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Disinfection Byproducts in Wastewater, Swimming Pools, and Tea: Identification, Quantification, and Drivers of Toxicity

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Disinfection Byproducts in Wastewater, Swimming Pools, and Tea: Identification, Quantification, and Drivers of Toxicity

by

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DEDICATION

I dedicate this dissertation to my wonderful mother (Alice), sisters (Emily and Leslie), brother (Adam), niece (Harper), nephew (Ethan), best friend (Caitlyn), and numerous cousins who encouraged and supported me throughout my academic career. I also dedicate this dissertation to all the friends I have made in my undergraduate and graduate school career. Thanks for all the amazing memories and for sharing in the ups and downs of school and research.

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ABSTRACT

Drinking water disinfection is considered one of the greatest scientific achievements of the 20th Century because it significantly reduced the number of deaths related to waterborne diseases. However, in 1974 J.J. Rook discovered that chlorine, a commonly used disinfectant, can react with natural organic matter to form disinfection byproducts (DBPs). Since then, more than 700 DBPs have been identified, with several epidemiological and toxicological studies linking DBPs to several adverse health effects such as bladder and colorectal cancer, adverse birth outcomes, and asthma. Due to their ubiquity in disinfected water and their adverse health effects, studying the formation and identifying new DBPs is critical to identifying the drivers of toxicity in disinfected water and ultimately improving water quality. The studies presented here utilized highly sensitive analytical methods and instruments to quantify DBPs in a variety of matrices, including wastewater treatment plant effluent and swimming pools. Further, the third study utilizes high-resolution mass spectrometry to identify unknown haloaromatic DBPs in tea.

Comprehensive DBP analysis of chlorinated wastewater treatment plant effluent and upstream/downstream river samples revealed that a variety of DBPs are formed during the disinfection step at wastewater treatment plants. Interestingly, *in vitro* studies reveal that nitrogenous DBPs (haloacetonitriles and haloacetamides) and haloketones are important drivers of cytotoxicity and genotoxicity, respectively, in samples collected downstream from wastewater treatment plants.

Extensive DBP analysis of conventional chlorine and salt water pool samples revealed that haloacetonitriles, haloacetic acids, and haloacetaldehydes were the primary drivers of calculated cytotoxicity, while haloacetic acids were the primary drivers of calculated genotoxicity. This study also provides an important comparison between conventional chlorine (liquid bleach) and electrochemically generated chlorine (salt water pool), two common disinfection techniques used in swimming pools. Results reveal the importance of maintaining a low residual of chlorine and ensuring proper ventilation to reduce swimmers' exposure to DBPs.

Unknowns analysis using high-resolution mass spectrometry revealed six newly identified DBPs in brewed tea, including two monochloro-hydroxyphenols, two monochloro-trihydroxybenzenes, and two dichloro-trihydroxybenznenes. The identification of haloaromatic DBPs is significant due to recent studies noting that they can be more toxic than aliphatic DBPs.

Overall, results from these studies reveal that nitrogenous DBPs are important drivers of toxicity in both river samples receiving wastewater treatment plant effluent and swimming pools. Additionally, unknowns analysis of tea emphasizes the importance of continuing to identify new DBPs due to their potential for elevated toxicity.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Chapter 1 investigates the formation and presence of disinfection byproducts (DBPs) in chlorinated wastewater treatment plant (WWTP) effluent and in upstream/downstream receiving bodies of water across the state of Minnesota. While DBPs have been extensively studied in drinking water, few studies have focused on the formation of DBPs in chlorinated WWTP effluent and their impact on the aquatic ecosystem. This chapter presents the most extensive study of DBPs in chlorinated effluent as well as the quantification of iopamidol, an anthropogenic contaminant that can serve as a precursor for the formation of toxic iodinated DBPs (I-DBPs). Overall, this study combines sensitive analytical techniques and *in vitro* cytotoxicity and genotoxicity assays to determine the drivers of toxicity in chlorinated effluent and downstream bodies of water. Results from Chapter 1 show that nitrogenous DBPs (N-DBPs) and iodinated DBPs (I-DBPs) are important drivers of toxicity in chlorinated WWTP effluent and that monitoring their formation is a critical step in improving the overall water quality of aquatic ecosystems.

Chapter 2 is a case study on the formation of DBPs at an indoor community pool in South Carolina while the pool was disinfecting with conventional liquid chlorine (sodium hypochlorite) and then after the implementation of electrochemically generated chlorine technology (salt water pool). In this study, the same indoor community pool was sampled and presented a unique opportunity for us to make a more direct comparison

between two different disinfection techniques. Results from this study show that although on average the overall level of DBPs increases in a salt water pool, the calculated cytotoxicity and genotoxicity decreases by 45% and 15%, respectively. Additionally, results reveal that even a small bromide impurity increases the formation of brominated DBPs (Br-DBPs).

Chapter 3 identifies unknown DBPs formed during the brewing process of making tea. The residual chlorine in tap water may react with ingredients like polyphenols present in tea to form high molecular weight DBPs. In this study, six unknown DBPs were identified for the first time in tea using GC-high-resolution mass spectrometry. Previous studies indicate that haloaromatic DBPs can be more toxic than aliphatic DBPs, showcasing the importance of continuing to identify new DBPs in a variety of matrices.

CHAPTER 2

NITROGENOUS DBPS AND IODO-ACIDS ARE IMPORTANT TOXICITY DRIVERS IN CHLORINATED WASTEWATER

[1] Granger, C.O.; Aziz, T.; Liberatore, H.K.; Ferrey, M.L.; Richardson, S.D. Nitrogenous DBPs and Iodo-acids are Important Toxicity Drivers in Chlorinated Wastewater. To be submitted to *Environ. Sci. Technol.*

ABSTRACT

While disinfection byproducts (DBPs) have been extensively studied in drinking water, few studies have investigated their formation in disinfected wastewater or in receiving rivers. Thus, the impact of DBPs on aquatic ecosystems is largely unknown. In this study, we report the most comprehensive investigation of DBPs to-date for wastewater treatment plants (WWTPs), with 60 DBPs quantified in chlorinated WWTP effluents and also upstream/downstream in receiving rivers across the state of Minnesota. Total DBPs (average) quantified for upstream, downstream, and effluent samples were 0.3, 4.2, and 9.6 μg/L, respectively, with the most dominant classes being haloacetic acids, trihalomethanes, and haloketones. We also report levels of iopamidol (up to 29,900 ng/L in WWTP effluent), an X-ray contrast media that may be an important precursor in the formation of I-DBPs observed (up to 100 ng/L) in the chlorinated effluents, as well as mammalian cell cytotoxicity and genotoxicity. *In vitro* toxicity assays reveal that three of the downstream samples were statistically ($P < 0.001$) more cytotoxic than their corresponding upstream samples while four of the downstream samples were statistically ($P < 0.001$) more genotoxic than their corresponding upstream samples. Interestingly, *in vitro* toxicity assays reveal that two of the upstream samples were statistically ($P < 0.001$) more cytotoxic and genotoxic than their corresponding downstream samples. *In vitro* cytotoxicity and genotoxicity assays also reveal that the toxicity of chlorinated WWTP effluent was primarily driven by haloacetonitriles and haloketones, while calculated toxicity revealed that iodoacetic acid plays an important role.

INTRODUCTION

Every day, wastewater treatment plants (WWTPs) across the United States treat approximately 34 billion gallons of industrial and household wastewater to remove contaminants and kill harmful pathogens before being discharged into the environment.[1] The effluent of these plants are treated with chemical disinfectants, which can react with natural organic matter and/or anthropogenic contaminants present in wastewater to form disinfection byproducts (DBPs) [2-5], which have been linked to several adverse health effects including colorectal cancer, bladder cancer, miscarriage, and birth defects.[5-15]

Removal of anthropogenic contaminants during wastewater treatment is a priority due to the impact they may have on aquatic ecosystems and drinking water quality downstream. Unfortunately, many contaminants (i.e., pharmaceuticals, pesticides, personal care products) are incompletely removed during conventional wastewater treatment and have been detected in rivers and lakes at ng/L to µg/L levels.[16-17,38] Of the pharmaceuticals detected in the environment, iodinated X-ray contrast media (ICM) are of particular interest because they undergo little to no removal during wastewater treatment and are detected in rivers and lakes across the world at levels > 1 µg/L (with levels as high as 100 µg/L).[18-23,38] Further, previous studies reveal that ICM, specifically iopamidol, can serve as a source of iodine for the formation of iodinated disinfection byproducts (I-DBPs) in the presence of natural organic matter (NOM) upon chlorination under drinking water treatment conditions.[24-29] I-DBPs are important to study due to their elevated levels of toxicity when compared to their brominated and chlorinated analogues.[30-34]

ICM are triiodinated benzene derivatives used to help image soft tissue (e.g., lungs, bladder, kidneys, etc.) in the human body. Approximately 200 g of ICM (100 g iodine) can be administered in one dose, which is then excreted from the human body 95% unmetabolized within 24 h of application.[35-37] ICM contain hydroxyl, carboxyl, and amide moieties to increase their solubility and polarity, thus increasing their stability in the human body and allowing them to be safe to use.[36] Global usage of ICM is approximately 3.5×10^6 kg/year.[37]

In a previous survey by the Minnesota Pollution Control Agency (MPCA), samples from 50 river and stream locations in Minnesota were analyzed for 146 pharmaceuticals and personal care products (PPCPs). Iopamidol was the most frequently detected PPCP, with a detection frequency of 78% and occurring at concentrations up to 1650 ng/L. Statistical evaluation of that data indicated that iopamidol was likely present in 82% of Minnesota's river miles, the highest of all the chemicals measured in that study.[41] Because of the tendency of iopamidol to react with chlorine to form I-DBPs, we hypothesized that I-DBPs might also form in chlorinated WWTP effluents when high levels of iopamidol are present. Preliminary data (unpublished) collected from a small pilot study of chlorinated WWTPs in Minnesota supported this hypothesis, with high levels of I-THMs (dichloroiodomethane and dibromoiodomethane) at one location when high levels of iopamidol were also present.

Our current study expanded on that effort by quantifying 60 DBPs and iopamidol in WWTP effluents and upstream/downstream samples of receiving rivers across the state of Minnesota. Nine WWTPs were sampled over three years for a total of 16 sampling events. In addition to six I-THMs, four iodoacetic acids (IAAs), four THMs, nine

haloacetic acids (HAAs), nine haloketones (HKs), four haloacetaldehydes (HALs), four halonitromethanes (HNMs), seven haloacetonitriles (HANs), and 13 haloacetamides (HAMs) were also measured, making this the most comprehensive study of DBPs in WWTP effluents to-date and one of the first to address downstream cytotoxicity and genotoxicity impacts. While DBPs have been extensively studied in drinking water, few studies have investigated their formation in WWTPs. Previous studies reported THMs and HAAs as dominant classes present in chlorinated wastewater effluent.[2,42]

Among the 60 DBPs included in this study are nitrogenous DBPs (N-DBPs) and iodinated DBPs (I-DBPs), which are much more toxic than carbonaceous DBPs (C-DBPs).[43-45] Regulated THMs and HAAs were also measured. In addition, *in vitro* cytotoxicity and genotoxicity were measured in these waters. Our results provide important insights into the toxicity drivers of chlorinated WWTP effluents and also consider DBPs together with iopamidol, a pharmaceutical that can serve as a precursor to DBPs.

MATERIALS AND METHODS

Chemicals and Reagents. Methanol (≥99.9%), methyl *tert*-butyl ether (99.9%), acetonitrile (≥99.9%), and ethyl acetate (≥99.9%) were of the highest purity and were purchased from VWR International (Radnor, PA), Sigma-Aldrich (St. Louis, MO), or Fisher Scientific (Waltham, MA). DBP standards were purchased from Sigma-Aldrich, CanSyn Chem. Corp. (Toronto, ON), and TCI America (Waltham, MA). Specific vendor information can be found in **Table A.1**. Diazald (99%) and CARBITOL (99%), used to derivatize iodoacetic acids and haloacetic acids, along with the internal standard (1,2-dibromopropane, 97%), were purchased from Sigma-Aldrich (St. Louis, MO).

Sample Collection. Grab samples were collected from the effluent outfall and upstream and downstream locations of nine WWTPs that chlorinate effluent in Minnesota. Samples were collected over three years (2019, 2020, and 2021) and WWTPs were selected based on a variety of factors, including population served, receiving body of water, presence and size of hospitals in the area, and average total volume of wastewater treated daily. Samples collected for the quantitative analysis of 60 DBPs were collected headspace-free in amber glass bottles with PTFE-lined polyethylene caps and shipped overnight on ice to the University of South Carolina and extracted immediately upon arrival. Due to the dechlorination step required at WWTPs (**Table A.2**), no quencher was added to the samples. Upstream and downstream samples (15 L) for mammalian cell cytotoxicity and genotoxicity measurements were collected headspace-free in amber glass bottles with PTFE-lined polyethylene caps and shipped overnight on ice to the University of South Carolina. Samples were extracted immediately, stored in amber vials at -20 °C until shipment to the University of Illinois for toxicity analysis. Samples for iopamidol analysis were collected in 500 mL amber glass bottles, filled headspace-free, and shipped on ice to SGS AXYS Analytical Services in Vancouver, BC for analysis.

Analytical Method for the Quantification of 60 DBPs. For each sample, four trihalomethanes (THMs), nine haloacetic acids (HAAs), nine haloketones (HKs), four haloacetaldehyde (HALs), four halonitromethanes (HNMs), seven haloacetonitriles (HANs), 13 haloacetamides (HAMs), six iodinated trihalomethanes (I-THMs), and four iodoacetic acids (IAAs) were quantified. Quantification was performed via standard addition due to the complexity of the wastewater sample matrices. To perform standard addition, at each site (upstream, downstream, and effluent) seven amber glass bottles with

PTFE-lined polyethylene caps were filled with 100 mL of sample. Of the seven bottles, two were set aside as non-spiked samples, while the remaining five were spiked with a mixture of DBPs. I-THMs, HKs, HNMs, HANs, HALs, HAMs, and THMs were spiked at 0.75, 1.5, 2.25, 3.0, and 6.0 µg/L using a 10 mg/L mixture, while IAAs/HAAs were spiked at 0.125/0.25, 0.25/0.5, 0.375/0.75, 0.5/1.0, and 1.0/2.0 µg/L using a 1.0/2.0 mg/L mixture, respectively. Samples were then extracted as described previously.[46-49] In brief, each sample was adjusted to pH < 1 by adding 1 mL of concentrated H_2SO_4. A liquid-liquid extraction (LLE) was performed by adding 30 grams of Na_2SO_4 and 5 mL of methyl *tert*-butyl ether (per extraction) as the extraction solvent. Samples were then shaken for 15 min and allowed to settle for 10 min, after which the top organic layer was removed and placed into a glass test tube. The extraction procedure was repeated twice (for a total extraction solvent volume of 15 mL), and the sample extract was dried by passing the sample though a column containing anhydrous Na_2SO_4. Once dried, the extracts were concentrated to 200 µL under a gentle stream of nitrogen, spiked with 4 µL of internal standard (1,2-dibromopropane), and split into two separate aliquots of 100 µL each. The first aliquot was used to quantify I-THMs, HKs, HNMs, HANs, HALs, HAMs, and THMs, while the second aliquot was used to quantify HAAs and IAAs. HAAs and IAAs were derivatized using freshly prepared diazomethane to convert carboxylic acids to methyl esters. Diazomethane was prepared according to a U.S. EPA Standard Operating Procedure.[50] In brief, 0.367 g of Diazald and 1.0 mL of CARBITOL were placed inside the inner tube of an Aldrich diazomethane generator. Then, 3.0 mL of methyl *tert*-butyl ether was placed in the outside portion of the diazomethane generator, and the entire generator was placed on ice. Next, 1.5 mL of 37% KOH was added

dropwise to the inner tube and allowed to react for 1 hr. After 1 hr, 50 µL of diazomethane (dissolved in the methyl *tert*-butyl ether in the outer portion of the generator) was added to 100 µL of sample extract and allowed to react for 30 min. After 30 min, excess diazomethane was quenched by adding 10 mg silica gel, and the samples were transferred into new amber vials before instrumental analysis.

Instrumental Analysis of 60 DBPs. The quantification of I-THMs, HKs, HNMs, HANs, HALs, HAMs, and THMs was conducted using an Agilent 7890 gas chromatograph-5977A mass spectrometer (GC-MS; Agilent Technologies, Santa Clara, CA) with electron ionization (EI) at 70 eV as previously described by Cuthbertson et al.[46] Sample extracts (1.0 µL) were injected into a multimode inlet (MMI) in pulsed splitless mode and chromatographically separated using a Restek Rtx-200 column (30 m x 0.25 mm x 0.25 µm film thickness; Restek Corporation, Bellefonte, PA). The GC temperature program was as follows: initial temperature of 35 °C for 5 min, increased to 220 °C at 9 °C/min, and then ramped at 20 °C/min to 280 °C and held for 15 min. Quantification of HAAs and IAAs were conducted on an Agilent GC-MS and a Thermo Trace 1310 GC-TSQ 9000 triple quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA) using the following GC temperature program: initial temperature held at 35 °C for 5 min, increased to 280 °C at 9 °C/min, and then held for 15 min. Samples were also chromatographically separated using a Restek Rtx-200 column. For both sets of analyses, the transfer line was held at 280 °C and the source temperature was held at 200 °C. Quantifier and qualifier ions for each DBP are listed in **Table A.1**.

Analytical Method for Iopamidol. Quantification of iopamidol was based on EPA method 1694.[51] In brief, samples were filtered (1.6 mm), adjusted to pH 2 with HCl, and

spiked with a surrogate, d8-iopamidol . Samples were then extracted via solid phase extraction (SPE) using an Oasis HLB cartridge (Waters, Milford, MA) and analyzed in duplicate using ultra-performance liquid chromatograph coupled to a triple quadrupole mass spectrometer (UPLC-QqQ-MS/MS) via electron spray ionization (ESI) in positive mode. Primary and secondary multiple reaction monitoring (MRM) transitions for iopamidol and d_8-iopamidol are shown in **Table A.3.**

XAD Resin Extraction. For mammalian cell cytotoxicity and genotoxicity testing, upstream and downstream samples (15 L) were collected in 1 L amber glass bottles and shipped overnight on ice to the University of South Carolina. Upon arrival, samples were extracted as previously described.[47,52-54] In brief, the samples were acidified to pH <1 with concentrated H_2SO_4 immediately upon arrival and passed through a glass column containing XAD-2 (Amberlite XAD-2, Sigma Aldrich) and DAX-8 (Supelite DAX-8, Sigma Aldrich). The resins were then eluted with ethyl acetate and dried using Na_2SO_4 before concentrating under high-purity nitrogen using a TurboVap® (Biotage, Sweden).

