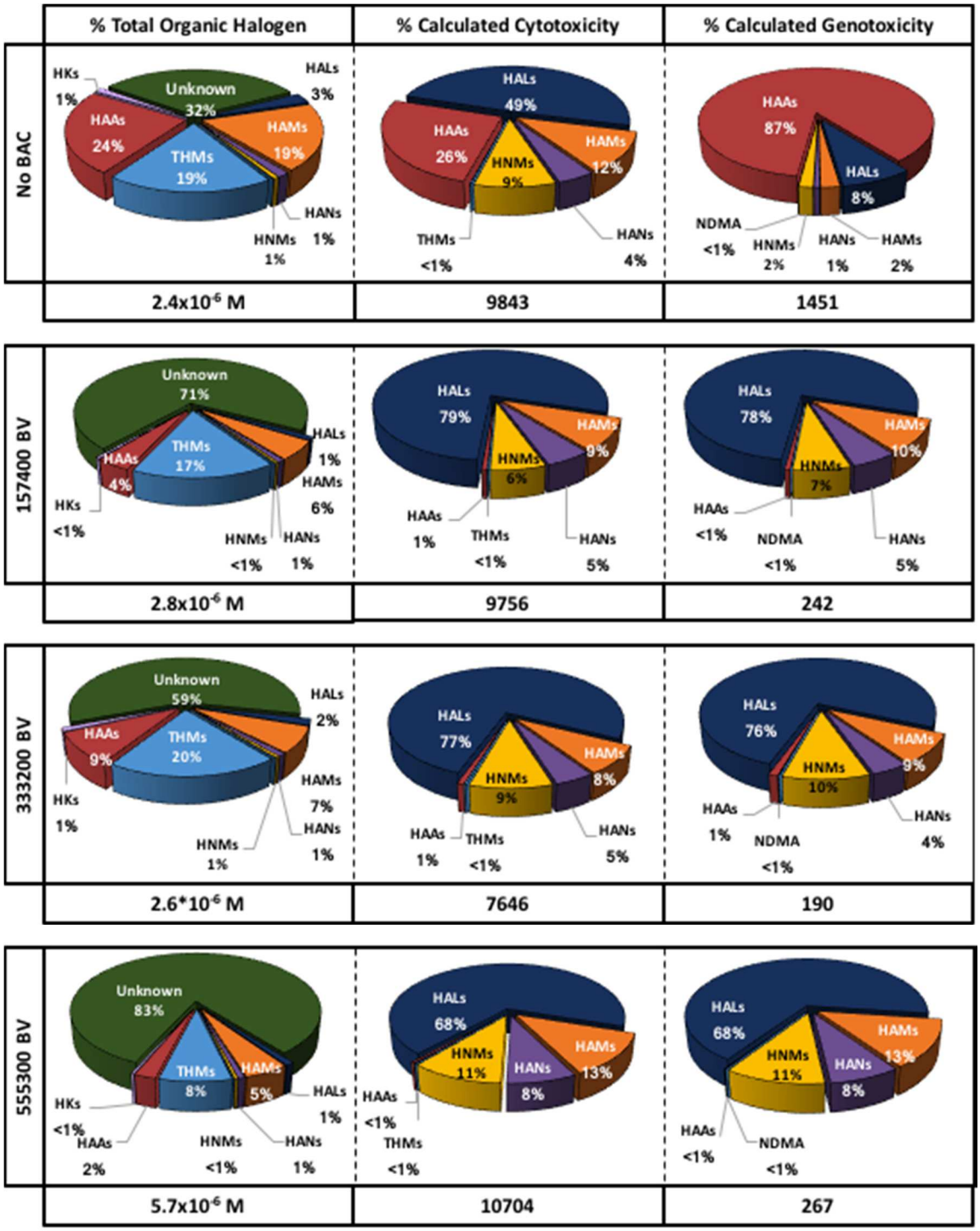


**Figure 5.5.** Plant B SDS: % Total organic halogen (% TOX) in M, % calculated cytotoxicity, and % calculated genotoxicity for each DBP class**.** Total calculated toxicity = $\sum([DBP] \times (C_{1/2})^{-1} \times 10^{6})$. Note that there is no data on cytotoxicity or genotoxicity in the literature for haloketones or tribromoacetonitrile.

**Table 5.5. GC-MS retention time (RT), vendor information, quantitative and qualifier ions, and method detection limits (MDLs) for priority DBPs quantified in this study, including haloacetaldehyde (HALs), haloacetonitriles (HANs), haloketones (HKs), halonitromethanes (HNMs), iodo-trihalomethanes (I-THMs), and haloacetamides (HAMs). DBPs are classified by their corresponding analytical method and DBP class.**