CHO Cell Chronic Cytotoxicity Assay. Chinese hamster ovary (CHO) cells (line AS52, clone 11-4-8) have been widely used to measure mammalian cell cytotoxicity of disinfected water samples.[33,34,43,44,53,55-57] CHO chronic cytotoxicity assays measure the reduction of cell density as a result of exposure to water extracts over 72 h.[32] In brief, CHO cells were exposed to varying concentrations of the sample extract (solvent exchanged into dimethylsulfonate) and a concentration-response curve was constructed. Using the concentration-response curve, cytotoxicity index values (CTI) were calculated for each sample. Further details about this assay can be found in **Appendix A.**

CHO Cell Single Cell Gel Electrophoresis Assay. CHO cells were exposed to sample extracts (solvent exchanged into DMSO) for 4 h at 37 °C with 5% CO_2. The cell suspensions were then transferred to a layer of low melting point agarose and allowed to solidify. Cell membranes were then removed via a lysing solution and each sample was electrophoresed and analyzed using a fluorescence microscope. Using the concentration-response curve generated, genotoxicity index values (GTI) were calculated.[32] Further detail of the SCGE assay is provided in **Appendix A.**

CHO Cell Cytotoxicity and Genotoxicity Statistical Analyses. For each sample extract, analysis of variance (ANOVA) tests were conducted to determine the lowest concentration factor (CF) required to induce a statistically significant reduction in cell density compared to the negative control. Using regression analysis, the LC_{50} was calculated for each water sample concentration-response curve. The average and standard error (SE) were calculated using multiple regression analyses using bootstrap statistics. LC_{50} values were converted to average cytotoxicity index values ($CTI = LC_{50}^{-1} \times 10^3$) so that a comparison among samples could be made easier (higher CTI, higher cytotoxicity). For each sample site, the upstream and downstream CTI values were statistically analyzed using a t-test and if $P \leq 0.001$, then the average CTI value of the two groups (upstream vs. downstream) is greater than would be expected by chance and therefore there is a statistically significant difference between the CTI values for each sample.

Using concentration-response curves and ANOVA tests, the lowest concentration that generated a statically significant reduction in genomic DNA damage was calculated. Multiple regression analyses and bootstrap statistics were used to calculate the average and standard error (SE) for the 50% Tail DNA and the genotoxicity index values (GTI =

50% Tail $DNA^{-1} \times 10^3$). For each sampling site, the upstream and downstream GTI values were compared using a t-test and if $P \leq 0.001$, then the average GTI value of the two groups (upstream vs. downstream) is greater than would be expected by chance and therefore, there is a statistically significant difference between the two samples. For more information, Wagner and Plewa published a detailed overview of the statistical analyses performed on cytotoxicity and genotoxicity data.[32]

DBP Correlation Statistics. Regression analyses were performed to determine if there was a statistically significant correlation between DBP concentrations and CTI or GTI values. A Pearson product-moment (r) was calculated and if $r > 0.90$, then DBP concentration and cytotoxicity or genotoxicity were said to have a very strong, positive correlation. If r was between 0.70 and 0.90, then they were considered to have a strong, positive correlation and if $r < 0.50$, they were considered to have a weak, positive correlation. P values (P) < 0.001, <0.01, and <0.05 were classified as very highly significantly correlated, highly significantly correlated, and significantly correlated, respectively.

Calculated Cytotoxicity and Genotoxicity. When mammalian cell cytotoxicity and genotoxicity testing was not possible, "TIC-Tox" was utilized. "TIC-Tox" is a metric used to calculate cytotoxicity and genotoxicity in order to predict potential toxicity drivers.[49,53,58-63] In brief, "TIC-Tox" estimates cytotoxicity and genotoxicity by multiplying the molar concentration of each DBP by their corresponding cytotoxicity or genotoxicity index values found in literature.[32,60] Assuming that the product of each cytotoxicity and genotoxicity calculation is additive[63], the predicted drivers of cytotoxicity and genotoxicity can be elucidated using eqs 1 and 2.

$$\text{total calculated cytotoxicity} = \Sigma([\text{DBP}] \times LC_{50}^{-1} \times 10^6) \quad (1)$$

$$\text{total calculated genotoxicity} = \Sigma([\text{DBP}] \times 50\%\ TDNA^{-1} \times 10^6) \quad (2)$$

where LC_{50}^{-1} is the inverse of the lethal concentration at 50% in molarity (M) and $TDNA^{-1}$ is the inverse of the 50% tail DNA measurement in molarity (M). Note that haloketones (HKs) are not included in "TIC-Tox" calculations because no cytotoxicity or genotoxicity index values are currently available.

RESULTS AND DISCUSSION

Overall Results for DBPs. Concentrations of the 60 DBPs quantified throughout this study can be found in **Table A.4-A.8.** The average total DBPs quantified in samples collected over the entirety of this project was 0.3, 4.2, and 9.6 µg/L in upstream, downstream, and effluent samples, respectively. All 9 classes of DBPs were detected in at least one downstream and effluent sample, with the most commonly detected DBP in downstream samples being trichloromethane (TCM) and the most commonly detected DBP in effluent samples being trichloroacetaldehyde (TCAL). This finding is consistent with previous studies that show a major degradation product of trichloroacetaldehyde is trichloromethane.[64,65]

In upstream samples, 10% of the DBPs measured were detected at least once, followed by 55% in downstream, and 73% in effluent samples. On average, there was a 2.3-fold decrease in the amount of DBPs present in downstream samples (due to dilution) compared to WWTP effluent, and a 14- and 32-fold increase in downstream and effluent concentrations, respectively, compared to upstream DBP concentrations. The predominant DBP classes quantified in upstream, downstream, and effluent samples were

THMs followed by HAAs. The dominant THM quantified was trichloromethane, with concentrations ranging from ND-0.6 µg/L, ND-3.5 µg/L, and ND-6.7 µg/L in upstream, downstream and effluent samples, respectively. The dominant HAA quantified was trichloroacetic acid (TCAA), with concentrations of ranging from ND-0.5 µg/L, ND-2.9 µg/L, and ND-6.7 µg/L in upstream, downstream and effluent samples, respectively. Although THMs and HAAs were also determined to be the dominant DBP classes in chlorinated WWTP effluent in a previous study[2], levels from this study were substantially lower. For example, Krasner et al. (2009) found that the median concentration of THMs and HAAs in chlorinated WWTP effluents were 57 and 70 µg/L, respectively. This discrepancy between studies could be due to a variety of factors, such as differing amount of dissolved organic carbon (DOC), chlorine contact time, or chlorine quenching differences at the WWTPs (discussed further in Quencher Effects section below).[63]

Haloketones (HKs) were the third most dominant DBP class quantified, with concentrations ranging from ND-0.7 µg/L and ND-1.3 µg/L, respectively, for downstream and effluent samples. The average concentrations of two HKs (1,1-dichloropropanone [11DCP] and 1,1,1-trichloropropanone [111TCP]) in chlorinated effluent in this study (0.5 and 0.3 µg/L, respectively) were lower than levels in previous studies that reported average concentrations of 0.8 and 1.5 µg/L, respectively.[42]

N-DBPs. Despite C-DBPs (THMs, HAAs, HKs, HALs) typically being the predominant DBP classes detected in downstream and effluent samples, the presence of N-DBPs (HANs, HAMs, HNMs) are of concern due their elevated levels of cytotoxicity and genotoxicity.[5,45] The average concentration of N-DBPs in downstream and effluent samples was 1.4 and 4.0 µg/L, respectively. Of the N-DBPs quantified, HANs were the

most frequently detected and occurred at the highest levels, with an average concentration of 0.8 and 2.4 µg/L in downstream and effluent samples, respectively. Of the HANs, dichloroacetonitrile (DCAN) was quantified most frequently and had maximum concentrations of 0.6 and 2.0 µg/L in downstream and effluent samples, respectively. A recent study reported dihalogenated haloacetonitriles (dichloro-, bromochloro-, dibromoacetonitrile) as important cytotoxicity drivers in drinking water.[47] For the remaining N-DBPs, HAMs had the second highest average concentrations in downstream (0.5 µg/L) and effluent (0.6 µg/L) samples, followed by HNMs, with an average effluent concentration of 0.1 µg/L. Low levels of HNMs are consistent with a previous study that found that effluent of WWTPs using free chlorine usually contained levels below 0.7 µg/L.[67]

I-DBPs. As part of our inquiry into DBPs formed in wastewater treatment, we investigated the role of iopamidol (see *Iopamidol Measurements*) as a source of iodine for I-DBP formation. In fact, I-DBPs were found in approximately 50% and 25% of chlorinated WWTP effluents and downstream samples, respectively. Total I-DBP levels ranged from 0.03 – 0.5 µg/L in effluent samples to 0.02 - 0.09 µg/L in downstream samples. In samples where I-THMs were detected above the limit of quantification (LOQ), average concentrations were 0.1 µg/L for both downstream and effluent samples. In samples where IAAs were detected above the LOQ, average concentrations were 0.02 µg/L in downstream samples and 0.05 µg/L in effluent samples.

Iopamidol Measurements. Overall, the average concentration of iopamidol (when present >RL) in effluent samples collected mid-week (Wednesday) was 5.7-fold higher when compared to samples collected on Monday (**Table A.9 and A.10**). For example,

iopamidol was <RL in every upstream, downstream, and effluent sample collected on a Monday (2019) with the exception of the effluent sample from WWTP 1 (1054 ng/L). Samples collected on a Wednesday, however, had an average effluent and downstream concentration of 1694 and 6008 ng/L, respectively. This increase in iopamidol concentrations later in the week is well documented because medical imaging procedures are typically conducted during weekdays.[18,20] This, combined with excretion rates of iopamidol and hydraulic retention time of WWTPs, can account for the increase in iopamidol in effluent and downstream samples when comparing Monday and Wednesday samples.

In 2020, three WWTPs were sampled on Wednesdays and average concentrations of iopamidol were 139, 1250, and 1673 ng/L in upstream, downstream, and effluent samples, respectively. The maximum concentration of iopamidol in effluent samples that year was 3,370 ng/L, more than 3x higher than the maximum effluent concentration detected in 2019. In 2021, eight WWTPs were also sampled on Wednesdays and iopamidol analysis was performed on downstream and effluent samples only. Average downstream and effluent concentrations were 2028 and 9260 ng/L, respectively, with average effluent concentrations of iopamidol driven by WWTP 6, which contained 29,900 ng/L, more than 18x higher than the previously reported maximum concentration in Minnesota.[41]

Quencher Effects. Before releasing treated effluent into the environment, WWTPs are required to dechlorinate (quench) their effluent because residual chlorine can be toxic to aquatic life.[68-70] Additionally, dechlorination prevents further formation of DBPs once the effluent is in the environment. In Minnesota, WWTPs chlorinate effluent from April to

November and have a chlorine residual limit of 0.038 mg/L in treated effluent.[71] In this study, WWTPs used either sodium bisulfite or sulfur dioxide (**Table A.2**), both of which have been shown to degrade DBPs via dehalogenation.[70] A recent study by Pan et al. noted that upon the addition of sodium bisulfite in chlorinated wastewater effluents, concentrations of di-haloacetic acids decreased, likely due to nucleophilic substitution of SO_3^{2-} on the alpha carbon and subsequent loss of halogen.[70] Dehalogenation of brominated and iodinated DBPs could lead to a reduction of toxicity in DBP mixtures, suggesting that dechlorination agents may be a viable option to decrease toxicity of chlorinated effluents.[70] Future studies are needed to examine the impact dechlorination agents have on DBPs in WWTP effluents.

CHO Cell Cytotoxicity and Genotoxicity. Mammalian cell cytotoxicity and genotoxicity results (**Table A.11 and A.12**) reveal that 3 of the downstream samples were statistically ($P < 0.001$) more cytotoxic than their corresponding upstream samples while 4 of the downstream samples were statistically ($P < 0.001$) more genotoxic than their corresponding upstream samples. Interestingly, *in vitro* toxicity assays reveal that 2 of the upstream samples were statistically ($P < 0.001$) more cytotoxic and genotoxic than their corresponding downstream samples. The most cytotoxic samples were from WWTP 2 in 2019, which had CTI values of 56.91 and 45.48 for upstream and downstream samples, respectively. The most genotoxic samples were from WWTP 4 in 2019, with GTI values of 0.182 and 0.847 upstream and downstream, respectively. CHO cell cytotoxicity concentration-response curves (Figure A.1-A.7) and CHO cell genomic DNA damage concentration-response curves (Figure A.8-A.14) are presented in Appendix A.

Although counterintuitive, several factors can account for an upstream sample being more cytotoxic and genotoxic than its corresponding downstream sample, including the presence of upstream contaminant sources like septic systems, stormwater, urban runoff, agricultural runoff, or industrial wastewater that can contribute contaminants such as antioxidants, pesticides, polyaromatic hydrocarbons (PAHs), and other contaminants.[72-75] Ultimately, these upstream anthropogenic contaminants can be significantly diluted by WWTP effluents released into the rivers, resulting in a less toxic downstream sample. For example, a recent study revealed that exposure to untreated stormwater containing a highly toxic quinone transformation product of *N*-(1,3-dimethylbutyl)-*N*′-phenyl-*p*-phenylenediamine (6PPD), a widely used tire rubber antioxidant, resulted in an increase in toxicity and mortality of adult coho salmon.[76] In addition, a stormwater study from Minnesota quantified 123 contaminants, with individual median concentrations ranging up to 900 ng/L.[73] Many of the samples contained PAHs, which are commonly present in stormwater and are genotoxic and/or carcinogenic in animal studies.[77-80] Thus, the dilution of upstream contaminants by WWTP effluents is a possible explanation for the decreased downstream toxicity at these two locations.

Cytotoxicity Correlations. For downstream samples, total N-DBPs and cytotoxicity demonstrated a very strong, positive correlation (Figure 2.1, Figure 2.3; $r = 0.99$, $P < 0.01$). Of these N-DBPs, HANs and HAMs also had very strong, positive correlations (Figure 2.3; $r = 0.99$, $P < 0.01$) with cytotoxicity. These results are consistent with higher cytotoxicity of N-DBPs compared to C-DBPs[3,5] and a previous report showing that N-

DBPs, particularly HANs, substantially contribute to additive cytotoxicity of chloraminated wastewater effluent organic matter.[81]

On the other hand, total DBPs in downstream samples did not correlate with cytotoxicity (Figure 2.3; $r = 0.51$, $P > 0.05$). Similarly, downstream concentrations of HALs and HKs did not significantly correlate with cytotoxicity (Figure 2.3; r = 0.20, $P > 0.05$).

Genotoxicity Correlations. HKs in downstream samples (excluding WWTP 3 from 2020) and genotoxicity showed a very strong, positive correlation (Figure 2.2, Figure 2.4, $r = 0.94$, $P < 0.05$). While there is no published data yet for genotoxicity of haloketones in CHO cells, haloketones were reported to be genotoxic to *E. coli*.[82] No significant correlation was observed for total DBPs, C-DBPs, or N-DBPs with genotoxicity in downstream samples (Figure 2.4, $P > 0.05$).

Calculated Cytotoxicity and Genotoxicity (2021). Because real toxicity measurements were not conducted for samples collected in 2021, calculated toxicity (also called "TIC-Tox")[60] was determined based on the 60 DBPs quantified. N-DBPs were the main contributors to calculated cytotoxicity in both downstream and effluent samples, contributing 54% and 48%, respectively, despite C-DBPs being the predominant classes formed (96% downstream and effluent). Of the N-DBPs, HANs were the most dominant contributors to calculated cytotoxicity in both downstream and effluent samples, contributing 54% and 46%, respectively. Dichloroacetonitrile (DCAN) was detected in 50% of the downstream and effluent samples collected and contributed 94% and 86% of the calculated cytotoxicity, respectively.

C-DBPs were the main contributors to calculated genotoxicity in downstream samples, with HAAs alone accounting for 48% of the calculated genotoxicity. In effluent samples, HNMs contributed 34% of the calculated genotoxicity while IAAs (<1%) contributed 10% of the calculated cytotoxicity and 32% of the calculated genotoxicity in downstream samples. Further, IAAs were a substantial driver of both calculated cytotoxicity and genotoxicity in effluent samples, contributing 16% and 33%, respectively. This finding is consistent with the high genotoxic potency of IAA.[30,32,55]

Overall, calculated cytotoxicity increased more than 400-fold and 210-fold in effluent and downstream samples compared to upstream. Likewise, calculated genotoxicity increased more than 2000-fold and 720-fold in effluent and downstream samples when compared to upstream samples. This increase in calculated cytotoxicity and genotoxicity was primarily driven by the presence of haloacetic acids, N-DBPs (particularly HANs), and iodoacetic acid.

Implications. Studying the formation of DBPs at WWTPs and identifying their potential precursors is important to understand and predict the toxicity of treated wastewater and its impacts on the aquatic ecosystem. This research combines sensitive analytical methods and *in vitro* cytotoxicity and genotoxicity assays to measure the largest number of DBPs in chlorinated WWTP effluent. The results demonstrate a large number of these DBPs exist in WWTP effluent and are drivers of aquatic toxicity. Data from this study also reveals that the predominant classes of DBPs present in chlorinated effluent and downstream samples are THMs, followed by HAAs, and haloketones. In addition, iopamidol, an X-ray contrast media found up to 29,900 ng/L in these WWTP effluents, may be an important precursor in the formation of I-DBPs observed in the chlorinated

effluents. Further, *in vitro* cytotoxicity assays reveal a strong, positive correlation between the levels of total N-DBPs and cytotoxicity in chlorinated effluent, while *in vitro* genotoxicity assays reveal a strong, positive correlation between haloketones and genotoxicity in chlorinated effluent. Calculated cytotoxicity and genotoxicity was driven by DBPs present at higher concentrations (i.e., HAAs) and by classes present at lower concentrations (i.e., haloacetonitriles and iodoacetic acids). These findings demonstrate that WWTPs contribute a significant amount of DBPs to receiving bodies of water and that analysis of DBPs released into the environment will aid in determining the toxicity drivers in the aquatic ecosystem.

Future research into understanding the WWTP conditions that limit N-DBP and I-DBP formation is crucial to limit their impact on aquatic ecosystems. Additionally, future research should study the impact that common dechlorination agents (e.g., sodium sulfite, sodium bisulfite) have on the stability of a variety of DBPs in these effluents, along with their potential transformation products and associated toxicities.

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Liberatore's participation in this study was done solely at the University of South Carolina prior to her employment by the U.S. EPA.

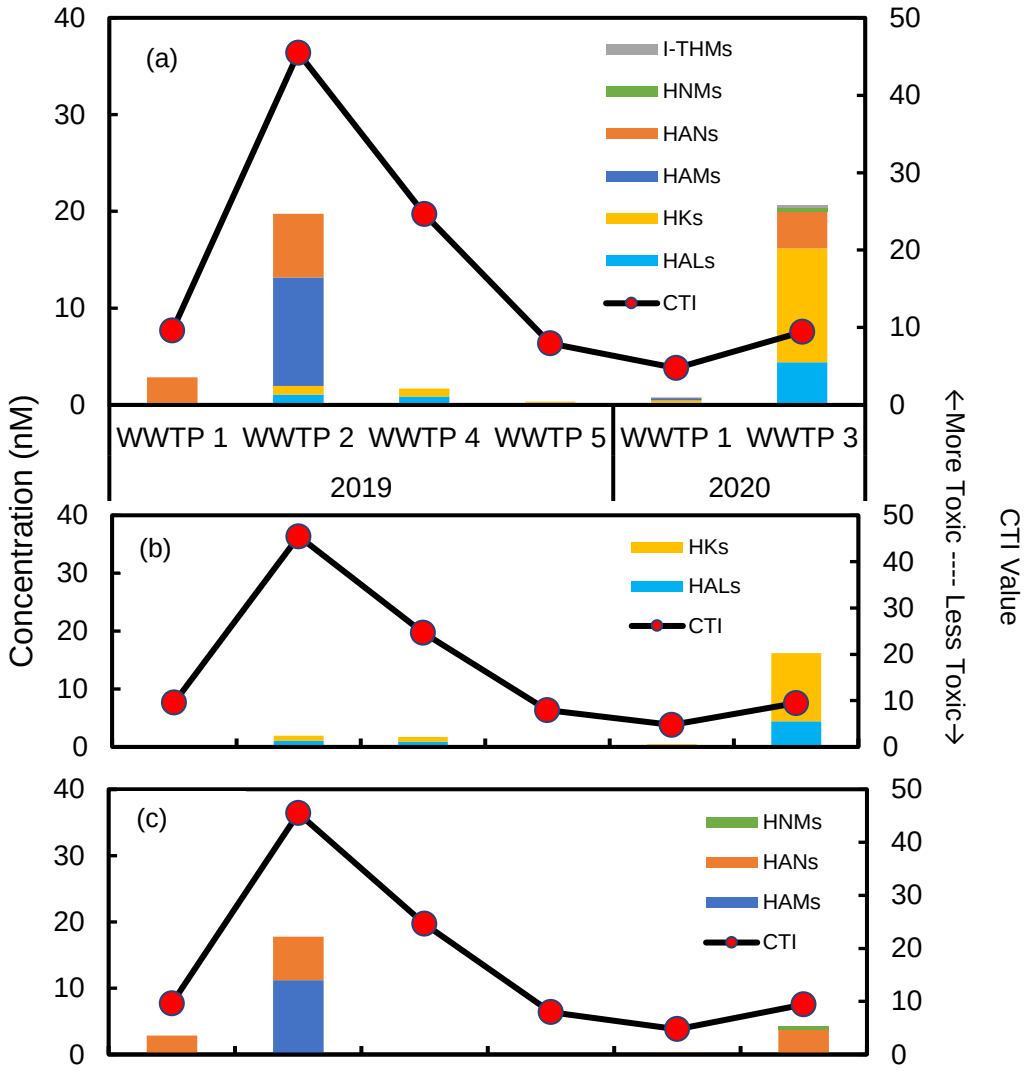

Figure 2.1 (a) Total DBPs, (b) total unregulated carbonaceous DBPs, and (c) total unregulated nitrogenous DBPs in downstream samples along with a line plot of cytotoxicity index values (CTI; LC_{50}^{-1} x 10^3).

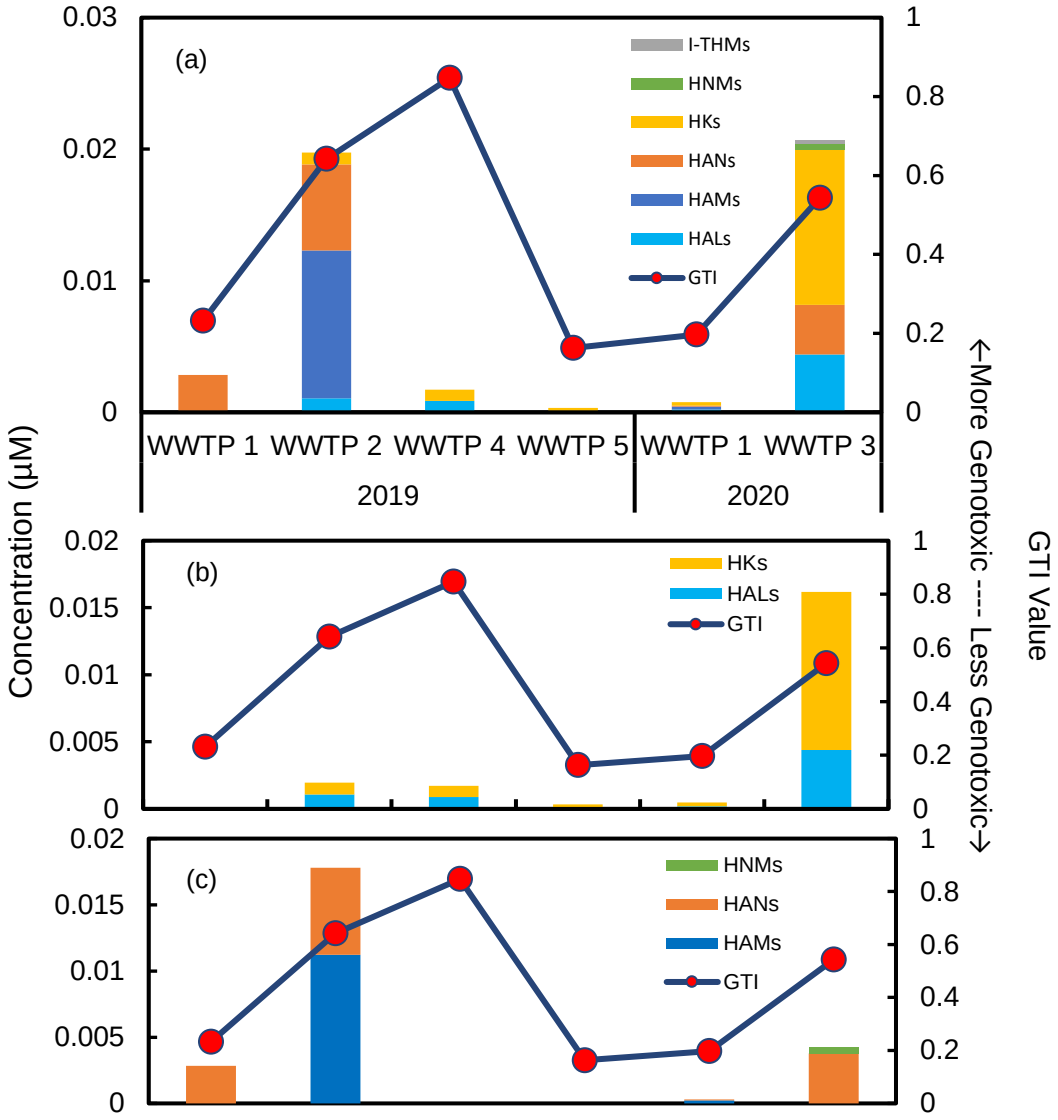

Figure 2.2 (a) Total DBPs, (b) total unregulated carbonaceous DBPs, and (c) total unregulated nitrogenous DBPs in downstream samples along with a line plot of genotoxicity index values (GTI = 50% Tail DNA^{-1} x 10^3).

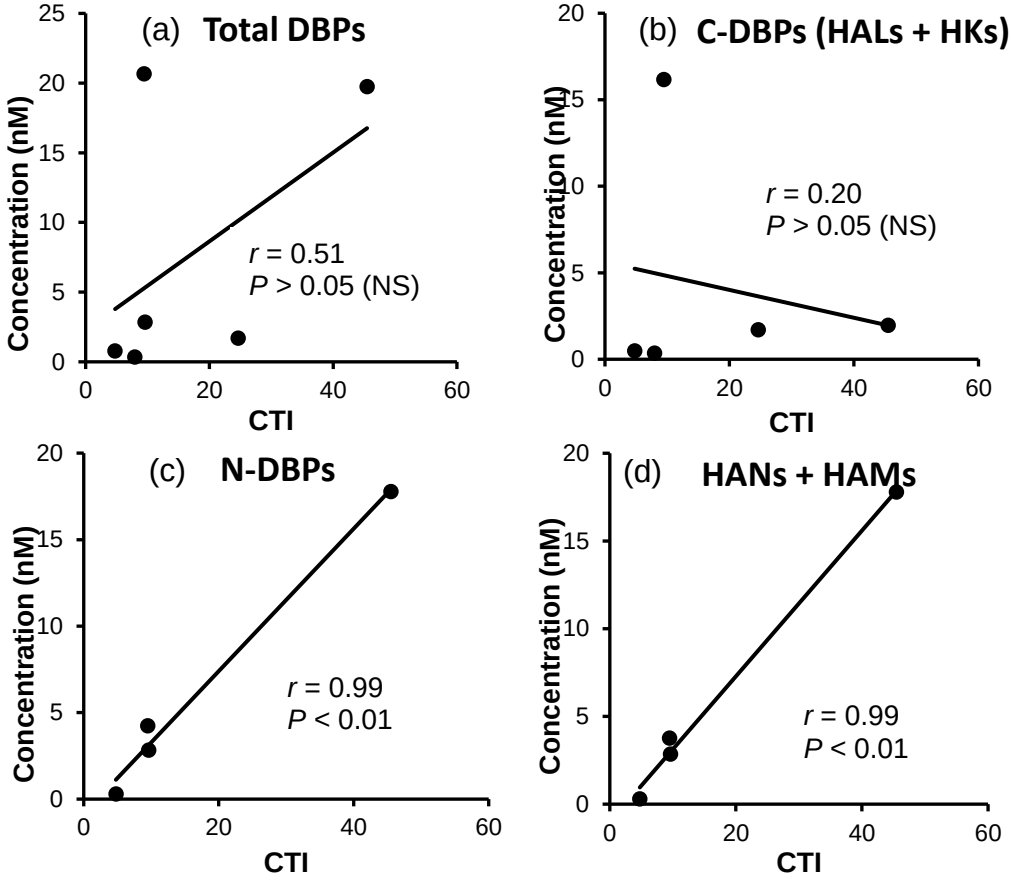

Figure 2.3 Correlation of mammalian cell cytotoxicity in downstream samples with (a) total DBPs, (b) summed carbonaceous DBPs, (c) summed nitrogenous DBPs, and (d) summed haloacetonitriles and haloacetamides. NS = not significant. Samples with NDs are excluded. Mammalian cell cytotoxicity index (CTI) values are defined as the LC_{50}^{-1} (10^3).

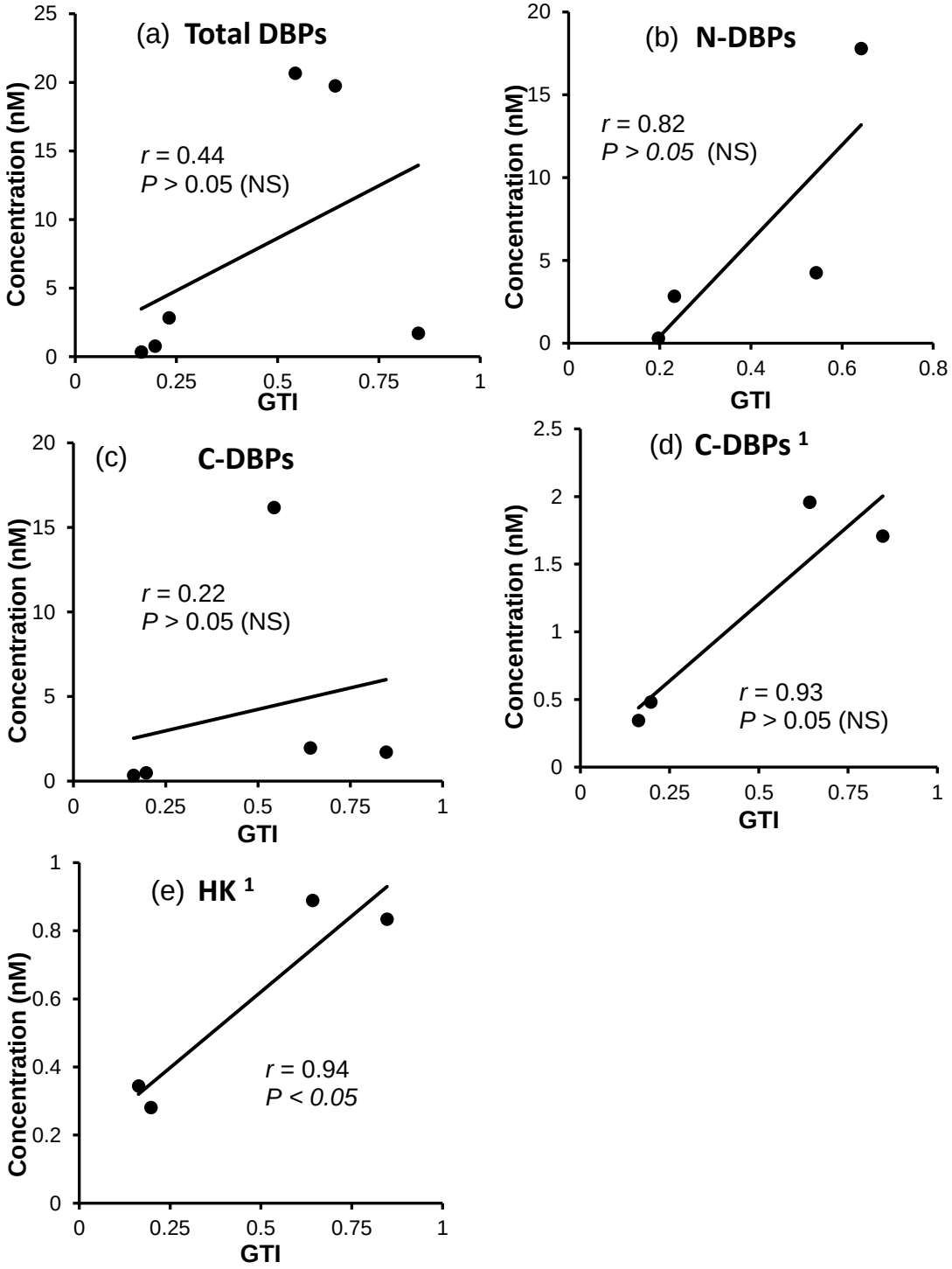

Figure 2.4 Correlation of genotoxicity in downstream samples with (a) total DBPs, (b) summed nitrogenous DBPs, (c) summed carbonaceous DBPs, (d) summed haloketones and haloacetaldehydes, and (e) summed haloketones. NS = not significant. Samples with NDs are excluded. [1] WWTP 3 from 2020 not included. Genotoxicity index (GTI) values are defined as the 50% Tail DNA^{-1} (10^3).

CHAPTER 3

DO DBPS SWIM IN SALT WATER POOLS? COMPARISON OF 60 DBPS FORMED BY ELECTROCHEMICALLY GENERATED CHLORINE VS. CONVENTIONAL CHLORINE

[2]Granger, C.O.; Richardson, S.D. Do DBPs Swim in Salt Water Pools? Comparison of 60 DBPs Formed by Electrochemically Generated Chlorine vs. Conventional Chlorine. In Press at *J. Environ. Sci.*

ABSTRACT

Disinfectants are added to swimming pools to kill harmful pathogens. Although liquid chlorine (sodium hypochlorite) is the most commonly used disinfectant, alternative disinfection techniques like electrochemically generated mixed oxidants or electrochemically generated chlorine, often referred to as salt water pools, are growing in popularity. However, these disinfectants react with natural organic matter and anthropogenic contaminants introduced to the pool water by swimmers to form disinfection byproducts (DBPs). DBPs have been linked to several adverse health effects, such as bladder cancer, adverse birth outcomes, and asthma. In this study, we quantified 60 DBPs using gas chromatography-mass spectrometry and assessed the calculated cytotoxicity and genotoxicity of an indoor community swimming pool before and after switching to a salt water pool with electrochemically generated chlorine. Interestingly, the total DBPs increased by 15% upon implementation of the salt water pool, but the calculated cytotoxicity and genotoxicity decreased by 45% and 15%, respectively. Predominant DBP classes formed were haloacetic acids, with trichloroacetic acid and dichloroacetic acid contributing 57% of the average total DBPs formed. Haloacetonitriles, haloacetic acids, and haloacetaldehydes were the primary drivers of calculated cytotoxicity, and haloacetic acids were the primary driver of calculated genotoxicity. Diiodoacetic acid, a highly toxic iodinated DBP, is reported for the first time in swimming pool water. Bromide impurities in sodium chloride used to electrochemically generate chlorine led to a 73% increase in brominated DBPs, primarily driven by bromochloroacetic acid. This study presents the most extensive DBP study to-date for salt water pools.

INTRODUCTION

In the United States, swimming is the fourth most popular recreational activity.[83] To limit swimmers' exposure to harmful viruses, bacteria, fungi, and algae, swimming pools are treated with disinfectants like chlorine, bromine, ultraviolet radiation (UV), or ozone.[84] However, these disinfectants react with natural organic matter (NOM) and anthropogenic contaminants introduced to the pool water by swimmers to form disinfection byproducts (DBPs).[53,85-87] Several epidemiologic studies have linked DBPs to bladder cancer, birth defects, miscarriage, and respiratory issues such as asthma.[5,11,88-95] Furthermore, studies have shown that dermal exposure to halogenated DBPs is an important exposure route to consider in swimming pool studies, due to the permeability of some DBPs across the skin.[96,97]

Studies quantifying DBPs in swimming pools have primarily focused on trihalomethanes (THMs) and haloacetic acids (HAAs). However, with more than 700 DBPs identified to date, many of which are more cytotoxic, genotoxic, or carcinogenic than THMs and HAAs, there is a clear need for the expansion of the classes of DBPs quantified in swimming pools. In recent years, brominated and iodinated DBPs have become of particular interest due to their elevated levels of toxicity when compared to chlorinated DBPs.[5,32,34] Additionally, nitrogenous DBPs (N-DBPs) are generally more toxic than carbonaceous DBPs (C-DBPs).[44] Recent studies completed in the United States and Australia have expanded on the DBPs quantified in pools using a variety of disinfection techniques to include priority, unregulated DBPs such as iodinated trihalomethanes (I-THMs), iodoacetic acids (IAAs), haloacetaldehydes (HALs), haloketones (HKs), haloacetamides (HAMs), haloacetonitriles (HANs), and

halonitromethanes (HNMs).[53,62] Despite chlorine being the most commonly used disinfectant in swimming pools around the world, alternative disinfection techniques are becoming more common. One such alternative disinfection technique is electrochemically generated chlorine (or salt water pools) which works by passing an electric current through a concentrated salt solution (sodium chloride) to produce hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) as the primary oxidants.[98] Previous studies have shown that when compared to pools disinfected with chlorine, salt water pools had lower levels of HAAs (dichloroacetic acid and trichloroacetic acid) and trichloroacetaldehyde, but higher levels of Br-DBPs, likely due to bromide impurities in the sodium chloride.[99,100] This increase in bromide is an important factor to monitor because previous studies show that dichloroacetic acid and trichloroacetic acid do not significantly contribute to the cytotoxicity or genotoxicity of pool waters.[101] However, there is currently no comprehensive DBP or toxicity data available that provides a direct comparison between conventional chlorine and salt water pools. To address this, a study of 60 DBPs was conducted at an indoor community pool in South Carolina while the pool was disinfecting with conventional liquid chlorine (sodium hypochlorite) and then after the implementation of electrochemically generated chlorine technology (salt water pools). Using DBP data collected over three sampling events, the calculated cytotoxicity and genotoxicity associated with each disinfection type was determined to better understand the impact each disinfection technology has on overall calculated toxicity, as well as the drivers of toxicity for each disinfection technique.