| DBP Class | DBP Name | Abbreviation | RT (min) | Quantitative Ion (m/z) | Qualifier Ion (m/z) | MDL (ng/L) |
|---|---|---|---|---|---|---|
| ***Analytical Method 1*** | | | | | | |
| HAL | Trichloroacetaldehyde[a] | TCAL | 3.82 | 82.0 | 110.9 | 3.0 |
| HAL | Bromodichloroacetaldehyde[b] | BDCAL | 5.17 | 83.0 | 111.0/163.8 | 3.0 |
| HAL | Dibromochloroacetaldehyde[b] | DBCAL | 7.17 | 128.9 | 127.9 | 3.0 |
| HAL | Tribromoacetaldehyde[a] | TBAL | 9.10 | 172.8 | 171.8 | 3.0 |
| HAN | Trichloroacetonitrile[a] | TCAN | 3.42 | 108.0 | 110.0 | 10 |
| HAN | Dichloroacetonitrile[a] | DCAN | 4.22 | 74.0 | 82.0 | 5.0 |
| HAN | Chloroacetonitrile[a] | CAN | 4.36 | 75.0 | 48.0 | 8.0 |
| HAN | Bromochloroacetonitrile[a] | BCAN | 6.00 | 74.0 | 155.0 | 5.0 |
| HAN | Bromoacetonitrile[a] | BAN | 6.16 | 118.9 | 120.9 | 48 |
| HAN | Dibromoacetonitrile[a] | DBAN | 8.17 | 117.9 | 199.0 | 38 |
| HAN | Iodoacetonitrile[a] | IAN | 8.85 | 167.0 | 126.9 | 31 |
| HK | 1,1-Dichloropropanone[a] | 11DCP | 4.63 | 43.0 | 83.0 | 26 |
| HK | Chloropropanone[a] | CP | 4.73 | 92.0 | 43.0 | 24 |
| HK | 1,1,1-Trichloropropanone[a] | 111TCP | 6.88 | 43.0 | 125.0 | 6.0 |
| HK | 1,1-Dibromopropanone[b] | 11DBP | 8.17 | 43.0 | 215.9 | 20 |
| HK | 1-Bromo-1,1-dichloropropanone[b] | 1B11DCP | 8.80 | 43.0 | 125.0 | 3.0 |
| HK | 1,3-Dichloropropanone[b] | 13DCP | 8.95 | 77.0 | 49.0 | 35 |
| HK | 1,1,3-Trichloropropnanone[a] | 113TCP | 10.02 | 77.0 | 83.0 | 2.0 |
| HK | 1,1,3,3-Tetrachloropropanone[b] | 1133TeCP | 10.92 | 83.0 | 85.0 | 9.0 |
| HK | 1,1,3,3-Tetrabromopropanone[c] | 1133TeBP | 16.13 | 200.8 | 119.9 | 22 |
| HNM | Trichloronitromethane[a] | TCNM | 4.67 | 116.9 | 119.0 | 6.0 |
| HNM | Dichloronitromethane[b] | DCNM | 4.77 | 83.0 | 85.0 | 28 |
| HNM | Bromochloronitromethane[b] | BCNM | 6.64 | 129.0 | 127.0 | 24 |
| HNM | Dibromonitromethane[b] | DBNM | 8.51 | 172.8 | 171.0 | 4.0 |
| I-THM | Dichloroiodomethane[b] | DCIM | 4.34 | 83.0 | 126.9 | 12 |
| I-THM | Bromochloroiodomethane[b] | BCIM | 5.95 | 128.9 | 126.9 | 39 |
| I-THM | Dibromoiodomethane[b] | DBIM | 7.83 | 172.8 | 299.7 | 15 |
| I-THM | Chlorodiiodomethane[b] | CDIM | 8.33 | 174.9 | 126.9 | 39 |
| I-THM | Bromodiiodomethane[b] | BDIM | 10.09 | 218.8 | 220.8 | 90 |
| I-THM | Iodoform[a] | TIM | 12.06 | 393.7 | 266.8 | 47 |

| DBP Class | DBP Name | Abbreviation | RT (min) | Quantitative Ion (m/z) | Qualifier Ion (m/z) | MDL (ng/L) |
|---|---|---|---|---|---|---|
| ***Analytical Method 2*** | | | | | | |
| HAM | Chloroacetamide[a] | CAM | 10.08 | 93.0 | 44.0 | 300 |
| HAM | Bromoacetamide[a] | BAM | 11.73 | 137.0 | 44.0 | 100 |
| HAM | Dichloroacetamide[d] | DCAM | 12.03 | 44.0 | 127.0 | 16 |
| HAM | Bromochloroacetamide[b] | BCAM | 13.38 | 44.0 | 173.0 | 22 |
| HAM | Trichloroacetamide[a] | TCAM | 13.92 | 44.0 | 82.0 | 7.0 |
| HAM | Iodoacetamide[a] | IAM | 14.07 | 185.0 | 58.0 | 400 |
| HAM | Dibromoacetamide[b] | DBAM | 14.08 | 44.0 | 217.0 | 41 |
| HAM | Chloroiodoacetamide[b] | CIAM | 15.15 | 92.0 | 219.0 | 38 |
| HAM | Bromodichloroacetamide[b] | BDCAM | 15.21 | 44.0 | 128.0 | 30 |
| HAM | Bromoiodoacetamide[b] | BIAM | 16.28 | 136.0 | 138.0 | 50 |
| HAM | Dibromochloroacetamide[b] | DBCAM | 16.44 | 44.0 | 128.0 | 23 |
| HAM | Tribromoacetamide[b] | TBAM | 17.59 | 44.0 | 173.0 | 40 |
| HAM | Diiodoacetamide[b] | DIAM | 17.91 | 184.0 | 311.0 | 23 |
| HAN | Tribromoacetonitrile[b] | TBAN | 9.11 | 197.8 | 195.8 | 100 |
| HNM | Bromodichloronitromethane[b] | BDCNM | 6.71 | 163.0 | 161.0 | 100 |
| HNM | Dibromochloronitromethane[b] | DBCNM | 8.87 | 206.8 | 209.0 | 100 |
| HNM | Tribromonitromethane[b] | TBNM | 10.81 | 251.0 | 253.0 | 100 |
| IAA | Iodoacetic acid[a] | IAA | 7.53 | 200>73 | 169>141 | 5.0 |
| IAA | Chloroiodoacetic acid[b] | CIAA | 9.68 | 234>79 | 234>107 | 5.0 |
| IAA | Bromoiodoacetic acid[b] | BIAA | 11.08 | 278>123 | 278>151 | 5.0 |
| IAA | Diiodoacetic acid[b] | DIAA | 12.91 | 326 >171 | 326>199 | 5.1 |
| ***Analytical Method 3*** | | | | | | |
| HAL | Chloroacetaldehyde[a] | CAL | 14.62 | 238.0 | 181.0/182.0 | 50 |
| HAL | Bromoacetaldehyde[c] | BAL | 15.87 | 287.0 | 238.0 | 25 |
| HAL | Iodoacetaldehyde[c] | IAL | 17.48 | 293.0 | 335.0 | 25 |
| HAL | Dichloroacetaldehyde[d] | DCAL | 15.49 | 272.0 | 181.0/182.0 | 100 |
| HAL | Bromochloroacetaldehyde[b] | BCAL | 16.80 | 272.0 | 238.0 | 50 |
| HAL | Dibromoacetaldehyde[b] | DBAL | 18.21 | 137.0 | 135.0 | 50 |