MATERIALS AND METHODS

Swimming pool sampling. Swimming pool samples were collected from an indoor community pool in South Carolina with an estimated total volume of 263,300 liters. This pool was chosen to study due to its consistent bather load (approximately 24 swimmers/day) throughout the year. The first sampling event occurred in May of 2021 when the swimming pool was using sodium hypochlorite (conventional chlorine) to disinfect the swimming pool. Two additional pool samples (November 2021 and January 2022) were collected after the implementation of an electrochemically generated chlorine system (Hayward Saline C 11.0 Commercial Salt Chlorine Generator; Rockville, MD) with a flow rate of 150 gallons per minute and a power supply rated to supply 72 amps. Additional details about the electrochemically generated chlorine system can be found in **Table B.1**.

Samples were collected in 1 L amber glass bottles, quenched with ammonium chloride, acidified to pH 3.5-4 with 1 M sulfuric acid (for sample preservation), and filled headspace free. Samples were then shipped overnight on ice to the University of South Carolina and extracted immediately upon arrival. Further sample details can be found in **Table 3.1**.

Chemical and reagents. All solvents (methanol, metyl *tert*-butyl ether, acetonitrile, ethyl acetate) were of the highest purity and were purchased from Sigma-Aldrich (St. Louis, MO) or VWR International (Radnor, PA). General reagents were of ACS reagent grade and were purchased from Sigma-Aldrich and Fisher Scientific (Waltham, MA). DBP standards were purchased from CanSyn Chem. Corp. (Toronto, ON), Sigma-Aldrich, Aldlab Chemicals (Woburn, MA), and TCI America (Waltham, MA) at the highest

purity. Specific vendor information can be found in **Appendix A**. The internal standard, 1,2-dibromopropane, along with the diazomethane derivatization reagents (Diazald, CARBITOL™) were purchased from Sigma-Aldrich.

DBP analysis. Quantification of 60 DBPs was performed in triplicate as described previously.[46-49,53] In brief, 100 mL of a sample was placed into a 125 mL amber bottle and acidified to pH <1 with concentrated sulfuric acid. Then, 5 mL of methyl *tert*-butyl ether was added to each sample along with 30 g of sodium sulfate. Samples were then shaken for 15 min, allowed to settle for 10 min, and the top organic layer was removed and placed into a test tube. This procedure was completed 2 more times for a total extract volume of 15 mL. The organic extract was then dried using sodium sulfate and concentrated to 200 µL using a gentle stream of nitrogen. The concentrated extract was spiked with 4 µL of an internal standard (1,2-dibromopropane) and split into two equal aliquots. The first aliquot was used to analyze for 4 trihalomethanes (THMs), 9 haloketones (HKs), 4 haloacetaldehyde (HALs), 4 halonitromethanes (HNMs), 7 haloacetonitriles (HANs), 13 haloacetamides (HAMs), and 6 iodinated trihalomethanes (I-THMs).

The second aliquot was derivatized using diazomethane for the analysis of 9 haloacetic acids (HAAs) and 4 iodoacetic acids (IAAs). Diazomethane derivatization converts carboxylic acids to methyl esters for analysis by gas chromatography (GC)-mass spectrometry (MS). The diazomethane derivatization was conducted as described by the U.S. Environmental Protection Agency Standard Operating Procedure.[50] In brief, 0.367 g of Diazald and 1.0 mL of CARBITOL™ were placed inside the inner tube of a diazomethane generator. Then, 3.0 mL of methyl *tert*-butyl ether was placed in the outer

tube of the generator, and the entire generator was placed in ice. Once on ice, 1.5 mL of 37% potassium hydroxide was added slowly (dropwise) to the inner tube and allowed to react for 1 hr. After 1 hr, 50 µL of diazomethane (dissolved in methyl *tert*-butyl ether) was spiked into a 100 µL organic extract aliquot and allowed to react for 30 min. After 30 min, the reaction was quenched with 10 mg of silica gel and transferred to new vials before analysis.

GC-MS analysis. Both extracts were analyzed using a gas chromatograph-mass spectrometer (Agilent 7890 GC, 5977A mass spectrometer, Agilent Technologies, Santa Clara, CA) with electron ionization (EI) at 70 eV in selection ion monitoring (SIM) mode. Sample extracts (1.0 µL) were injected into a multimode inlet (MMI) in pulsed splitless mode. Analytes were chromatographically separated using a Restek Rtx-200 column (30 m x 0.25 mm x 0.25 µm film thickness; Restek Corporation, Bellefonte, PA). This column provides improved separation and detection limits for iodo-THMs and haloacetamides, which tend to tail and give lower responses using a DB-5 column.[46] The GC temperature program for the analysis of the 4 THMs, 9 HKs, 4 HALs, 4 HNMs, 7 HANs, 13 HAMs, 6 I-THMs was as follows: initial temperature of 35 °C for 5 min, increased to 220 °C at 9 °C/min, ramped at 20 °C/min to 280 °C, and held for 15 min. The GC temperature program for the analysis of the 9 HAAs and 4 IAAs was as follows: initial temperature held at 35 °C for 5 min, increased to 280 °C at 9 °C/min, and held for 15 min. Both methods held the transfer line at 280 °C and source temperature at 200 °C. Quantifier and qualifier ions along with limits of quantification (LOQ) for each DBP are listed in **Appendix B.**

Bromide and iodide measurements. To quantify the amount of bromide and iodide present in the sodium chloride used for salt water pools, a solid sodium chloride sample used at this community pool was collected and dissolved in ultrapure water for analysis. Bromide and iodide were quantified via a Dionex Integrion high performance ion chromatography (HPIC) system (Sunnyvale, CA) with an IonPac AS20 guard column and an IonPac AS20 analytical column. The system included a 500 µL sample loop and 50 mM NaOH as the eluent. An external calibration curve was prepared in ultrapure water (1, 5, 10, 20, and 30 µg/L) using sodium bromide and sodium iodide. The limits of quantification (LOQs) for both bromide and iodide are 1.0 µg/L.

"TIC-Tox": Calculated cytotoxicity and genotoxicity. "TIC-Tox" is a metric previously used in several studies to calculate cytotoxicity and genotoxicity of water samples and predict the drivers of toxicity.[49,53,58-62] In brief, "TIC-Tox" calculates cytotoxicity and genotoxicity by multiplying the molar concentration of each individual DBP by their corresponding cytotoxicity or genotoxicity index values for Chinese hamster ovary (CHO) cells reported in literature.[32,60] Each product is then multiplied by a normalization factor (10^6) and summed together (eqs 1 and 2).

$$\text{total calculated cytotoxicity} = \Sigma([\text{DBP}] \times \text{LC}_{50}^{-1} \times 10^6) \quad (1)$$

$$\text{total calculated genotoxicity} = \Sigma([\text{DBP}] \times 50\%\ \text{TDNA}^{-1} \times 10^6) \quad (2)$$

where LC_{50}^{-1} is the inverse of the lethal concentration at 50% in molarity (M) and TDNA^{-1} is the inverse of the 50% tail DNA measurement in molarity (M). "TIC-Tox" assumes that the toxicity of individual DBPs is additive, an assumption shown to be accurate in a recently published study.[63] Note that haloketones (HKs) are not included in

"TIC-Tox" calculations because there are no cytotoxicity or genotoxicity index values currently available in the literature. Additional details about 'TIC-Tox" and the determination of cytotoxicity and genotoxicity index values can be found in previous studies.[32,60,102-105]

RESULTS AND DISCUSSION

Overall findings. Upon implementation of an electrochemically generated chlorine system, there was a 15% increase in average total DBPs compared to the conventionally chlorinated pool sample. Of the 60 DBPs measured in this study, 68% were detected at least once. **Table 3.2** shows the 60 DBPs quantified during each sampling event. The dominant DBP classes quantified were haloacetic acids (HAAs), followed by haloacetaldehydes (HALs), and trihalomethanes (THMs) (**Figure 3.1**). HAAs, which accounted for 63% of the average total DBPs present, are known to accumulate in pools due to their lack of volatility.[53,87,106,107] Total HAA concentrations ranged from 1066 to 2425 µg/L and were dominated by Cl-HAAs (sum of chloroacetic acid, dichloroaceitc acid, and trichloroacetic acid). Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) were the dominant HAAs detected with an average concentration of 1332 and 277 µg/L, respectively. This finding matches well with previously published data from our lab in which another indoor pool had DCAA and TCAA at levels as high as 1230 and 275 µg/L, respectively.[53] Of the haloacetaldehydes quantified in this study, trichloroacetaldehyde (TCAL) was the most commonly detected, and accounted for 19% of the average total DBPs formed. Trichloromethane (TCM) was the second most abundant DBP quantified with an average concentration of 447 µg/L. Interestingly, the January salt water pool sample contained the highest level of total DBPs (3251 µg/L)

despite having the lowest residual chlorine measured (**Table 3.1**). Prior to the January sampling, an exercise class was offered, thus the bather load was higher compared to the other two sampling events (**Table 3.1**). An increase in turbulence in the pool prior to sampling resulted in lower THMs levels (260 µg/L) but higher levels of non-volatile DBPs, particularly HAAs (2425 µg/L). This finding matches well with previous studies that noted as the turbulence in the swimming pool increases, so does the THM concentration in air samples collected at indoor pools,[108,109] thus, decreasing THM levels in the water.

Iodinated trihalomethanes (I-THMs), iodoacetic acids (IAAs), and halonitromethanes (HNMs) were present at the lowest levels. On average, these classes represented <1% of the average DBPs present in all pool samples. Trichloronitromethane (TCNM) was the most frequently detected HNM in pool samples, with average levels ranging from 2.1 µg/L in the salt water pool samples to 4.6 µg/L in the conventional chlorinated pool sample. The only I-THM detected in this study was bromodiiodomethane (BDIM), which was found in the conventional chlorinated pool sample at 0.5 µg/L. Iodoacetic acid (IAA) was also detected in the conventional chlorinated pool at 0.2 µg/L and diiodoacetic acid (DIAA) and chloroiodoacetic acid (CIAA) were detected in the January salt water pool sample, both at 0.3 µg/L. This is the first report of DIAA in swimming pool water and is a significant finding due to its elevated level of toxicity. For example, DIAA is 1.8x more cytotoxic than DBAA. Ion chromatography analysis of the salt used in the salt water pool revealed that iodide was not present as an impurity, suggesting that the presence of the I-DBPs was the result of iodide in disinfected source water used to fill the pool.

Conventional chlorine vs. salt water: C-DBPs

Haloacetic acids (HAAs). The 15% increase in the average total DBPs in the salt water pool samples was driven by HAAs. The January salt water pool sample had the lowest residual chlorine but the highest bather load prior to sampling. This overall increase in HAAs was driven by the January sample which had the lowest residual chlorine but the highest bather load prior to sampling. This increase in DBP formation was driven by dichloroacetic acid and trichloroacetic acid, which saw a 124% and 25% increase, respectively. Dichloroacetic acid accounted for 69% (730 µg/L) of the HAAs formed in the conventional chlorine pool and 77% (1633 µg/L) of the HAAs present in the salt water pool, a 124% increase. Trichloroacetic acid accounted for 22% (238 µg/L) of HAAs present in the conventional chlorine pool sample and 14% (297 µg/L) in salt water pool samples, a 25% increase. A previous study noted a similar increasing trend when comparing levels of dichloroacetic acid (196% increase) and trichloroacetic (229% increase) in conventional chlorine and salt water pools.[101]

Haloacetaldehydes (HALs). The concentration of trichloroacetaldehyde (TCAL) was 580 µg/L in the conventional chlorine pool and 490 µg/L on average in the salt water pool, a 16% decrease. Lee et al. (2010) also noted a decrease (40%) in the formation of trichloroacetaldehyde between conventional chlorine and salt water pools.[100] This decrease in trichloroacetaldehyde is an important finding when considering previous studies have cited trichloroacetaldehyde as the primary driver of calculated chronic cytotoxicity in pools.[62]

Trihalomethanes (THMs). Trichloromethane concentrations in the conventional chlorine and salt water pool samples were 764 µg/L and 288 µg/L, respectively. Notably,

764 µg/L of trichloromethane in the conventional chlorinated indoor pool is the third highest reported level of trichloromethane in the literature, second to only 980 µg/L reported in a study conducted by Lahl et al. (1981).[110] Elevated levels of trichloromethane in indoor pools underlines the importance of maintaining a low residual chlorine and having adequate ventilation in indoor pools to decrease swimmers' exposure to volatile DBPs via inhalation[12], especially when there is an increase in bather load like during the January sampling.

Haloketones (HKs). The average concentration of haloketones (HKs) decreased by 76% in salt water pool samples (8.4 µg/L) compared to the conventional chlorine pool sample (34.5 µg/L). Of the 8 HKs detected in one or more pool samples, 7 of them decreased in concentration, ranging from a 10% decrease to a 100% decrease, with the exception of 1,1,3,3-tetrabromopropanone (1133TeBP), which was not detected in conventional chlorinated waters, but had an average concentration of 2.4 µg/L in salt water pool samples. The formation of 1133TeBP indicates the presence of a bromide impurity in the salt used in the salt water pool, which would lead to the formation of Br-DBPs. Further discussion of bromide levels and resulting Br-DBPs can be found in a following section (Br-DBPs in pool samples).

Conventional chlorine vs. salt water: N-DBPs

Haloacetamides (HAMs). This study presents the first report of the quantification of two haloacetamides (dichloroacetamide and bromodichloroacetamide) in a salt water pool. Of the 13 HAMs quantified in this study, only 3 were detected above the limit of quantification. Of those, trichloroacetamide (TCAM) was quantified at the highest level, with an average concentration of 23.0 µg/L, followed by dichloroacetamide (DCAM) at

8.3 µg/L and bromodichloroacetamide (BDCAM) at 1.3 µg/L. For trichloroacetamide and dichloroacetamide, maximum concentrations occurred in the conventional chlorinated pool water at 37.0 µg/L and 21.5 µg/L, respectively. The maximum concentration of bromodichloroacetamide occurred in the November salt water pool sampling event at 1.9 µg/L. On average, salt water pool samples showed a decrease in trichloroacetamide (57%), dichloroacetamide (92%), and bromodichloroacetamide (17%) when compared to the conventional chlorinated pool sample.

Haloacetonitriles (HANs). Dichloroacetonitirle (DCAN) and chloroacetonitrile (CAN) were present at the highest level of all HANs quantified in this study, with an average concentration of 4.9 µg/L and 3.4 µg/L, respectively. HANs in salt water pool samples consistently decreased when compared to conventional chlorine pool samples, with the exception of bromochloroacetonitrile (BCAN) and dibromoacetonitrile (DBAN), likely due to the presence of bromide in the salt used in the salt water pool.

Br-DBPs in pool samples. Brominated DBPs (Br-DBPs) are of interest due to their elevated levels of toxicity compared to their chlorinated analogues.[5,32] Previous salt water pool studies, which measured a smaller number of DBPs, have attributed the formation of Br-THMs, Br-HAAs, and Br-HANs to bromide present in sodium chloride and emphasized the importance of using high purity sodium chloride.[99,100,111,112] Ion chromatography analysis of the sodium chloride used in the salt water pool in this study revealed that the salt contained approximately 0.05% bromide. Assuming the salinity of salt water pools are typically maintained around 3,000 to 5,000 mg/L, at 4,000 mg/L salinity, a 0.05% bromide impurity will contribute approximately 118 µg/L of bromide to the pool. This impurity is a significant contribution to the bromide levels in this pool,

considering that the tap water in the city where the community pool is located only contained 22 µg/L of bromide. Overall, the switch to a salt water pool led to a 73% increase in Br-DBPs, primarily driven by bromochloroacetic acid (BCAA), which saw a 268% increase to an average of 54.9 µg/L.

Calculated cytotoxicity. Calculated cytotoxicity decreased by 45% after implementation of the salt water pool system. Overall, the calculated cytotoxicity in pool samples was driven by HANs, HAAs, and HALs, which accounted for 34%, 30%, and 26%, respectively, of the average calculated cytotoxicity in conventional chlorinated and salt water pool samples combined (**Figure 3.2**). In pool waters disinfected with conventional chlorine, the calculated cytotoxicity was driven by HANs (53%), followed by HALs (19%) and HAAs (17%). The 45% decrease in calculated cytotoxicity of salt water pools was primarily driven by HANs. Although the concentration of BCAN and DBAN increased in salt water pools, the concentration of BAN increased in conventional chlorine pool samples compared to salt water pool samples (3.1 µg/L and ND, respectively).Therefore, despite the overall increase in Br-DBPs upon implementing the electrochemically generated chlorine system, an increase in the formation of BAN (contributing 0.1% of the total DBPs formed) in conventional chlorine pools resulted in a substantial increase in calculated cytotoxicity and accounted for 40% of the calculated cytotoxicity in the conventional chlorine sample.

Trichloroacetaldehyde, despite being the least cytotoxic haloacetaldehyde quantified, contributed 22% of the average calculated cytotoxicity of all pool samples. In the conventional chlorinated pool sample, trichloroacetaldehyde accounted for 17% of the total calculated cytotoxicity and 26% in the salt water pool samples. Although to a

lesser degree, this finding is consistent with a previous study in which trichloroacetaldehyde was cited as a significant driver of calculated cytotoxicity in swimming pools (Carter et al., 2019). Unlike HANs and HALs, HAAs did not have a clear driver of cytotoxicity in conventional chlorinated pool samples, but was driven by several HAAs like chloroacetic acid (4%), bromoacetic acid (5%), dichloroacetic acid (4%), and trichloroacetic acid (3%).

In salt water pools, the calculated cytotoxicity was driven by HAAs (41%), HALs (33%), and HANs (17%). On average, dichloroacetic acid contributed 39% to the calculated cytotoxicity for HAAs despite contributing 77% of the HAAs detected. Chloroacetic acid, which contributed 31% of the calculated cytotoxicity of the HAAs, only accounted for 5% of the average HAAs formed. Trichloroacetaldehyde contributed to 26% of the total calculated cytotoxicity but only 17% of the average total DBPs in salt water pool samples. Dichloroacetonitrile (7%) and dibromoacetonitrile (4%) were the primary HANs contributing to calculated cytotoxicity in salt water pool samples. The cases described above further showcase the importance of utilizing "TIC-Tox" to determine drivers of calculated cytotoxicity rather than inferring toxicity based on total DBP concentrations.

All classes of DBPs saw a decrease in calculated cytotoxicity when comparing the conventional chlorine pool to the salt water pool, with the exception of HAAs that saw an increase of 31%. When compared to conventional chlorinated pool samples, Br-HAAs and Br/Cl-HAAs were major contributors to the increase in calculated cytotoxicity. The calculated cytotoxicity contributed by bromochloroacetic acid and dibromoacetic acid saw a 268% and 250% increase, respectively, in the salt water pool. Interestingly,

bromoacetic acid was not detected in salt water pool samples but was present at low levels (1.2 µg/L) in the conventional chlorinated pool sample and contributed 5% of the total calculated cytotoxicity of that sample. Chloroacetic acid, dichloroacetic acid, and trichloroacetic acid also saw an increase in calculated cytotoxicity (56%, 124%, and 25%, respectively) when compared to the conventional chlorine pool sample, likely due to the increase in bather load prior to the January sampling event (**Table 3.1**).

Calculated genotoxicity. Calculated genotoxicity decreased by 15% upon implementation of a salt water system. Primary drivers of calculated genotoxicity were HAAs, which accounted for 80% of the average calculated genotoxicity in all pool samples. The calculated genotoxicity of the conventional chlorine pool samples was driven by a combination of HAAs (62%) and HANs (23%). Chloroacetic acid (47%) and bromoacetic acid (14%) were the main contributors to calculated genotoxicity (**Figure 3.3**), despite only contributing 3% and <1% of the total DBPs in the conventional chlorine pool sample, respectively. Like with calculated cytotoxicity, bromoacetonitrile (18%) was also the primary driver of calculated genotoxicity in the conventional chlorine pool sample, despite contributing <1% of the total DBPs formed. Chloroacetic acid (86%) was the calculated genotoxicity driver in salt water pools samples, despite only contributing 4% of the total DBPs formed.

All classes of DBPs saw a decrease in calculated genotoxicity in salt water pool samples when compared to the conventional chlorinated pool, with the exception of HALs and HAAs, which saw an increase of 89% and 24%, respectively. IAAs (99%), HANs (89%), and HNMs (59%) saw the largest percent reduction in calculated genotoxicity, but were only responsible for 7% of the total calculated genotoxicity in the

salt water pool. Bromochloroacetic acid and dibromoacetic acid saw the largest increase in calculated genotoxicity, with a 268% and 250% increase, respectively. However, this increase in Br-HAAs only contributed 4% of the total genotoxicity of the pool samples. Furthermore, bromoacetic acid was not detected in salt water pool samples, but was present at low levels (1.2 µg/L) in the conventional chlorine pool sample, contributing 14% of the total calculated genotoxicity of that sample.

CONCLUSIONS

This study provides an extensive analysis of 60 DBPs in the same indoor community pool treated with either conventional chlorine or electrochemically generated chlorine (salt water). Of the 60 DBPs measured, 68% were detected at least once, with dominant DBP classes including HAAs, HALs, and THMs, with average concentrations of 1763 µg/L, 522 µg/L, and 453 µg/L, respectively. DBP levels were consistent with previous studies that reported these 3 classes, with the exception of trichloromethane, which was present at 764 µg/L in the conventional chlorine pool sample, likely due to a high residual chlorine (6.1 mg/L). This finding highlights the importance of maintaining a lower residual chlorine (1.0 to 2.0 mg/L) and ensuring adequate ventilation in indoor pools to decrease swimmers' exposure to volatile DBPs. The switch from conventional chlorine to a salt water system saw a 15% increase in the average total DBPs present, driven by trichloroacetic acid and dichloroacetic acid. The overall increase in total DBPs in the salt water pool samples was driven by the January sample which was collected after an exercise class and contained 28% and 24% more total DBPs compared to the conventional chlorine sample and the November salt water pool sample, respectively.

However, the implementation of a salt water system led to a 45% and 15% decrease in calculated cytotoxicity and genotoxicity, respectively. Calculated cytotoxicity values for both conventional chlorine and salt water pool samples were driven by HALs, HANs, and HAAs. This decrease in calculated cytotoxicity and genotoxicity further indicates that maintaining a low residual chlorine is also just as important as limiting the bather load. Further, our calculated cytotoxicity findings match well with a previous drinking water study that demonstrated a statistically significant correlation between the concentration of N-DBPs and cytotoxicity.[53] Therefore, limiting the formation of N-DBPs by reducing the amount of nitrogen sources like sweat and urine in swimming pools will be crucial in reducing the overall toxicity of swimming pools.[101,113,114]

I-THMs, HNMs, HAMs, and THMs contributed only 9% on average to the total calculated cytotoxicity of all three pool samples. IAAs, despite their elevated levels of toxicity, only contributed 1% of the calculated cytotoxicity, due to their presence at low or non-detect levels. Trichloroacetaldehyde was the primary driver of calculated cytotoxicity, contributing 22% of the calculated cytotoxicity on average.

Calculated genotoxicity values for both conventional chlorine and salt water pool samples were driven by HNMs, HANs, and HAAs, with chloroacetic acid contributing 72% on average, despite only accounting for 3% of the average total DBPs. HAMs, HALs, and I-THMs were not significant contributors to calculated genotoxicity due to their presence at low or non-detect levels. Further, despite their high levels, THMs are not genotoxic in CHO cells[32], so they did not contribute to the calculated genotoxicity for these pool samples.

Ion chromatography analysis of the sodium chloride used in the salt water pool system revealed a 0.05% bromide impurity. Based on the average salinity required for salt water pools, this 0.05% impurity results in an increase of bromide levels by more than 100 µg/L. As a result, the concentration of Br-DBPs and Br/Cl-DBPs increased from 49.9 µg/L to 86.1 µg/L, a 73% increase. This increase in Br-DBPs was primarily driven by bromochloroacetic acid, which increased by 268% to 54.9 µg/L, but did not substantially contribute to the calculated genotoxicity.

This study provides important insights for pools utilizing conventional chlorine vs electrochemically generated chlorine (salt water). Overall, the change from a conventional chlorinated pool to a salt water pool system reduced the calculated cytotoxicity and genotoxicity despite the presence of a bromide impurity and the increase in bather load prior to the second (January) salt water sample. Due to the increasing popularity of salt water pools, future work focusing on controlled lab reactions and measurement of whole water toxicity of salt water pools using a variety of sodium chloride salts would be beneficial to better understand the impact bromide impurities may have on the toxicity of the pool water. Additionally, future research studying a larger number of both indoor and outdoor pools (utilizing both salt water and conventional chlorine) will aid in a more robust understanding of the factors that drive toxicity in each treatment technique.

ACKNOWLEDGEMENTS

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TABLES AND FIGURES

Table 3.1. Sampling information (date, time, disinfectant technology used), water quality parameters (pH, residual chlorine), estimated bather load, THM levels, HAA levels, and total DBPs.

Sample ID	Sample Collection Time	Disinfectant	pH	Residual Chlorine (mg/L)	Exercise Class	THMs (µg/L)	HAAs (µg/L)	Total DBPs (µg/L)
May sample (5/17/2021)	1:00 PM	12% liquid sodium hypochlorite	7.4	6.1	No, typical bather load	777	1066	2541
November sample (11/18/2021)	12:30 PM	Electrochemically generated chlorine*	7.5	3.2	No, typical bather load	322	1798	2613
January sample (1/12/2022)	1:10 PM	Electrochemically generated chlorine*	7.3	2.0	Yes, 8-10 participants	260	2425	3251

*salt water pool

Table 3.2. DBPs quantified in conventional chlorine or salt water pools (µg/L).[a, *]

			conventional chlorine	salt water	
DBP class	**Name**	**Abbreviation**	**May**	**November**	**January**
HALs	Trichloroacetaldehyde	TCAL	580±15.0	448±10.7	532±58.3
	Bromodichloroacetaldehyde	BDCAL	1.0±0.0	ND	4.8±0.2
	Dibromochloroacetaldehyde	DBCAL	0.2±0.0	ND	0.1±0.0
	Tribromoacetaldehyde	TBAL	ND	ND	0.1±0.0
HANs	Trichloroacetonitrile	TCAN	0.6±0.0	0.3±0.0	0.5±0.0
	Dichloroacetonitrile	DCAN	5.2±0.3	5.4±0.2	4.2±0.4
	Chloroacetonitrile	CAN	6.3±0.1	2.6±0.2	1.4±0.0
	Bromochloroacetonitrile	BCAN	0.2±0.0	0.3±0.0	0.5±0.0
	Bromoacetonitrile	BAN	3.1±0.0	ND	ND
	Dibromoacetonitrile	DBAN	0.2±0.0	0.1±0.0	0.4±0.0
	Iodoacetonitrile	IAN	ND	ND	ND
HKs	1,1-Dichloropropanone	11DCP	2.8±0.1	ND	ND
	Chloropropanone	CP	18.0±1.0	4.6±0.2	3.5±0.4
	1,1,1-Trichloropropanone	111TCP	8.2±0.3	1.0±0.0	0.9±0.0

			conventional chlorine	salt water	
DBP class	**Name**	**Abbreviation**	**May**	**November**	**January**
HKs	1,1-Dibromopropanone	11DBP	ND	ND	ND
	1-Bromo-1,1-dichloropropanone	1B11DCP	0.3±0.0	0.2±0.0	0.3±0.0
	1,3-Dichloropropanone	13DCP	2.8±0.0	0.7±0.1	0.7±0.1
	1,1,3-Trichloropropanone	113TCP	1.6±0.1	0.2±0.0	ND
	1,1,3,3-Tetrachloropropanone	1133TeCP	0.8±0.0	ND	ND
	1,1,3,3-Tetrabromopropanone	1133TeBP	ND	3.1±0.3	1.7±0.4
HNMs	Trichloronitromethane	TCNM	4.6±0.1	2.4±0.1	1.7±0.1
	Dichloronitromethane	DCNM	0.4±0.0	ND	ND
	Bromochloronitromethane	BCNM	0.2±0.0	ND	ND
	Dibromonitromethane	DBNM	ND	ND	ND
THMs	Trichloromethane	TCM	764±14.8	318±29.0	257±38.1
	Tribromomethane	TBM	0.3±0.0	ND	ND
	Dibromochloromethane	DBCM	0.9±0.1	0.2±0.0	0.3±0.0
	Bromodichloromethane	BDCM	11.8±0.1	3.5±0.1	3.0±0.1

			conventional chlorine	salt water	
DBP class	**Name**	**Abbreviation**	**May**	**November**	**January**
I-THMS	Dichloroiodomethane	DCIM	ND	ND	ND
	Bromochloroiodomethane	BCIM	ND	ND	ND
	Dibromoiodomethane	DBIM	ND	ND	ND
	Chlorodiiodomethane	CDIM	ND	ND	ND
	Bromodiiodomethane	BDIM	0.5±0.0	ND	ND
	Iodoform	TIM	ND	ND	ND
HAMs	Chloroacetamide	CAM	ND	ND	ND
	Bromoacetamide	BAM	ND	ND	ND
	Dichloroacetamide	DCAM	21.5±0.9	ND	3.4±0.2
	Bromochloroacetamide	BCAM	ND	ND	ND
	Iodoacetamide	IAM	ND	ND	ND
	Trichloroacetamide	TCAM	37.0±1.8	23.1±0.8	9.0±0.7
	Dibromoacetamide	DBAM	ND	ND	ND
	Bromodichloroacetamide	BDCAM	1.5±0.1	1.9±0.0	0.6±0.0
	Chloroiodoacetamide	CIAM	ND	ND	ND

			conventional chlorine	salt water	
DBP class	**Name**	**Abbreviation**	**May**	**November**	**January**
HAMs	Bromoiodoacetamide	BIAM	ND	ND	ND
	Dibromochloroacetamide	DBCAM	ND	ND	ND
	Tribromoacetamide	TBAM	ND	ND	ND
	Diiodoacetamide	DIAM	ND	ND	ND
HAAs	Chloroacetic acid	CAA	67.5±6.8	120±5.6	90.9±5.5
	Bromoacetic acid	BAA	1.2±0.0	ND	ND
	Dichloroacetic acid	DCAA	730±113	1298±150	1969±32.5
	Trichloroacetic acid	TCAA	238±23.4	317±17.6	277±24.6
	Bromochloroacetic acid	BCAA	14.9±0.7	40.7±3.6	69.1±2.7
	Bromodichloroacetic acid	BDCAA	7.0±0.2	9.0±0.3	6.5±0.4*
	Dibromoacetic acid	DBAA	2.8±0.1	7.5±0.5	12.0±0.7*
	Dibromochloroacetic acid	DBCAA	3.9±0.1	4.2±0.0	0.5±0.0*
	Tribromoacetic acid	TBAA	ND	1.6±0.0	0.2±0.0*
IAAs	Iodoacetic acid	IAA	0.2±0.0	ND	ND
	Chloroiodoacetic acid	CIAA	ND	ND	0.3±0.1*

			conventional chlorine	salt water	
DBP class	**Name**	**Abbreviation**	**May**	**November**	**January**
	Bromoiodoacetic acid	BIAA	ND	ND	ND
IAAs	Diiodoacetic acid	DIAA	ND	ND	0.3±0.0*

[a] Values reported as average ± standard deviation of triplicate measurements; *Values reported as average ± standard error of duplicate measurements; ND = not detected.

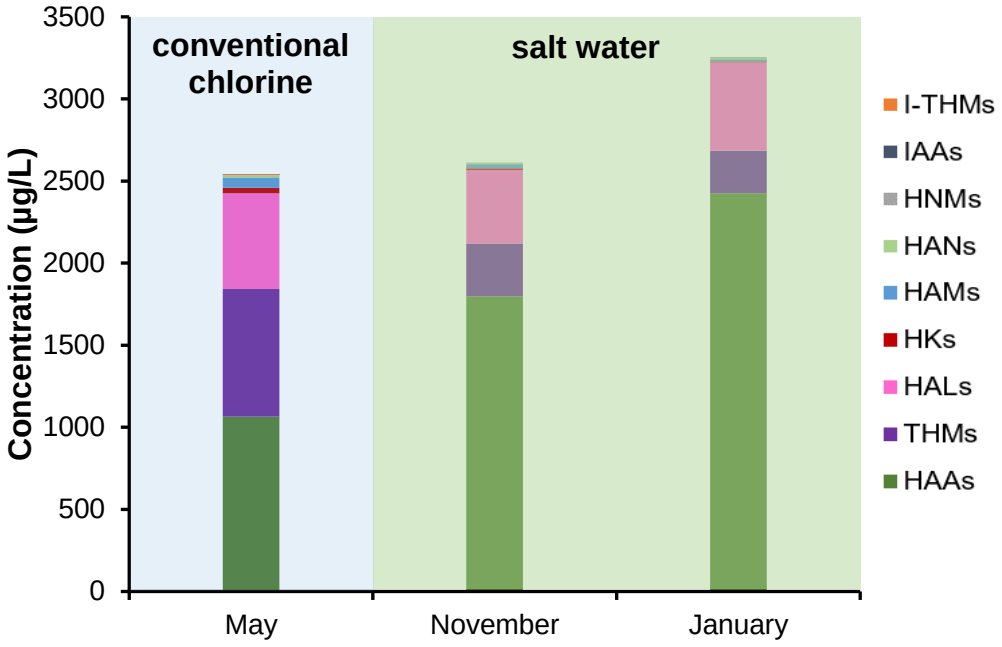

Figure 3.1. Total concentration of DBPs by class in conventional chlorine and salt water pool samples (µg/L).

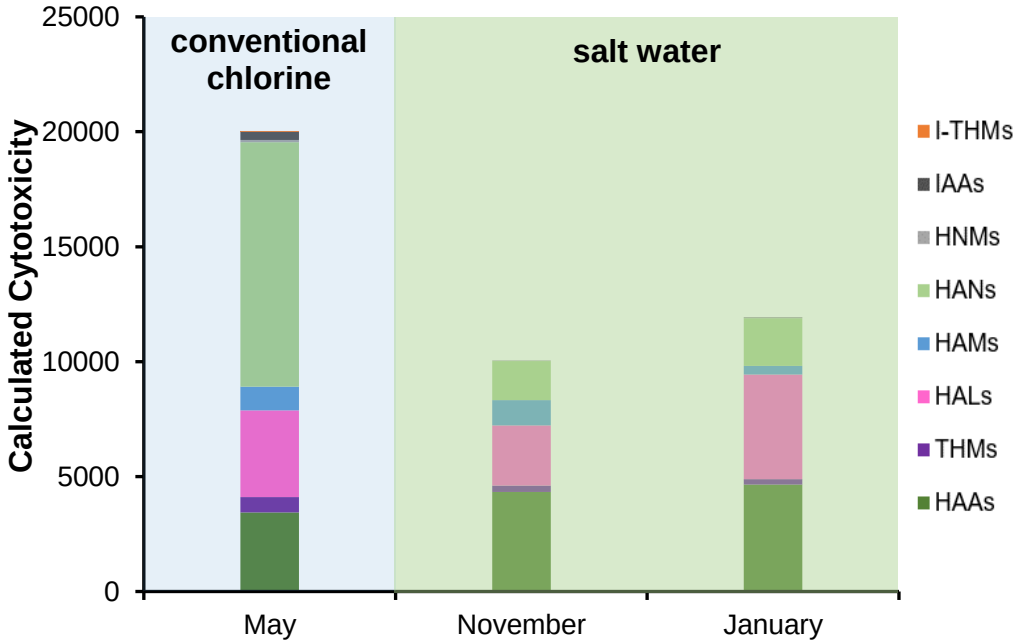

Figure 3.2. Calculated cytotoxicity of DBPs by class in conventional chlorine and salt water pool samples. Note that cytotoxicity data for haloketones (HKs) are currently not available in literature.