[a] Sigma Aldrich, [b] CanSyn Chem. Corp., [c] Aldlab Chemicals, [d] TCI America

**Table 5.6. List of analytes and experimental methods used for its monitoring.**

| Analyte | Method |
|---|---|
| Residual Chloramine (as chlorine) | *N,N*-diethyl-*p*-phenylendiamine titrimetric method |
| Dissolved organic carbon (DOC) | Standard Method (SM) 5310 B[a] |
| Fluorescence excitation emission matrices (EEMs) | McKnight et al., 2001; using a Horiba Scientific Aqualog |
| Free chloramine, high range (>10 mg-$Cl_2$/L) | Modified 4500-Cl B |
| Free chloramine, low range (<10 mg-$Cl_2$/L) | SM 4500-Cl G; using a Hach Pocket Colorimeter II, Chlorine kit |
| Ammonia | SM 4500-NH G |
| Nitrite | SM 4500-$NO^{2-}$ |
| Nitrate | SM 4500-$NO^{3-}$ |
| pH | SM 4500-H+ B |
| Total dissolved nitrogen (DN) | Using a Shimadzu TOC-TN Analyzer[a] |
| Total Chloramine (as chlorine) | SM 4500-Cl G; using a Hach Pocket Colorimeter II, Chlorine kit |
| UV254 | SM 5910 B[a] |

**Table 5.7. Plant A: Effect of GAC age on preformed DBP and TOX removal.**

| DBP Class | DBP | No GAC (µg/L) | 46,700 BV (µg/L) | 93,500 BV (µg/L) | 140,200 BV (µg/L) |
|---|---|---|---|---|---|
| HAL | **TCAL** | 1.1±0.09 | 0.2±0.02 | 0.3±0.01 | 0.4±0.06 |
| HAL | **BDCAL** | 0.5±0.0008 | ND | ND | ND |
| HAL | **DCAL** | 0.1±0.1 | ND | ND | 0.1± 0.0 |
| HAM | **DCAM** | 3.3±0.1 | 1.1±0.2 | 1.4±0.001 | ND |
| HAM | **BCAM** | 0.4±0.01 | ND | ND | ND |
| HAM | **TCAM** | 0.2±0.0005 | ND | ND | 0.2 |
| HAM | **IAM** | 2.4±0.5 | 2.5±0.2 | 2.8±0.6 | 1.2 |
| HAM | **DIAM** | 2.4±0.02 | ND | ND | ND |
| HAN | **DCAN** | 0.3±0.03 | ND | ND | 0.2±0.04 |
| HK | **11DCP** | 0.8±0.06 | 0.2±0.01 | 0.2±0.03 | 0.2±0.05 |
| HK | **111TCP** | 0.7±0.02 | 0.3±0.01 | 0.4±0.02 | 0.4±0.01 |
| HK | **1133TeCP** | 0.6±0.01 | 0.5±0.0006 | 0.5±0.004 | 0.5±0.003 |
| HNM | **BDCNM** | 0.8±0.0008 | ND | ND | 1.0±0.3 |
| HNM | **DCNM** | 0.2±0.007 | 0.2±0.02 | 0.2±0.02 | 0.3±0.006 |
| HNM | **TCNM** | 0.2±0.001 | ND | ND | ND |
| IAA | **IAA** | 0.02±0.003 | 0.004±0.0008 | 0.003±0.001 | 0.01±0.005 |
| IAA | **CIAA** | 0.009±0.002 | ND | ND | ND |
| I-THM | **DCIM** | 0.1 ± 0.006 | <0.1 | <0.1 | <0.1 |
| NA | **NDMA** | 0.008 | <2.5 | <2.5 | <2.5 |
| THM | **TCM** | 10.9 | 11.0 | 10.6 | 10.7 |
| THM | **BDCM** | 1.8 | 1.8 | 1.8 | 1.8 |
| THM | **DBCM** | 0.5 | 0.2 | 0.2 | 0.3 |
| HAA | **CAA** | 2.0 | 0.2 | ND | 2.1 |
| HAA | **BAA** | 0.2 | ND | ND | ND |
| HAA | **DCAA** | 11.9 | ND | 0.3 | 0.3 |
| HAA | **TCAA** | 3.6 | 2.3 | 1.8 | 0.8 |
| HAA | **BCAA** | 1.4 | ND | ND | ND |
| HAA | **BDCAA** | 1.7 | 1.3 | 1.3 | 1.3 |
| HAA | **DBCAA** | 2.3 | 1.5 | 1.0 | 1.1 |
| HAA | **TBAA** | 4.7 | 1.0 | 0.9 | 1.3 |
| TOX | **TOCl as Cl** | 84.3±1.9 | 101.0±4.1 | 68.5±19.7 | 71.1±1.3 |
| TOX | **TOBr as Br** | 9.0±0.3 | 7.1±0.01 | 7.9±0.2 | 8.4±0.01 |
| TOX | **TOI as I** | 5.1±0.4 | 2.2±0.04 | 2.3±0.2 | 3.2±0.2 |