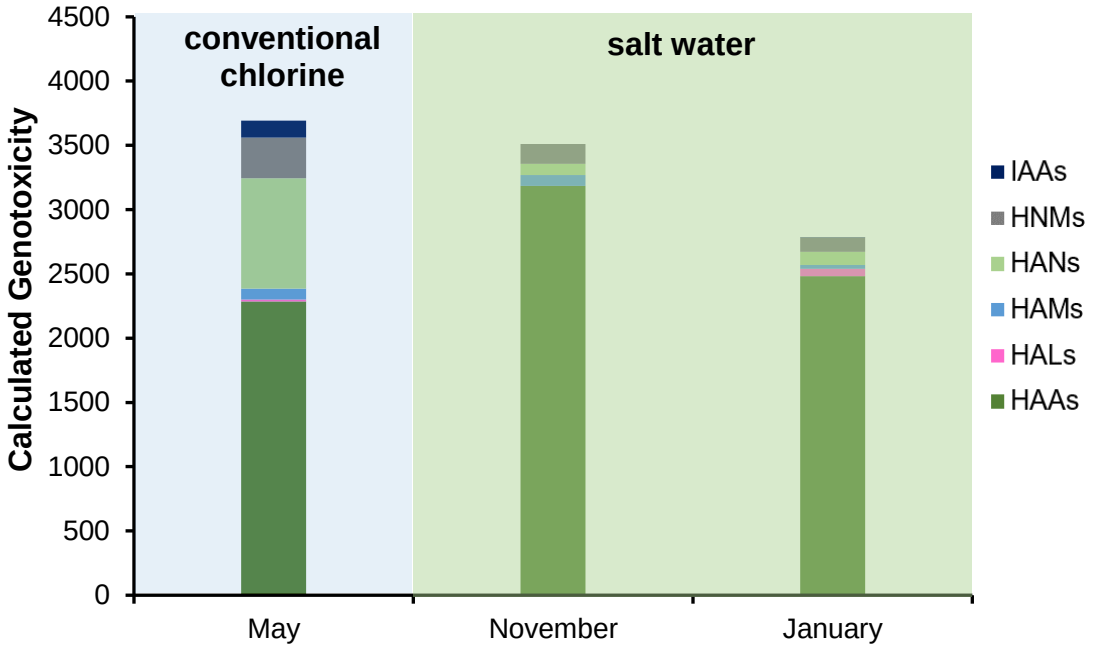

Figure 3.3. Calculated genotoxicity of DBPs by class in conventional chlorine and salt water pool samples. Note that genotoxicity data for HKs are currently not available in literature. I-THMs and THMs are not shown in this figure due to their presence at low or non-detect levels and/or their low genotoxicity values reported in literature.

CHAPTER 4

USING GAS CHROMATOGRAPHY- HIGH RESOLUTION-MASS SPECTROMETRY TO IDENTIFY UNKNOWN DBPS IN TEA

[3]Li, J.; Aziz, T.; Granger, C.O.; Richardson, S.D. Are Disinfection Byproducts (DBPs) Formed in My Cup of Tea? Regulated, Priority, and Unknown DBPs. *Environ. Sci. Technol.* **2021**, 55, 19, 12994–13004.

ABSTRACT

Globally, tea is the second most consumed non-alcoholic beverage and is an important pathway of disinfection by-product (DBP) exposure. When boiled tap water is used to brew tea, residual chlorine can produce DBPs by the reaction of chlorine with tea compounds. In a previous study, 60 regulated and priority, unregulated DBPs were measured in Twinings green tea, Earl Grey tea, and Lipton tea that was brewed using tap water or simulated tap water (nanopure water with chlorine). Total organic halogen (TOX) measurements of brewed tea reveal that the 60 regulated and priority, unregulated DBPs only accounted for 4% of TOX, with 96% of the unknown TOX likely being unidentified halogenated DBPs. Much of this unknown TOX may be high molecular weight haloaromatic compounds, likely formed by the reaction of chlorine with polyphenols present in tea leaves. The identification of six haloaromatic DBPs, including two monochloro-hydroxyphenols, two monochloro-trihydroxybenzenes, and two dichloro-trihydroxybenznenes using gas chromatography (GC)-high resolution-mass spectrometry (MS) indicates this may be the case. Further studies on the identity and formation of these aromatic DBPs should be conducted, since haloaromatic DBPs can have significant toxicity.

INTRODUCTION

Globally, tea is the second most consumed non-alcoholic beverage next to drinking water.[115,116] World production of tea was ~4.8 million tons in 2012, and per capita consumption is ~100 g/year.[115,117] Popular types of tea include green and black tea, both of which use leaves from the *Camellia sinesis* plant.[118] Green tea is not fermented (oxidized), while black tea has undergone fermentation (oxidation). In East Asian

countries, tea is generally brewed using boiled water and loose tea leaves, and in Western countries, tea bags are popular.[119] Both green tea and black tea contain approximately 500 compounds, including polyphenols, amino acids, caffeine, pigments, esters, polysaccharides, vitamins, minerals, and aromatic substances.[118,120,121] Some of these compounds have functional groups that can react with chlorine to form disinfection by-products (DBPs). For example, chlorine is well known to undergo electrophilic aromatic substitution reactions with phenols to form DBPs.[122,123]

To protect drinking water safety, disinfection is widely used to control waterborne pathogens.[124] Although disinfection is important, one downside is DBP formation.[5,125] Epidemiologic studies show that bladder cancer, colorectal cancer, and adverse birth outcomes are associated with DBPs in drinking water.[6,8,11,13-15,93,126-135] Eleven DBPs are currently regulated in the U.S.,[136] and DBPs are also regulated in other countries.[125] Thus, DBPs are a global concern. While chloramine and ozone are often used for disinfection, chlorine is still the most commonly used chemical disinfectant for drinking water.[137] To control the regrowth of microorganisms, residual chlorine is typically maintained in drinking water distribution systems.[119,138] In the U.S. and China, up to 4 mg/L residual chlorine is allowed, and up to 0.5 mg/L is generally maintained in the UK.[119,136,139,140] Boiling water in a kettle removes only 5-19% of the chlorine residual.[119] Therefore, a significant chlorine residual remains, which can form DBPs when this water is used to make tea.

In this study, we performed large scale solid phase extractions of three popular green and black teas in the U.S. (Twinings green tea, Earl Grey tea, and Lipton tea) using XAD resins. These extracts were then analyzed using gas chromatography-high resolution-

mass spectrometry to identify unknown halogenated DBPs. In completing this study, six DBPs were identified for the first time in tea. Further studies on the identity and formation of these aromatic DBPs should be conducted, since haloaromatic DBPs can have significant toxicity.

MATERIALS AND METHODS

Chemicals and Reagents. Ethyl acetate and methanol were purchased from Sigma-Aldrich and Honeywell International (Muskegon, MI) at the highest purity. Twinings green tea, Twinings Earl Grey (black) tea, and Lipton (black) tea (all in tea bags, 2.0 g, 2.0 g, and 4.1 g, respectively) were purchased from a local supermarket. Earl Grey and Lipton teas were both fermented black teas. Experiments were conducted using nanopure water (18.2 MΩ).

Experimental Design. For the analysis of unknown DBPs, a higher residual chlorine level (4 mg/L, before boiling) was used to allow somewhat higher levels of DBPs to enable their detection by gas chromatography (GC)-full-scan mass spectrometry, and to simulate real tap water with the maximum level of residual chlorine allowed by regulation.[141] In addition, 200 μg/L sodium bromide as bromide and 20 μg/L sodium iodide as iodide were added to the simulated tap water (nanopure water with chlorine) to mimic real source waters that can have high bromide and iodide.[30,141-143] In these cited studies, bromide in Rolla, MO tap water was 10.1 μg/L,[142] and iodide in Hong Kong tap water was 0.1-0.4 μg/L.[143] Tea was brewed in 1 L and 2 L beakers (1 L water per tea bag), then the tea was cooled at room temperature (combined brew and cooling time of 30 min). Control experiments were also conducted using boiled nanopure water. Experiments for unknown (non-target) DBP identification were conducted in duplicate.

Total Organic Halogen (TOX). Total organic chlorine (TOCl), bromine (TOBr), and iodine (TOI) were analyzed using a Mitsubishi TOX analyzer (Mitsubishi Chemical Analytech, Chigasaki, Japan; Cosa Xentaur, Yaphank, USA). Procedures were based on published papers, with a few modifications described in Appendix C.[53,61,137] Briefly, acidified samples (pH < 2) were adsorbed onto activated carbon, washed with sodium nitrate, and combusted at 1000 °C in the presence of oxygen and argon as the carrier gas. Combusted gases were collected in an aqueous solution containing 0.03% hydrogen peroxide, which was analyzed for chloride, bromide, and iodide using a Dionex 1600 ion chromatograph (Dionex, Sunnyvale, CA).

Non-Target Identification of Unknown DBPs in Tea. For the non-target analysis of unknowns, samples were extracted using pre-cleaned XAD resins as described in our previous research.[56] Briefly, 5.0 L tea was acidified to pH <1 using concentrated H_2SO_4. A glass column was packed with two kinds of XAD resins (XAD-2 and XAD-8, 9 mL each). XAD resins were preconditioned with 25 mL of nanopure water, 12.5 mL of 0.1 M HCl, 12.5 mL of 0.1 M NaOH, 25 mL of 0.1 M HCl, and 25 mL nanopure water, in sequence. Afterwards, aqueous samples were passed through the XAD resins and allowed to drain completely. Adsorbed compounds (i.e., DBPs) were eluted with 70 mL ethyl acetate. The eluent was collected, residual water removed using a separatory funnel, further dried with Na_2SO_4, and concentrated to 0.2 mL with high-purity nitrogen.

A LECO Pegasus GC-HRT time-of-flight (TOF) high resolution mass spectrometer (GC-HRT-TOF-HRMS; 50,000 resolution; LECO Corp., St. Joseph, MI) was used for these non-target analyses with electron ionization (EI) at 70 eV in full-scan mode (*m/z* 33 to 530). Procedures were similar to a previously published paper from our lab.[56] Extracts

(1.5 μL) were injected into the inlet in pulsed spitless mode. The GC oven temperature program was as follows: initial temperature held at 35 °C for 4 min, increased to 280 °C at 9 °C/min, and then held for 20 min. Samples were chromatographically separated using a Restek Rxi-5ms GC column (30m × 0.25 mm ID × 0.25 μm). The transfer line was held at 280 °C and the source temperature was held at 225 °C.

The numbers and types of halogens were determined using characteristic isotopic patterns, and high resolution-MS was used to determine empirical formulas for the molecular ions and fragment ions. Based on the fragmentation patterns and molecular formulas, possible structures were proposed. Library database searching (NIST) was also utilized.

Quality Assurance and Quality Control. To ensure data quality, solvent blanks and procedural blanks (boiled nanopure water brewed tea) were used to confirm the formation of DBPs. Results revealed that none of the DBPs identified in the study were detected in either solvent blanks or procedural blanks.

RESULTS AND DISCUSSION

Role of Tea in DBP Formation and Total Organic Halogen. Tea is rich in polyphenols,[141] which contain activated benzene rings that can readily react with chlorine,[144-147]producing halogenated (poly)phenols and other compounds that might be stable end products under the conditions used for brewing tea. As a result, higher molecular weight DBPs might be more dominant than the 60 lower molecular weight DBPs quantified in the previous study. To test this hypothesis, total organic chlorine, bromine, and iodine (TOCl, TOBr, and TOI) were measured in tea brewed using boiled

simulated tap water. Total organic halogen (TOX) in simulated tap water brewed tea was 66 μg/L (as Cl^-). Results revealed that the 60 regulated and priority unregulated DBPs only account for 3.7% of the TOX in tea brewed using simulated tap water (Figure 4.1). This finding confirms that most of TOX in tea is due to unknown halogenated DBPs.

TOCl was the dominant contributor to TOX in simulated tap water brewed tea with an average contribution of 94%. In a previously published study on simulated tap water treated tea, 164-196 μg/L (as Cl^-) of TOX was generated when instant tea reacted with 4 mg/L chlorine for 24 h.[141] These higher TOX levels are likely due to a higher chlorine level and longer contact time compared to our present study. TOI was not detected above the limit of quantification (5 μg/L) and TOBr was present at 0.8 μg/L in simulated tap water brewed tea.

Non-Target Identification of Unknown DBPs. For the identification of unknown tea DBPs, simulated tap water (containing only bromide, iodide, and chlorine) was used as the water source to investigate DBPs formed directly from tea precursors only. Six unknown DBPs were identified using GC with high resolution-MS as halogenated dihydroxybenzenes and halogenated trihydroxybenzenes. These six DBPs are reported here for the first time as tea DBPs. Haloaromatic DBPs are important to study due to their elevated levels of toxicity compared to aliphatic DBPs (THMs and HAAs).[148] Han et al. (2021) recently published a study that revealed haloaromatic DBP fractions of chlorinated water samples were more toxic than the corresponding aliphatic DBP fractions (THMs and HAAs).[149] Given this, research into identifying new routes of exposure to haloaromatic DBPs is important to improve the overall safety of commonly consumed beverages.

Two haloaromatic DBPs (DBP-1 and DBP-2) had full-scan mass spectral matches in the NIST library and could be tentatively assigned as two mono-chloro-hydroxyphenols. High resolution data for the molecular ions, as well as isotopic patterns and fragment ions, supported these structural assignments (Table 4.1). Structures of DBP-1 and DBP-2 were not confirmed, due to high number (six) of possible isomers and difficulty in obtaining some of the standards.

Four haloaromatic DBPs (DBP-3, DBP-4, DBP-5, and DBP-6) were not present in the NIST library database, and their structures were proposed as described below. DBP-3 and DBP-4 at RT 17.19 and 20.29 min, respectively, have the same molecular ion at *m/z* 160/162, with an isotopic abundance ratio of 3:1, indicating that these compounds contain 1 chlorine atom (**Figure 4.2**). Their accurate masses and isotopic abundance ratios matched well with those of monochloro-trihydroxybenzenes (theoretical and observed *m/z* of 159.9922 and 159.9922, respectively; Δ=0.0 ppm). Fragment ions *m/z* 141.9818/143.9789, 117.9817, 85.9918, and 71.9762 are due to $[M\text{-}H_2O]^+$, $[M\text{-}C(OH)CH]^+$, $[M\text{-}C_2H_2O_3]^+$, and $[M\text{-}C_3H_4O_3]^+$ respectively (Table 4.1). DBP-5 and DBP-6 at RT 20.10 and 20.49 min have the same molecular ion at *m/z* 194/196/198, and an isotopic abundance ratio of 9:6:1, indicating that these compounds contain 2 chlorine atoms (**Figure 4.2**). A molecular formula of $C_6H_4Cl_2O_3$ was indicated by the accurate mass of *m/z* 193.9534 (theoretical *m/z* of 193.9532; Δ=1.03 ppm). These compounds are proposed to be dichloro-trihydroxybenzenes. Accurate mass data indicate that fragment ions *m/z* 175.9433/177.9399, 119.9528, and 112.9789 are due to $[M\text{-}H_2O]^+$, $[M\text{-}C_2H_2O_3]^+$, and $[M\text{-Cl-CHOOH}]^+$, respectively (Table 4.1). This is the first time

halogenated trihydroxybenzenes have been reported as DBPs in both drinking water and tea.

Semi-quantitative Analysis of 6 Haloaromatic DBPs. Semi-quantitative analysis of the six newly identified was conducted. Due to the lack of standards, their concentrations were calculated using a one-point calculation curve of 4-chlorophenol (4CP; 10 mg/L) to obtain a 4CP-equivalent concentration (Table 4.2). The total concentrations were 356.8, 788.4, and 4036.2 ng/L for Earl Grey tea, Lipton tea, and green tea, respectively, with DBP-6 being the dominant DBP formed. Future work focusing on synthesizing standards of the newly identified DBPs is critical for quantitative analysis.

IMPLICATIONS

Tea is the second most globally consumed non-alcoholic beverage, accounting for a large portion of the daily consumption of tap water. Residual chlorine in tap water may react with the polyphenols present in tea leaves to form aromatic DBPs. TOX analysis of brewed tea revealed that 96% of halogenated DBPs in tea are unknown. To identify unknown DBPs, tea was brewed using simulated tap water and extracted using XAD resins. Analysis of the tea extracts via gas chromatography-high resolution-mass spectrometry reveals the presence of six newly identified haloaromatic DBPs in tea. The newly identified DBPs include two monochloro-hydroxyphenols, two monochloro-trihydroxybenzenes, and two dichloro-trihydroxybenznenes. Further studies on the identity and formation of these aromatic DBPs should be conducted, since haloaromatic DBPs can have significant toxicity when compared to aliphatic DBPs.[149]

To lower DBP/TOX levels, chlorine-free bottled water could be used as a substitute for tap water when brewing tea. Recent studies also indicate that adding ascorbate or lemon slices (rich in Vitamin C) before boiling could reduce the toxicity of DBPs in chlorinated tap water.[150,151] This may be another simple way to mitigate DBP risks when preparing hot tea.