**Table 5.8. Plant A simulated distribution system (SDS) results: Effect of GAC age on DBP and TOX removal.**

| DBP Class | DBP | No GAC (µg/L) | 46,700 BV (µg/L) | 93,500 BV (µg/L) | 140,200 BV (µg/L) |
|---|---|---|---|---|---|
| HAL | **TCAL** | 1.3±0.002 | 0.2±0.002 | 0.2±0.003 | 0.3±0.005 |
| HAL | **CAL** | 0.2±0.06 | 0.3±0.2 | 0.3±0.03 | 0.2±0.08 |
| HAL | **DBAL** | ND | 2.0±0.3 | ND | 1.5±0.2 |
| HAL | **DCAL** | 3.7±0.2 | 0.7±0.1 | 1.0±0.06 | 1.1±0.09 |
| HAM | **DCAM** | 3.0±1.6 | 2.4±0.4 | 1.9±0.6 | 2.7±0.2 |
| HAM | **BCAM** | ND | 0.4±0.1 | 0.2±0.08 | 0.4±0.02 |
| HAN | **DCAN** | 0.2±0.003 | ND | ND | 0.2±0.04 |
| HAN | **BCAN** | 0.4±0.002 | 0.4±0.02 | 0.4±0.006 | 0.4±0.003 |
| HK | **11DCP** | 0.7±0.02 | 0.3±0.008 | 0.3±0.05 | 0.4±0.02 |
| HNM | **BDCNM** | 0.7±0.0002 | ND | ND | ND |
| HNM | **DBCNM** | 1.5±0.0006 | ND | ND | ND |
| HNM | **DCNM** | 0.2±0.007 | 0.1±0.0002 | 0.1±0.003 | 0.1±0.02 |
| HNM | **TCNM** | 0.5±0.01 | 0.2±0.007 | 0.3±0.002 | 0.3±0.008 |
| I-THM | **DCIM** | 0.1±0.01 | <0.1 | <0.1 | <0.1 |
| IAA | **IAA** | 0.006±0.0004 | 0.007±0.005 | 0.008 | LOST |
| IAA | **CIAA** | 0.002±0.0 | ND | 0.001 | LOST |
| NA | **NDMA** | 0.050 | 0.088 | 0.043 | 0.055 |
| THM | **TCM** | 11.4 | 8.5 | 8.3 | 8.5 |
| THM | **BDCM** | 1.8 | 1.3 | 1.3 | 1.3 |
| THM | **DBCM** | 0.4 | 0.3 | 0.3 | 0.4 |
| THM | **TBM** | 0.4 | 0.5 | 0.4 | 0.5 |
| HAA | **CAA** | 1.8 | 1.3 | 1.1 | 1.6 |
| HAA | **DCAA** | 17.5 | 6.5 | 7.0 | 8.2 |
| HAA | **TCAA** | 3.4 | 0.8 | 2.3 | 2.7 |
| HAA | **BCAA** | 2.3 | 1.4 | 1.1 | 1.6 |
| HAA | **BDCAA** | 1.4 | 1.2 | 1.3 | 1.3 |
| HAA | **DBCAA** | 0.9 | 0.8 | 0.9 | 0.9 |
| HAA | **TBAA** | 2.1 | 0.4 | 2.4 | 0.6 |
| TOX | **TOCl as Cl** | 91.7±2.6 | 74.3±15.8 | 65.0±7.7 | 63.2±2.3 |
| TOX | **TOBr as Br** | 8.6±0.5 | 6.8±0.3 | 6.4±0.03 | 7.3±0.4 |
| TOX | **TOI as I** | 1.8±1.0 | 1.3±1.0 | 1.4±1.0 | 2.0±0.7 |

**Table 5.9. Plant B: Effect of GAC age on preformed DBP removal.**

| DBP Class | DBPs | No GAC (µg/L) | 157,400 BV (µg/L) | 333,200 BV (µg/L) | 555,300 BV (µg/L) |
|---|---|---|---|---|---|
| HAL | **TCAL** | 0.8±0.01 | 0.2±0.01 | 0.6±0.21 | 0.3±0.01 |
| HAM | **DCAM** | 1.1±1.19 | 0.4±0.16 | 1.1±0.53 | 1.0±0.23 |
| HAM | **TCAM** | 0.3±0.15 | 0.1±0.03 | 0.2±0.05 | 0.2±0.03 |
| HAM | **DBCAM** | ND | 0.1±0.03 | ND | 0.1±0.00 |
| HAN | **DCAN** | 0.3±0.04 | ND | 0.1±0.03 | ND |
| HK | **11DCP** | 0.7±0.02 | ND | 0.3±0.12 | 0.1±0.01 |
| HK | **111TCP** | 0.2±0.03 | ND | 0.2±0.11 | ND |
| HNM | **BDCNM** | 0.8±0.00 | ND | ND | ND |
| HNM | **DBCNM** | 1.4±0.00 | ND | ND | ND |
| HNM | **TCNM** | 7.4±1.6598 | 1.2±0.4 | 3.5±0.1648 | 2.2±0.1529 |
| IAA | **IAA** | 0.014±0.003 | 0.002±0.0002 | 0.005±0.0005 | 0.001±0.0001 |
| IAA | **CIAA** | 0.004±0.001 | ND | 0.001±0.0001 | ND |
| NA | **NDMA** | 0.03 | 0.005 | 0.005 | 0.004 |
| THM | **TCM** | 15.4 | 11.4 | 12.0 | 11.7 |
| THM | **BDCM** | 2.4 | 2.0 | 2.0 | 2.1 |
| THM | **DBCM** | 0.2 | 0.3 | 0.4 | 0.4 |
| HAA | **CAA** | 1.3 | 0.5 | 1.3 | 1.2 |
| HAA | **DCAA** | 27.3 | 6.8 | 16.6 | 9.3 |
| HAA | **TCAA** | 7.4 | 4.0 | 5.6 | 5.5 |
| HAA | **BCAA** | 2.7 | 1.8 | 2.3 | 1.8 |
| HAA | **BDCAA** | 1.1 | 1.1 | 1.1 | 1.1 |
| HAA | **DBAA** | ND | 0.2 | ND | 0.1 |
| HAA | **DBCAA** | 0.8 | 1.3 | 0.9 | 0.8 |
| HAA | **TBAA** | 0.7 | 0.5 | 1.0 | 0.7 |
| TOX | **TOCl as Cl** | 67.5±1.3 | 48.3±2.1 | 62.2±3.3 | 65.6±6.7 |
| TOX | **TOBr as Br** | 8.0±0.09 | 5.9±0.08 | 7.4±0.2 | 6.5±0.2 |
| TOX | **TOI as I** | 1.9±0.2 | 1.5±0.2 | 1.9±0.6 | 1.2±0.08 |

**Table 5.10. Plant B simulated distribution system results: Effect of GAC age on DBP and TOX removal. CAA not quantified due to instrumental interference**.

| Class | DBPs | No GAC (µg/L) | 157,400 BV (µg/L) | 333,200 BV (µg/L) | 555,300 BV (µg/L) |
|---|---|---|---|---|---|
| HAL | **TCAL** | 2.4±0.04 | ND | 1.3±0.08 | 1.2±0.04 |
| HAL | **CAL** | 1.3±0.8 | 2.1±0.07 | 1.6±0.6 | 2.0±0.4 |
| HAL | **DCAL** | 0.3±0.2 | 0.1±0.004 | 0.2±0.08 | 0.2±0.05 |
| HAM | **DCAM** | 25.4±0.6 | 7.4±0.15 | 10.8±1.2 | 14.3±0.9 |
| HAM | **BCAM** | 3.0±0.7 | 2.0±0.06 | 1.5±0.10 | 3.2±0.2 |
| HAM | **TCAM** | 1.2±0.01 | 0.4±0.00 | 0.4±0.04 | 0.5±0.03 |
| HAM | **DBAM** | ND | 0.6±0.02 | 0.2±0.01 | 0.5±0.02 |
| HAN | **DCAN** | 1.8±0.03 | 0.9±0.01 | 1.2±0.03 | 1.8±0.03 |
| HAN | **BCAN** | 0.2±0.01 | 0.5±0.01 | 0.2±0.00 | 0.8±0.02 |
| HK | **11DCP** | 2.0±0.10 | 0.7±0.06 | 1.6±0.02 | 1.2±0.04 |
| HNM | **DCNM** | 1.2±0.01 | 0.8±0.01 | 0.9±0.02 | 1.5±0.04 |
| NA | **NDMA** | 0.070 | 0.011 | 0.045 | 0.035 |
| THM | **TCM** | 15.5 | 16.3 | 15.6 | 15.5 |
| THM | **BDCM** | 2.6 | 2.4 | 2.6 | 2.5 |
| THM | **DBCM** | 1.0 | 0.3 | 5.9 | 0.4 |
| HAA | **BAA** | 3.0 | ND | ND | ND |
| HAA | **DCAA** | 17.2 | 1.6 | 8.4 | 3.3 |
| HAA | **TCAA** | 7.0 | 3.9 | 5.4 | 4.3 |
| HAA | **BCAA** | 1.8 | 0.2 | 1.1 | 0.0 |
| HAA | **BDCAA** | 2.2 | 1.5 | 1.5 | 1.3 |
| HAA | **DBAA** | 0.0 | 0.2 | 0.0 | 0.0 |
| HAA | **DBCAA** | 12.6 | 0.9 | 0.9 | 0.9 |
| HAA | **TBAA** | 0.0 | 0.3 | 0.0 | 0.0 |
| TOX | **TOCl as Cl** | 81.5±32.8 | 94.1±12.4 | 89.4±8.6 | 198.0±99.4 |
| TOX | **TOBr as Br** | 8.2±0.8 | 8.4±0.4 | 6.8±0.05 | 8.3±0.05 |
| TOX | **TOI as I** | 1.6±0.2 | 1.1±0.01 | 1.28±0.0 | 1.2 ± 0.01 |