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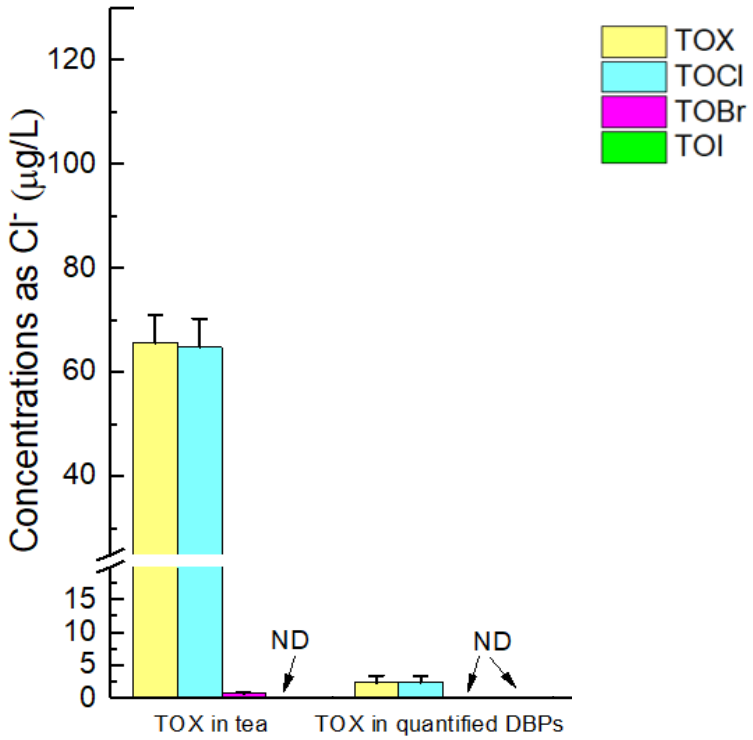

Figure 4.1. Total organic halogen in simulated tap water brewed tea (ND= not detectable)

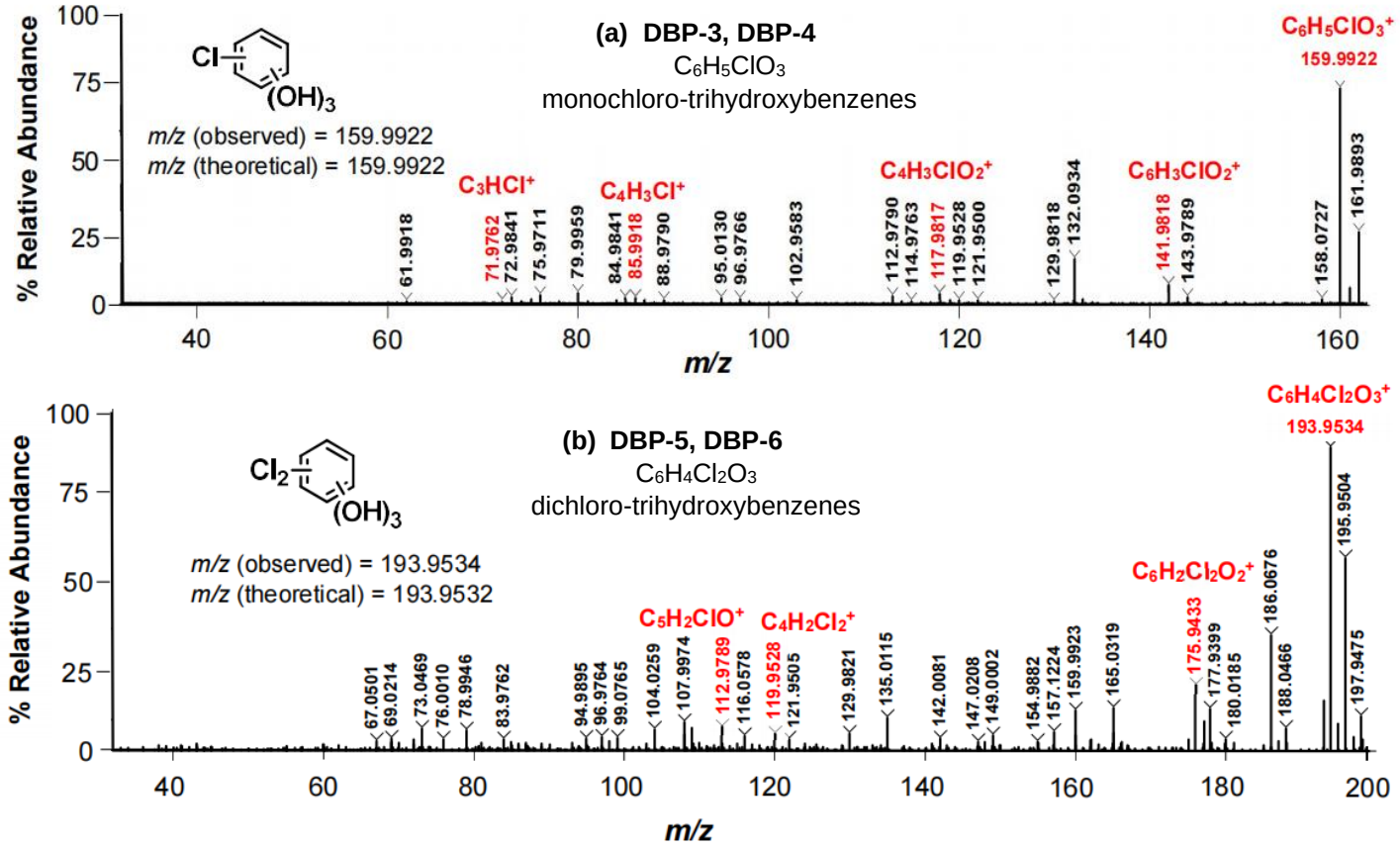

Figure 4.2. High resolution mass spectra of unknown DBPs in tea.

Table 4.1. Unknown DBPs identified in simulated tap water brewed tea.[a]

DBP	Formula	Retention time (min)	Theoretical m/z			Observed m/z			Δ(ppm)		
			M	M+2	M+4	M	M+2	M+4	M	M+2	M+4
DBP-1	$C_6H_5ClO_2$	13.28	143.9973	145.9943	--	143.9974	145.9944	--	0.69	0.68	--
DBP-2	$C_6H_5ClO_2$	14.07	143.9973	145.9943	--	143.9974	145.9946	--	0.69	2.05	--
DBP-3	$C_6H_5ClO_3$	17.19	159.9922	161.9892	--	159.9923	161.9894	--	0.63	1.23	--
DBP-4	$C_6H_5ClO_3$	20.29	159.9922	161.9892	--	159.9922	161.9893	--	0	0.62	--
DBP-5	$C_6H_4Cl_2O_3$	20.10	193.9532	195.9503	197.9477	193.9534	195.9504	197.9475	1.03	0.51	-1.01
DBP-6	$C_6H_4Cl_2O_3$	20.49	193.9532	195.9503	197.9477	193.9533	195.9503	197.9474	0.52	0	-1.52

[a] Unknown DBPs were detected in all tea samples.

Table 4.2. Semi-quantitative concentrations of haloaromatic DBPs in simulated tap water brewed tea (ng/L).[a]

DBP	Lipton tea	Green tea	Earl Grey
DBP-1 (4CP-eq)	38.8±15.8	4.4±0.6	1.3±1.3
DBP-2 (4CP-eq)	94.6±14.8	7.3±1.1	21.6±3.4
DBP-3 (4CP-eq)	109±43.5	43.5±46.4	69.0±3.1
DBP-4 (4CP-eq)	263±181	663±393	40.8±34.3
DBP-5 (4CP-eq)	102±66.1	1115±180	20.1±7.6
DBP-6 (4CP-eq)	181±3.2	2203±882	204±86.8

[a]Duplicate analyses; for DBP-10, DBP-11, DBP-12, DBP-13, DBP-14, and DBP-15, due to lack of corresponding standards, their concentrations were calculated using a one-point calibration curve (10 ppm 4-chlorophenol (4CP)) to obtain a 4CP-equivalent concentration).

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APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 2

Chinese Hamster Ovary (CHO) Cell Chronic Cytotoxicity Assay.

Chronic cytotoxicity was measured using XAD extracts collected at each sampling location. XAD extracts were solvent exchanged from ethyl acetate to dimethyl sulfoxide (DMSO) using nitrogen to blow to near dryness. A 96-well flat-bottomed microplate was used with one column serving as a blank control (200 µL of F12 and 5% fetal bovine serum (FBS)), another column serving as a concurrent negative control ($3x10^3$ CHO cells, F12, and FBS), and the remaining columns contained $3x10^3$ CHO cells, F12, FBS, and a known volume of sample extract for a total volume of 200 µL. Once the microplate was prepped, it was covered with AlumnaSeal™ and placed on a rocking platform at 37 °C for two 5 min-periods with the microplate turning 90° after the first 5 min interval. This step is important as it ensures that there is an even distribution of cells across the bottom of the microplate wells. The cells were then incubated for 72 hr at 37 °C (5% CO_2). After the 72 hr, each cell was aspirated and the cells were fixed in methanol for 5 min and stained for 5 min using 1% crystal violet solution in 50% methanol. The unattached crystal violet was then removed via a washing step and the microplate was dried. Each well on the microplate received 50 µL of a DMSO/methanol mixture (3:1 v/v) and was then incubated at room temperature for 5 min. The microplate was analyzed using a SpectraMax microplate reader at 595 nm. The assay was calibrated and there was a direct relationship between the absorbance of the crystal violet dye associate with cell

density and the number of viable cells.[152] Every well was blank subtracted using the average of the blank and the average blank corrected absorbance value of the negative control was set to 100%. The absorbency for each treatment group well was converted into a percentage of the negative control. For each sample, a range finding experiment along with two independent experiments were conducted and used to construct a concentration-response curve. Regression analysis was applied to each concentration-response curve and a median lethal concentration (LC_{50}) value was calculated. The LC_{50} is the concentration of sample that induced a cell density that was 50% of the negative control. The average and standard error (SE) were derived from multiple regression analyses using bootstrap statistics.[32,153-155] LC_{50} values were then converted to cytotoxicity index (CTI), defined as $LC_{50}^{-1}(10^3)$, thus making the comparison between samples easier (a higher CTI, higher cytotoxicity).

Chinese Hamster Ovary (CHO) Cell Single Cell Gel Electrophoresis (SCGE) Assay.

The day before analysis, $4x10^4$ CHO cells were added to a microplate containing 200 µL of F12 medium and 5% fetal bovine serum (FBS) and incubated at 37 °C for 16-20 hrs. Cells were then rinsed with Hank's balanced salt solution (HBSS) and treated with a range of concentrations from each sample extract in F12 medium (total volume of 25 µL) without FBS present for 4 h at 37 °C with 5% CO_2. With each experiment conducted, there was a negative control along with a positive control (3.8 mM ethyl methanesulfonate, EMS). The microplates were covered with AlumnaSeal™ to prevent contamination. After the incubation period, the cells were washed two times with HBSS and harvested with 50 µL of 0.01% tryspin and 53 µM EDTA. The tryspin was inactivated with 70 µL of F12 and FBS. A 10 µL aliquot of cell suspension was then

removed and mixed with 10 µL of 0.05% trypan blue vital dye in phosphate buffered saline (PBS) to measure acute cytotoxicity.[156] If the cell suspension had acute cytotoxicity above 30%, SCGE data was not used. Prior to the experiment, a clear microscope slide coated with 1% normal melting point agarose was prepared using deionized water and allowed to dry overnight. Then, the cell suspension was embedded in a layer of low melting point agarose prepared using PBS and placed onto the slides for analysis. The samples were transferred to a tray and placed on ice to solidify. Once solidified, a layer of 0.5% low melting point agarose was placed as the final layer. To remove the cellular membranes, samples were placed in a lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, 1% sodium sarcosinate, 1%Triton X-100, and 10% DMSO) at 4°C overnight. The following day, samples were removed from the lysing solution and placed in an alkaline buffer (pH 13.5) in an electrophoresis tank to denature the DNA for 20 min. The microgels were electrophoresed at 25 V, 300 mA (0.72 V/cm) for 40 min at 4 °C. The microgels were then removed from the electrophoresis tank and neutralized with Tris buffer (pH 7.5) and rinsed with cold water. The samples were then dehydrated with cold methanol and dried at 50 °C and stored at room temperature in a covered slide box. To analyze via a microscope, the microgels were hydrate with cold deionized water for 20-30 min, stained with ethidium bromide (65 uL, 20 ug/mL) for 3 min, rinsed with cold water, and analyzed using a Zeiss fluorescence microscope with an excitation filter of 546/10 nm and a barrier filter of 590 nm. Two microgels were prepared per treatment group, and 25 randomly chosen nuclei were analyzed in each microgel using a charged coupled device (CCD) camera. The %Tail DNA (the amount of DNA that migrated from the nucleus into the microgel, a measure of DNA damage) was

determined using an image analysis software (Comet IV, Perspective Instruments, Ltd, Suffolk, UK).[157] Experiments were repeated 2-4 times for each sample concentrate. Concentration-response curves were generated for each sample extract along with an analysis of variance (ANOVA) test used to determine the lowest concentration that generated a significant reduction in genomic DNA damage. The average and standard error (SE) were derived from multiple regression analyses using bootstrap statistics.[154] Genotoxicity index values (GTI; 50% Tail $DNA^{-1}(10^3)$) were also calculated.

Table A.1 Vendor information, retention time (RT), molecular mass, and quantifier and qualifier ions for 60 DBPs quantified in this study.

DBP class	Name	Abbreviation	Molecular mass (Da)	RT (min)	Quantifier ion (*m/z*)	Qualifier ion (*m/z*)
Haloacetaldehydes (HALs)	Trichloroacetaldehyde [a]	TCAL	147.39	5.39	82.0	110.9
	Bromodichloroacetaldehyde [b]	BDCAL	191.84	7.61	83.0	111/163.8
	Dibromochloroacetaldehyde [b]	DBCAL	236.29	9.77	128.9	127.9
	Tribromoacetaldehyde [a]	TBAL	280.74	11.69	172.8	171.8
Haloacetonitriles (HANs)	Trichloroacetonitrile [a]	TCAN	144.39	6.99	108.0	110.0
	Dichloroacetonitrile [a]	DCAN	109.94	6.14	74.0	82.0
	Chloroacetonitrile [a]	CAN	75.50	6.47	75.0	77.0
	Bromochloroacetonitrile [a]	BCAN	154.39	8.49	153.0	155.0
	Bromoacetonitrile [a]	BAN	119.95	8.72	118.90	120.9
	Dibromoacetonitrile [a]	DBAN	198.84	10.70	117.9	199.0
	Iodoacetonitrile [a]	IAN	166.95	11.44	167.0	126.9
Haloketones (HKs)	1,1-Dichloropropanone [a]	11DCP	126.97	6.92	63.0	83.0
	Chloropropanone [a]	CP	92.52	7.05	92.0	94.0
	1,1,1-Trichloropropanone [a]	111TCP	161.41	9.50	125.0	127.0
	1,1-Dibromopropanone [b]	11DBP	215.87	10.79	216.0	218.0

DBP class	Name	Abbreviation	Molecular mass (Da)	RT (min)	Quantifier ion (*m/z*)	Qualifier ion (*m/z*)
Haloketones (HKs)	1-Bromo-1,1-dichloropropanone [b]	1B11DCP	205.87	11.39	125.0	127.0
	1,3-Dichloropropanone [b]	13DCP	126.97	11.49	77.0	49.0
	1,1,3-Trichloropropanone [a]	113TCP	161.41	12.54	77.0	83.0
	1,1,3,3-Tetrachloropropanone [b]	1133TeCP	195.86	13.43	83.0	85.0
	1,1,3,3-Tetrabromopropanone [c]	1133TeBP	373.66	18.76	200.8	119.9
Halonitromethanes (HNMs)	Trichloronitromethane [a]	TCNM	164.38	6.98	116.9	119.0
	Dichloronitromethane [b]	DCNM	129.93	7.07	83.0	85.0
	Bromochloronitromethane [b]	BCNM	174.38	8.40	129.0	127.0
	Dibromonitromethane [b]	DBNM	218.83	10.75	172.8	171.0
Trihalomethanes (THMs)	Trichloromethane [a]	TCM	119.38	3.45	83.0	85.0
	Tribromomethane [a]	TBM	252.73	7.83	173.0	252.0
	Dibromochloromethane [a]	DBCM	208.28	5.77	129.0	127.0
	Bromodichloromethane [a]	BDCM	163.83	4.23	83.0	129.0
Iodinated-trihalomethanes (I-THMs)	Dichloroiodomethane [b]	DCIM	210.83	6.35	83.0	126.9
	Bromochloroiodomethane [b]	BCIM	255.28	8.41	128.9	126.9
	Dibromoiodomethane [b]	DBIM	299.73	7.83	172.8	299.7

DBP class	Name	Abbreviation	Molecular mass (Da)	RT (min)	Quantifier ion (*m/z*)	Qualifier ion (*m/z*)
Iodinated-trihalomethanes (I-THMs)	Chlorodiiodomethane [b]	CDIM	302.28	10.80	174.9	126.9
	Bromodiiodomethane [b]	BDIM	346.73	15.20	218.8	220.8
	Iodoform [a]	TIM	393.73	14.46	393.7	266.8
Haloacetamides (HAMs)	Chloroacetamide [a]	CAM	93.51	12.38	93.0	44.0
	Bromoacetamide [a]	BAM	137.96	14.48	137.0	44.0
	Dichloroacetamide [b]	DCAM	127.96	14.66	44.0	127.0
	Bromochloroacetamide [b]	BCAM	172.41	16.05	44.0	173.0
	Iodoacetamide [a]	IAM	184.96	16.75	158.0	85.0
	Trichloroacetamide [a]	TCAM	162.4	16.45	44.0	82.0
	Dibromoacetamide [d]	DBAM	216.86	17.29	44.0	217.0
	Bromodichloroacetamide [b]	BDCAM	206.85	17.85	44.0	128.0
	Chloroiodoacetamide [b]	CIAM	206.85	17.85	92.0	219.0
	Bromoiodoacetamide [b]	BIAM	263.86	18.69	136.0	138.0
	Dibromochloroacetamide [b]	DBCAM	251.3	19.06	44.0	128.0
	Tribromoacetamide [b]	TBAM	295.76	19.11	44.0	295.0
	Diiodoacetamide [b]	DIAM	310.86	20.75	184.0	311.0

DBP class	Name	Abbreviation	Molecular mass (Da)	RT (min)	Quantifier ion (*m/z*)	Qualifier ion (*m/z*)
Haloacetic acids (HAAs)	Chloroacetic acid [a]	CAA	94.5	6.45	108.0	77.0
	Dichloroacetic acid [a]	DCAA	128.94	7.41	83.0	85.0
	Trichloroacetic acid [a]	TCAA	163.39	8.42	119.0	117.0
	Bromoacetic acid [a]	BAA	138.95	7.42	152	154
	Dibromoacetic acid [a]	DBAA	217.84	9.91	173.0	175.0
	Tribromoacetic acid [a]	TBAA	296.74	12.62	251.0	253.0
	Bromochloroacetic acid [a]	BCAA	173.39	8.72	129.0	127.0
	Dibromochloroacetic acid [a]	DBCAA	252.29	12.25	207.0	209.0
	Bromodichloroacetic acid [a]	BDCAA	207.84	9.84	163.0	161.0
Iodoacetic acids (IAAs)	Iodoacetic acid [a]	IAA	185.95	8.67	200.0	73.0
	Chloroiodoacetic acid [b]	CIAA	220.39	10.43	234.0	175.0
	Bromoiodoacetic acid [b]	BIAA	264.84	11.54	151.0	278.0
	Diiodoacetic acid [b]	DIAA	311.85	13.00	326.0	199.0

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

Table A.2. Sampling dates and wastewater treatment plant details.

WWTP ID	Sampling Dates [a]	Dechlorination agent	Origin of water	Treatment
WWTP 1	M (9/23/2019)	sodium bisulfite	Domestic and industrial	AS
	W (9/16/2020)			
	W (9/15/2021)			
WWTP 2	M (9/9/2019)	sulfur dioxide	Domestic and industrial	TF
	W (9/30/2020)			
	W (8/25/2021)			
WWTP 3	M (10/7/2019)	sodium bisulfite	Domestic and industrial	TF
	W (10/14/2020)			
	W (9/22/2021)			
WWTP 4	M (9/16/2019)	sulfur dioxide	Domestic and industrial	TF
	W (8/11/2021)			
WWTP 5	M (9/30/2019)	sulfur dioxide	Domestic and industrial	AS
WWTP 6	W (10/6/2021)	sodium bisulfite	Domestic and industrial	AS

WWTP ID	Sampling Dates [a]	Dechlorination agent	Origin of water	Treatment
WWTP 7	W (8/4/2021)	sulfur dioxide	Domestic and industrial [b]	OD
WWTP 8	W (9/1/2021)	sulfur dioxide	Domestic and industrial	TF-AS
WWTP 9	W (10/13/2021)	sulfur dioxide	Domestic and industrial [b]	AS

Notes: [a] where M = Monday, W = Wednesday; [b] indicates small contribution "AS" = activated sludge; "TF" = trickling filters; "OD" = oxidation ditch

Table A.3. Primary and secondary multiple reaction monitoring (MRM) transitions for iopamidol and d_8-iopamidol

Target Analyte	RT (min)	Primary MRM Transition	Secondary MRM Transition
Iopamidol	**3.50**	794.8 > 777.9	794.8 > 558.9
Iopamidol-d8	**3.51**	802.9 > 785.8	802.9 > 562.9

Table A.4. DBP concentrations (µg/L) in upstream (U), downstream (D), and effluent (Eff) samples from 2019 (WWTP 1,2, and 3).