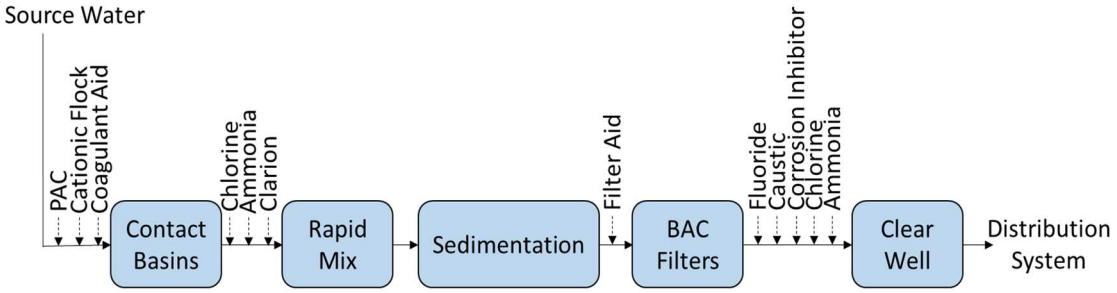


**Figure 5.6.** Process flow diagram of Plant A. PAC is powdered activated carbon.

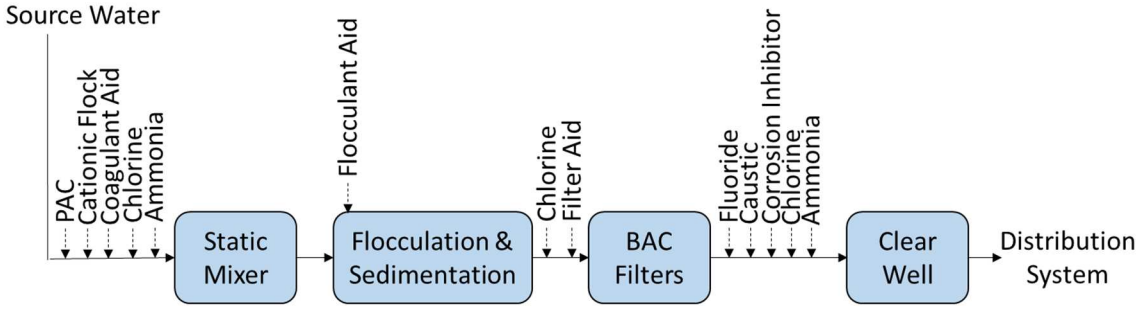


**Figure 5.7.** Process flow diagram of Plant B. PAC is powdered activated carbon.

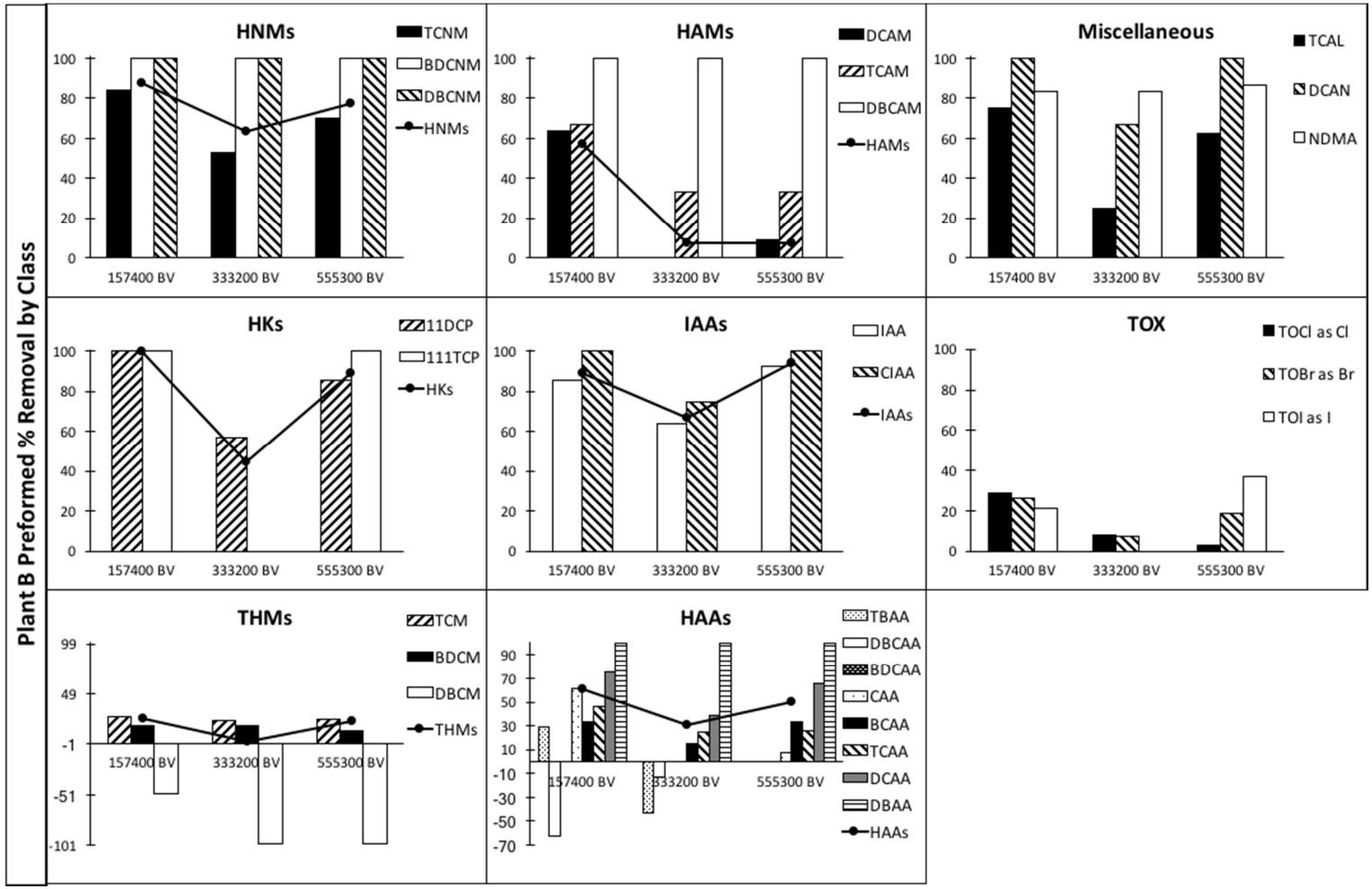


**Figure 5.8.** Plant B: % Removal of preformed DBPs by BAC grouped by class and total organic halogen (TOX). Effect of BAC age in bed volumes (BV). Percent removal of classes based on summed concentrations and not average removal.

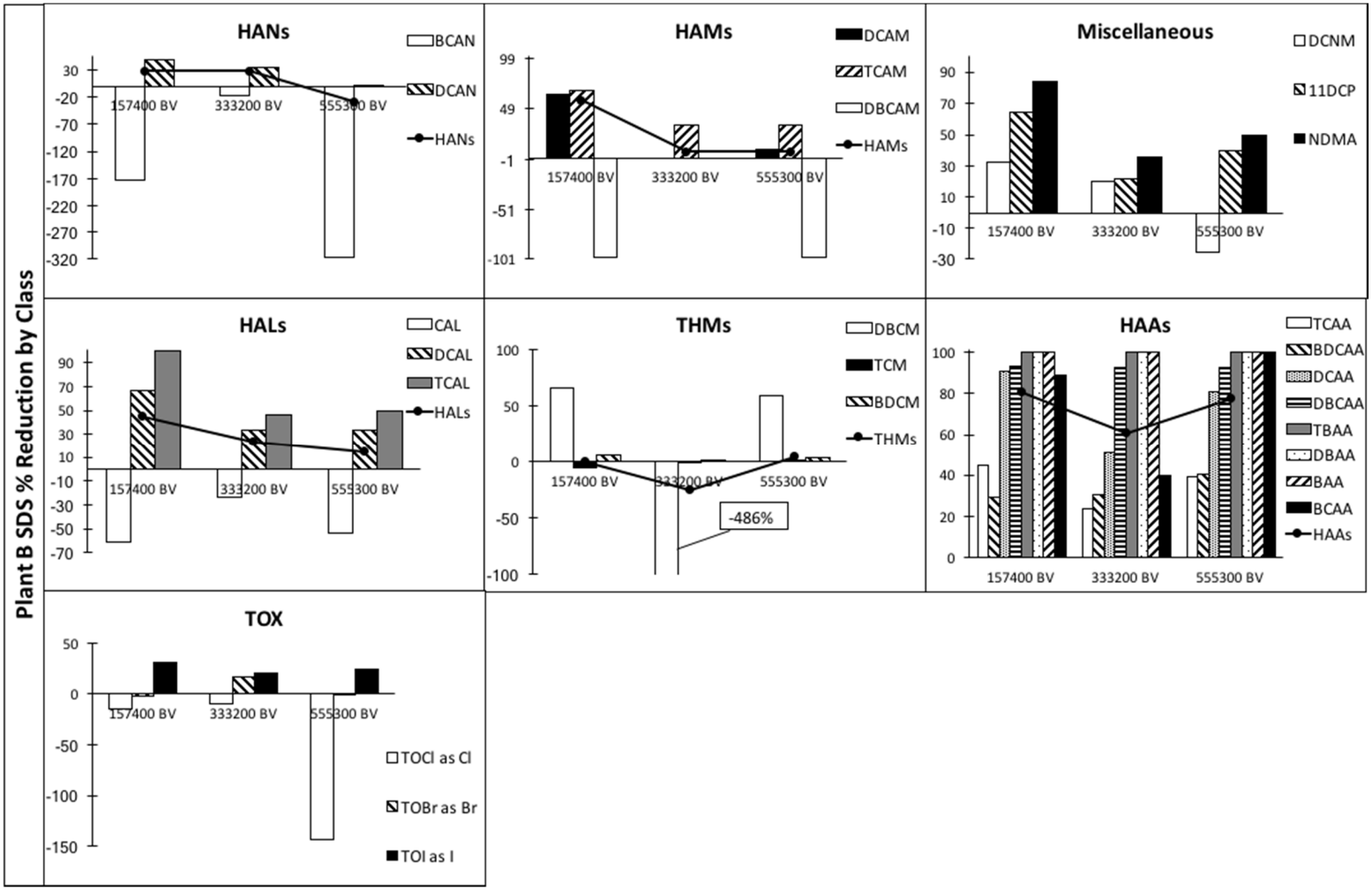


**Figure 5.9.** Plant B SDS: Effect of BAC age by bed volumes (BV) on DBP removal- breakthrough of all quantified DBPs by class and total organic halogen (TOX). Percent removal of classes based on summed concentrations and not average removal.

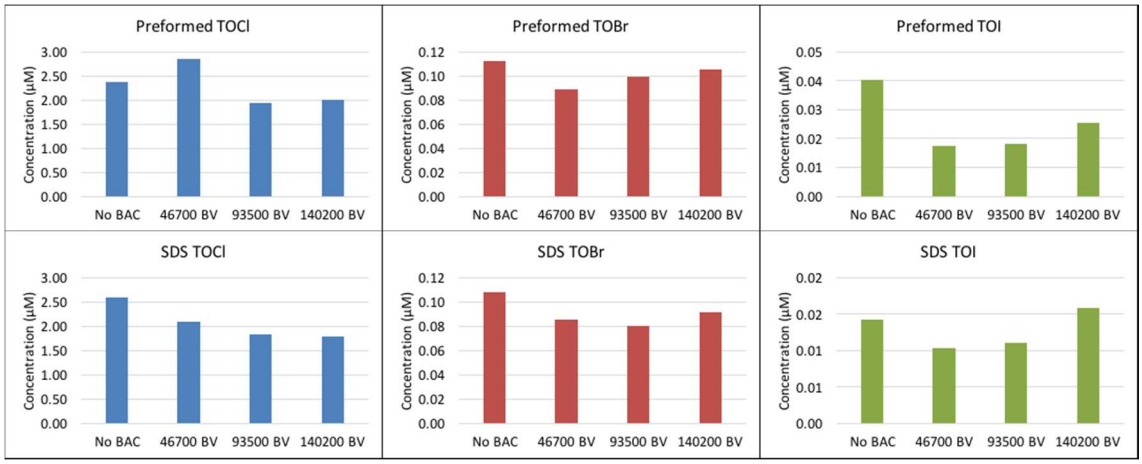


**Figure 5.10**. Plant A preformed and SDS: Effect of BAC age in bed volumes (BV) on TOCl, TOBr, and TOI removal in molarity.

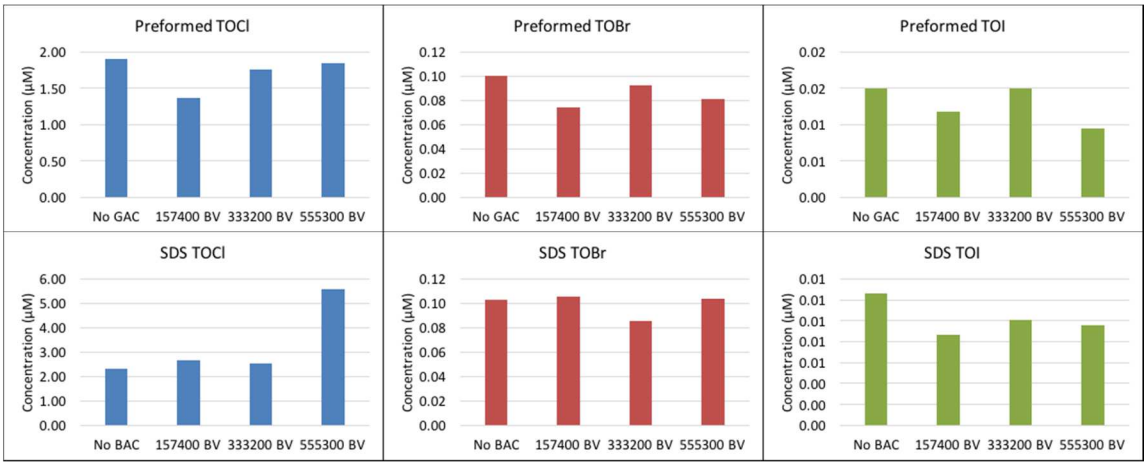


**Figure 5.11.** Plant B preformed and SDS: Effect of BAC age in bed volumes (BV) on TOCl, TOBr, and TOI removal in molarity.

# APPENDIX A

# FIRST AUTHOR PUBLISHED WORKS

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# Showering in Flint, MI: Is there a DBP problem?

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ABSTRACT

Lead contamination in the City of Flint, MI has been well documented over the past two years, with lead levels above the EPA Action Level until summer 2016. This resulted from an ill-fated decision to switch from Detroit water (Lake Huron) with corrosion control, to Flint River water without corrosion control. Although lead levels are now closer to normal, reports of skin rashes have sparked questions surrounding tap water in some Flint homes. This study investigated the presence of contaminants, including disinfection by-products (DBPs), in the hot tap water used for showering in the homes of residents in Flint. Extensive quantitative analysis of 61 regulated and priority unregulated DBPs was conducted in Flint hot and cold tap water, along with the analysis of 50 volatile organic compounds and a nontarget comprehensive, broadscreen analysis, to identify a possible source for the reported skin rashes. For comparison, chlorinated hot and cold waters from three other cities were also sampled, including Detroit, which also uses Lake Huron as its source water. Results showed that hot water samples generally contained elevated levels of regulated and priority unregulated DBPs compared to cold water samples, but trihalomethanes were still within regulatory limits. Overall, hot shower water from Flint was similar to waters sampled from the three other cities and did not have unusually high levels of DBPs or other organic chemicals that could be responsible for the skin rashes observed by residents. It is possible that an inorganic chemical or microbial contaminant may be responsible.


## Introduction

The Flint Water Crisis began in April 2014 when the City of Flint made an unfortunate decision, driven by costs, to switch source waters from Lake Huron in Detroit to the Flint River, while eliminating corrosion control (Pieper et al., 2017; Del Toral, 2015; Croft et al., 2015). This switch immediately led to violations for bacteria (including legionella), then total trihalomethanes (TTHMs), unprecedented corrosion of iron mains, main breaks, and elevated lead in drinking water (Del Toral, 2015; Croft et al., 2015; Edwards, 2015; Smith, 2015; Washington Post, 2016). While Lake Huron source water is regarded as a relatively pristine source of drinking water, the Flint River is highly corrosive, containing approximately eight times the normal level of chloride (Pieper et al., 2017). Corrosion inhibitors, such as orthophosphate, are commonly added to distribution systems to prevent the leaching of lead from pipes, but this practice was discontinued following the

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# The DBP exposome: Development of a new method to simultaneously quantify priority disinfection by-products and comprehensively identify unknowns

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ABSTRACT

Disinfected drinking water contains hundreds of disinfection by-products (DBPs) that are formed by the reaction of disinfectants with natural and anthropogenic organic matter, bromide, and iodide. Understanding what these DBPs are is important because millions of people worldwide consume drinking water every day, and human epidemiologic studies have reported cancer, miscarriage, and birth defects from consuming such waters. While more than 600 DBPs are reported in the literature, very few studies quantify complete classes of chlorinated, brominated, and iodinated DBPs. Also, very few studies conduct comprehensive non-target analyses of unknown DBPs to characterize the complete DBP exposure (the exposome). We developed a new gas chromatography (GC)-mass spectrometry (MS) method that simultaneously quantifies 39 priority unregulated DBPs from six different chemical classes (haloacetaldehydes, haloketones, haloacetamides, haloacetonitriles, halonitromethanes, and iodinated-trihalomethanes) and analyzes unknown DBPs with mass accuracy <600 ppm under full-scan conditions. Using a new type of time-of-flight (TOF) mass spectrometer, which combines selected ion monitoring (SIM)-level sensitivity with mass accuracy of ±0.05 Da, method detection limits of 3–61 ng/L were achieved. These levels were found to be quite comparable to those of a widely used single quadrupole mass spectrometer (2–90 ng/L) operated in SIM mode. However, analysis using this TOF mass spectrometer offers two additional advantages over traditional quadrupole-MS: (1) full-scan data, which provides additional confidence for target analytes, as well as complete mass spectra for unknown analysis, and (2) two decimal place mass accuracy, which allows additional confidence for target analytes and importantly, molecular formula indication for unknowns. High resolution accurate mass TOF was also used to validate identification of selected compounds. This new method was demonstrated on finished drinking waters from three different drinking water plants, where target quantification and non-target unknown analyses were performed simultaneously during the same run. This enabled the quantification of 39 DBPs, along with the non-target identification of many other drinking water contaminants, including two additional non-target DBPs: *N,N*-dimethylacetamide and *N*-nitrosodibutylamine.



## 1. Introduction

Water disinfection is used worldwide to protect public health against harmful pathogens that cause waterborne diseases. However, disinfectants can also react with constituents found in natural waters (i.e., natural and anthropogenic organic matter, bromide, and iodide) to unintentionally form DBPs that have been associated with several adverse health effects from long-term exposure, including bladder and colorectal cancer and adverse birth outcomes (Bove et al., 2007; Costet et al., 2011; Grazuleviciene et al., 2013; Nieuwenhuijsen et al. 2000, 2013; Righi et al., 2012; Savitz et al., 2005; Smith et al., 2016; Villanueva et al. 2004, 2007; Waller et al., 1998). DBPs represent one class of chemical exposure

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# Does Granular Activated Carbon with Chlorination Produce Safer Drinking Water? From Disinfection Byproducts and Total Organic Halogen to Calculated Toxicity

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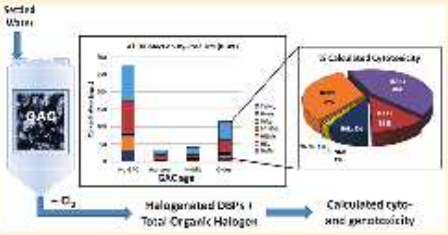
Supporting Information

**ABSTRACT:** Granular activated carbon (GAC) adsorption is well-established for controlling regulated disinfection byproducts (DBPs), but its effectiveness for unregulated DBPs and DBP-associated toxicity is unclear. In this study, GAC treatment was evaluated at three full-scale chlorination drinking water treatment plants over different GAC service lives for controlling 61 unregulated DBPs, 9 regulated DBPs, and speciated total organic halogen (total organic chlorine, bromine, and iodine). The plants represented a range of impacts, including algal, agricultural, and industrial wastewater. This study represents the most extensive full-scale study of its kind and seeks to address the question of whether GAC can make drinking water safer from a DBP perspective. Overall, GAC was effective for removing DBP precursors and reducing DBP formation and total organic halogen, even after >22 000 bed volumes of treated water. GAC also effectively removed preformed DBPs at plants using prechlorination, including highly toxic iodoacetic acids and haloacetonitriles. However, 7 DBPs (mostly brominated and nitrogenous) increased in formation after GAC treatment. In one plant, an increase in tribromonitromethane had significant impacts on calculated cytotoxicity, which only had 7−17% reduction following GAC. While these DBPs are highly toxic, the total calculated cytotoxicity and genotoxicity for the GAC treated waters for the other two plants was reduced 32−83% (across young−middle−old GAC). Overall, calculated toxicity was reduced post-GAC, with preoxidation allowing further reductions.




## INTRODUCTION

Drinking water disinfection is vital for prevention of waterborne illness. Since its introduction in the U.S. in the early 1900s, disinfection is reported to have contributed significantly to an estimated 29-year increase in life expectancy.[1] An unintended consequence of disinfection is the formation of disinfection byproducts (DBPs), which have been associated with adverse health effects, including bladder cancer, colon cancer, miscarriage, and birth defects.[2−10] In the U.S., regulations are enforced for four trihalomethanes (THMs), five haloacetic acids (HAAs), bromate, and chlorite under the Stage 2 Disinfectants and DBP Rule.[11] Several recent studies indicate that, while THMs and HAAs are the dominant DBPs formed upon chlorination, they are not necessarily drivers of toxicity associated with DBP formation.[12−14]

DBPs are formed by the reaction of disinfectants with natural organic matter (NOM), bromide, and iodide.[6,15] Many NOM fractions can react to form THMs and HAAs, while phenolic NOM structures have been shown to form haloacetaldehydes (Table S1).[16] Free and combined amino acids, aldehydes, and aromatic NOM have been shown to form

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# APPENDIX B

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