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
TCAL	N.D.	N.D.	0.05±0.00	N.D.	0.16±0.00	0.01±0.00	N.D.	0.26±0.00	0.90±0.05
BDCAL	N.D.	N.D.	0.03±0.01	N.D.	N.D.	N.D.	N.D.	0.05±0.00	0.50±0.02
DBCAL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.05±0.00
TBAL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAN	N.D.	N.D.	N.D.	N.D.	0.55±0.03	1.2±0.1	N.D.	0.16±0.00	0.49±0.04
CAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCAN	N.D.	0.42±0.02	0.08±0.01	N.D.	0.24±0.04	0.84±0.06	N.D.	N.D.	0.25±0.01
BAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBAN	N.D.	0.02±0.00	0.10±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	0.03±0.00
IAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
11DCP	N.D.	N.D.	N.D.	N.D.	N.D.	0.02±0.00	N.D.	0.07±0.00	0.37±0.02
CP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
111TCP	N.D.	N.D.	0.08±0.00	N.D.	0.11±0.00	0.20±0.00	N.D.	0.22±0.01	0.59±0.02

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
11DBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1B11DCP	N.D.	N.D.	N.D.	N.D.	0.05±0.00	N.D.	N.D.	N.D.	N.D.
13DCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
113TCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01±0.00
1133TeCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.07±0.00
1133TeBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.03±0.00	N.D.
DCNM	N.D.	N.D.	0.07±0.00	N.D.	N.D.	N.D.	N.D.	0.01±0.00	0.05±0.00
BCNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCM	NM	NM	NM	NM	NM	NM	N.D.	0.71±0.01	1.75±0.20
TBM	NM	NM	NM	NM	NM	NM	N.D.	N.D.	N.D.
DBCM	NM	NM	NM	NM	NM	NM	N.D.	N.D.	N.D.
BDCM	NM	NM	NM	NM	NM	NM	N.D.	N.D.	N.D.
DCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
BCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBIM	N.D.	N.D.	N.D.	N.D.	N.D.	0.46±0.01	N.D.	N.D.	N.D.
CDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAM	N.D.	N.D.	N.D.	N.D.	0.52±0.03	0.59±0.03	N.D.	N.D.	N.D.
BCAM	N.D.	N.D.	N.D.	N.D.	0.84±0.07	1.0±0.1	N.D.	N.D.	0.17±0.01
IAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.042±0.00
DBAM	N.D.	N.D.	N.D.	N.D.	0.49±0.03	0.94±0.02	N.D.	N.D.	N.D.
BDCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
DBCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TBAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
BAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
DCAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
TCAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
BCAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
BDCAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
DBAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
DBCAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
TBAA	NM	NM	NM	NM	NM	NM	NM	NM	NM
IAA	N.D.	N.D.	N.D.	N.D.	N.D.	0.05±0.00	N.D.	0.02	0.02±0.00
CIAA	N.D.	N.D.	N.D.	N.D.	N.D.	0.003±0.000	N.D.	N.D.	0.028±0.009
BIAA	N.D.	N.D.	N.D.	N.D.	N.D.	0.009±0.001	N.D.	N.D.	N.D.

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
DIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a] Values reported as average ± standard error of duplicate measurements; N.D. : non-detect

Table A.5. DBP concentrations (µg/L) in upstream (U), downstream (D), and effluent (Eff) samples from 2019 (WWTP 4 and 5).

	WWTP 4			WWTP 5		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**
TCAL	N.D.	0.13±0.00	0.09±0.00	N.D.	N.D.	2.5±0.6
BDCAL	N.D.	N.D.	N.D.	N.D.	N.D.	2.2±0.6
DBCAL	N.D.	N.D.	N.D.	N.D.	N.D.	1.3±0.3
TBAL	N.D.	N.D.	N.D.	N.D.	N.D.	0.11±0.01
TCAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAN	N.D.	N.D.	0.18±0.01	N.D.	N.D.	2.0±0.7
CAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCAN	N.D.	N.D.	N.D.	N.D.	N.D.	1.1±0.4
BAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBAN	N.D.	N.D.	N.D.	N.D.	N.D.	0.16±0.05
IAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
11DCP	N.D.	N.D.	0.13±0.01	N.D.	N.D.	0.68±0.16
CP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
111TCP	N.D.	0.13±0.00	0.17±0.01	N.D.	N.D.	0.70±0.19
11DBP	N.D.	N.D.	N.D.	N.D.	N.D.	0.11±0.02

	WWTP 4			WWTP 5		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**
1B11DCP	N.D.	N.D.	N.D.	N.D.	0.07±0.00	0.63±0.16
13DCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
113TCP	N.D.	N.D.	N.D.	N.D.	N.D.	0.08±0.02
1133TeCP	N.D.	N.D.	N.D.	N.D.	N.D.	0.24±0.02
1133TeBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCNM	N.D.	N.D.	N.D.	N.D.	N.D.	0.15±0.02
BCNM	N.D.	N.D.	N.D.	N.D.	N.D.	1.3±0.3
DBNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCM	N.D.	N.D.	N.D.	N.D.	0.51±0.01	4.0
TBM	N.D.	N.D.	N.D.	N.D.	N.D.	0.03
DBCM	N.D.	N.D.	0.99±0.12	N.D.	N.D.	N.D.
BDCM	N.D.	N.D.	N.D.	N.D.	N.D.	2.0
DCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 4			WWTP 5		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**
CDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCAM	N.D.	N.D.	N.D.	N.D.	N.D.	0.43±0.05
IAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCAM	N.D.	N.D.	N.D.	N.D.	N.D.	0.19±0.02
DBAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDCAM	N.D.	N.D.	N.D.	N.D.	N.D.	0.12±0.01
CIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BIAM	N.D.	N.D.	0.06±0.01	N.D.	N.D.	N.D.
DBCAM	N.D.	N.D.	0.03±0.02	N.D.	N.D.	N.D.
TBAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 4			WWTP 5		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**
CAA	NM	NM	NM	NM	NM	NM
BAA	NM	NM	NM	NM	NM	NM
DCAA	NM	NM	NM	NM	NM	NM
TCAA	NM	NM	NM	NM	NM	NM
BCAA	NM	NM	NM	NM	NM	NM
BDCAA	NM	NM	NM	NM	NM	NM
DBAA	NM	NM	NM	NM	NM	NM
DBCAA	NM	NM	NM	NM	NM	NM
TBAA	NM	NM	NM	NM	NM	NM
IAA	N.D.	N.D.	0.03±0.00	N.D.	N.D.	0.04±0.02
CIAA	N.D.	N.D.	N.D.	N.D.	N.D.	0.03±0.00
BIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a] Values reported as average ± standard error of duplicate measurements; N.D.: non-detect; NM: not measured

Table A.6. DBP concentrations (µg/L) in upstream (U), downstream (D), and effluent (Eff) samples from 2020.

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
TCAL	N.D.	0.03±0.00	0.05±0.00	N.D.	0.10±0.00	0.25±0.00	N.D.	0.54±0.01	0.85±0.01
BDCAL	N.D.	N.D.	0.03±0.00	N.D.	0.05±0.00	0.05±0.00	N.D.	0.12±0.00	0.22±0.01
DBCAL	N.D.	N.D.	0.03±0.00	N.D.	N.D.	N.D.	N.D.	0.02±0.00	0.22±0.00
TBAL	N.D.	N.D.	0.01±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	0.02±0.00
TCAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAN	N.D.	0.01±0.00	0.07±0.01	N.D.	0.11±0.01	0.31±0.00	N.D.	0.34±0.01	0.55±0.00
CAN	N.D.	N.D.	N.D.	N.D.	0.05±0.00	0.08±0.00	N.D.	N.D.	N.D.
BCAN	N.D.	N.D.	0.03±0.00	N.D.	0.01±0.001	0.16±0.00	N.D.	0.11±0.01	0.28±0.00
BAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBAN	N.D.	N.D.	N.D.	N.D.	0.05±0.00	N.D.	N.D.	N.D.	0.08±0.00
IAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
11DCP	N.D.	N.D.	0.13±0.00	N.D.	0.13±0.00	0.29±0.00	N.D.	0.70±0.02	1.3±0.0
CP	N.D.	N.D.	0.06±0.00	N.D.	0.05±0.01	0.23±0.00	N.D.	0.20±0.00	0.27±0.00
111TCP	N.D.	0.02±0.00	0.05±0.03	N.D.	0.13±0.00	0.19±0.00	N.D.	0.46±0.02	0.60±0.00

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
11DBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.10±0.00
1B11DCP	N.D.	0.03±0.00	0.06±0.00	N.D.	0.13±0.02	0.25±0.02	N.D.	0.25±0.03	0.40±0.01
13DCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
113TCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.25±0.00
1133TeCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.07±0.00
1133TeBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.06±0.00
DCNM	N.D.	N.D.	0.02±0.00	N.D.	0.002±0.000	0.06±0.000	N.D.	0.06±0.00	0.09± 0.00
BCNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.03±0.00
DBNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCM	0.10±0.00	0.30±0.01	1.2±0.1	N.D.	0.78±0.01	1.5±0.1	N.D.	2.5±0.00	3.1±0.6
TBM	0.04±0.00	0.02±0.00	0.10±0.00	N.D.	0.52±0.01	1.1±0.00	N.D.	0.02±0.00	0.05±0.00
DBCM	N.D.	0.13±0.01	0.401±0.02	N.D.	1.3±0.0	2.9±0.1	N.D.	0.37±0.02	0.58±0.01
BDCM	N.D.	0.13±0.01	0.46±0.02	N.D.	1.3±0.03	2.6±0.0	N.D.	1.0±0.0	2.1±0.0
DCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.07±0.00

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
BCIM	N.D.	N.D.	0.03±0.00	N.D.	0.06±0.00	0.01±0.00	N.D.	0.06±0.00	0.08±0.00
DBIM	N.D.	N.D.	0.03±0.00	N.D.	0.04±0.00	0.01±0.00	N.D.	N.D.	N.D.
CDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAM	N.D.	N.D.	0.31±0.03	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCAM	N.D.	N.D.	0.02±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBAM	N.D.	N.D.	N.D.	N.D.	N.D.	0.17±0.00	N.D.	N.D.	N.D.
BDCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BIAM	0.06±0.00	0.06±0.02	0.10±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
DBCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TBAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAA	N.D.	N.D.	N.D.	N.D.	N.D.	0.27±0.03	N.D.	0.91±0.13	0.33±0.03
BAA	N.D.	N.D.	N.D.	0.02±0.00	0.30±0.01	0.64±0.13	0.03±0.00	2.1±0.4	2.6±0.1
DCAA	N.D.	N.D.	N.D.	N.D.	0.23±0.01	0.79±0.25	N.D.	1.2±0.0	2.2±0.1
TCAA	N.D.	N.D.	N.D.	N.D.	0.39±0.00	0.60±0.10	N.D.	1.25±0.01	1.4±0.04
BCAA	N.D.	N.D.	N.D.	N.D.	0.40±0.00	0.80±0.27	N.D.	0.61±0.06	1.2±0.02
BDCAA	N.D.	N.D.	N.D.	N.D.	0.32±0.01	0.62±0.06	N.D.	0.74±0.02	0.71±0.10
DBAA	N.D.	N.D.	N.D.	N.D.	0.49±0.02	1.1±0.3	N.D.	0.32±0.01	0.46±0.11
DBCAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TBAA	N.D.	N.D.	N.D.	N.D.	0.02±0.00	0.04±0.00	N.D.	N.D.	N.D.
IAA	N.D.	N.D.	N.D.	N.D.	N.D.	0.01±0.00	N.D.	N.D.	0.04±0.00
CIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01±0.00

	WWTP 1			WWTP 2			WWTP 3		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
DIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a] Values reported as average ± standard error of duplicate measurements; N.D.: non-detect; NM: not measured

Table A.7 DBP concentrations (µg/L) in upstream (U), downstream (D), and effluent (Eff) samples from 2021 (WWTP 1-4)

	WWTP 1			WWTP 2			WWTP 3			WWTP 4		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
TCAL	N.D.	N.D.	0.01±0.00	N.D.	0.54±0.01	1.5±0.0	N.D.	0.43±0.00	0.84±0.01	N.D.	0.08±0.02	0.21±0.01
BDCAL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.22±0.01	0.46±0.02	N.D.	N.D.	N.D.
DBCAL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.03±0.00	N.D.	N.D.	N.D.
TBAL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAN	N.D.	N.D.	N.D.	N.D.	0.62±0.04	0.77±0.03	N.D.	0.24±0.02	0.42±0.01	N.D.	0.03±0.00	0.22±0.02
CAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCAN	N.D.	N.D.	N.D.	N.D.	0.04±0.00	0.18±0.01	N.D.	N.D.	0.02±0.00	N.D.	N.D.	N.D.
BAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
11DCP	N.D.	N.D.	0.14±0.00	N.D.	0.34±0.01	0.66±0.05	N.D.	0.68±0.01	1.3±0.0	N.D.	N.D.	N.D.
CP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
111TCP	N.D.	N.D.	0.04±0.00	N.D.	0.36±0.01	0.61±0.00	N.D.	0.45±0.00	0.83±0.00	N.D.	0.08±0.02	0.17±0.01

	WWTP 1			WWTP 2			WWTP 3			WWTP 4		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
11DBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1B11DCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.07±0.01	N.D.	N.D.	N.D.
13DCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
113TCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.13±0.00	N.D.	N.D.	N.D.
1133TeCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.05±0.00	N.D.	N.D.	N.D.
1133TeBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.02±0.00	N.D.	N.D.	N.D.
DCNM	N.D.	N.D.	N.D.	N.D.	0.10±0.00	0.10±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCM	N.D.	0.51±0.03	2.5±0.3	N.D.	N.D.	6.7±0.8	N.D.	3.5±0.3	3.9±0.5	N.D.	1.8±0.4	3.5±0.1
TBM	N.D.	N.D.	0.08±0.01	N.D.	0.21±0.01	0.33±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBCM	N.D.	0.05±0.00	0.61±0.01	N.D.	2.6±0.1	2.8±0.01	N.D.	0.13±0.00	0.20±0.00	N.D.	0.14±0.02	0.15±0.00
BDCM	N.D.	0.10±0.01	0.63±0.01	N.D.	5.0±0.4	4.4±0.1	N.D.	0.74±0.02	1.1±0.00	N.D.	1.6±0.4	0.49±0.01
DCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.03±0.01	N.D.	N.D.	N.D.

	WWTP 1			WWTP 2			WWTP 3			WWTP 4		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
BCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01±0.00	N.D.	N.D.	N.D.
DBAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 1			WWTP 2			WWTP 3			WWTP 4		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
DBCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TBAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.59±0.19	0.86±0.04
BAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAA	N.D.	0.36±0.02	0.83±0.09	N.D.	2.0±0.2	1.7±0.1	N.D.	2.0±0.0	2.3±0.1	N.D.	1.4±0.5	1.6±0.1
TCAA	N.D.	0.87±0.08	2.8±0.2	N.D.	2.9±0.4	2.2±0.3	N.D.	1.6±0.0	1.9±0.1	N.D.	N.D.	N.D.
BCAA	N.D.	0.19±0.01	0.86±0.12	N.D.	2.0±0.3	1.8±0.2	N.D.	0.42±0.01	1.4±0.0	N.D.	0.14±0.05	0.26±0.00
BDCAA	N.D.	0.03±0.00	0.24±0.02	N.D.	0.61±0.13	0.45±0.00	N.D.	0.14±0.01	0.25±0.02	N.D.	0.09±0.03	0.18±0.00
DBAA	N.D.	0.13±0.00	0.27±0.01	N.D.	0.15±0.015	0.29±0.03	N.D.	0.02±0.00	0.05±0.01	N.D.	0.02±0.00	0.04±0.00
DBCAA	N.D.	N.D.	0.09±0.01	N.D.	0.26±0.07	0.23±0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TBAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IAA	N.D.	0.02±0.00	0.07±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CIAA	N.D.	N.D.	0.02±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 1			WWTP 2			WWTP 3			WWTP 4		
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**
DIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a] Values reported as average ± standard error of duplicate measurements; N.D.: non-detect

Table A.8 DBP concentrations (µg/L) in upstream (U), downstream (D), and effluent (Eff) samples from 2021 (WWTP 6-9)

	WWTP 6			WWTP 7			WWTP 8			WWTP 9	
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**Lake**	**Eff**
TCAL	N.D.	N.D.	0.02±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	0.12±0.00	N.D.	0.05±0.00
BDCAL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBCAL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TBAL	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAN	N.D.	N.D.	N.D.	N.D.	0.24±0.01	0.09±0.01	N.D.	N.D.	N.D.	N.D.	N.D.
CAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IAN	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
11DCP	N.D.	N.D.	0.45±0.02	N.D.	N.D.	0.43±0.02	N.D.	N.D.	0.10±0.02	N.D.	N.D.
CP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
111TCP	N.D.	N.D.	0.05±0.00	N.D.	N.D.	0.14±0.00	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 6			WWTP 7			WWTP 8			WWTP 9	
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**Lake**	**Eff**
11DBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1B11DCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
13DCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
113TCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1133TeCP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1133TeBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCNM	N.D.	N.D.	0.28±0.02	N.D.	N.D.	N.D.	N.D.	N.D.	0.28±0.03	N.D.	N.D.
DCNM	N.D.	N.D.	0.06±0.00	N.D.	N.D.	N.D.	N.D.	N.D.	0.36±0.03	N.D.	N.D.
BCNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBNM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCM	0.59±0.03	0.55±0.00	0.76±0.21	N.D.	0.08±0.01	0.99±0.04	N.D.	0.56±0.11	2.26±0.00	0.11±0.01	4.6±0.9
TBM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBCM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDCM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 6			WWTP 7			WWTP 8			WWTP 9	
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**Lake**	**Eff**
BCIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DBIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TIM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TCAM	N.D.	N.D.	N.D.	N.D.	N.D.	0.07±0.00	N.D.	N.D.	N.D.	N.D.	N.D.
DBAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BDCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 6			WWTP 7			WWTP 8			WWTP 9	
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**Lake**	**Eff**
DBCAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TBAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DIAM	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DCAA	N.D.	0.21±0.02	1.36±0.05	N.D.	0.13±0.01	1.08±0.06	N.D.	N.D.	0.55±0.01	N.D.	0.38±0.02
TCAA	0.49±0.00	0.67±0.02	2.14±0.20	N.D.	0.14±0.00	0.86±0.073	N.D.	0.54±0.083	6.7±0.4	N.D.	1.6±0.2
BCAA	N.D.	N.D.	N.D.	N.D.	N.D.	0.17±0.01	N.D.	N.D.	0.04±0.00	N.D.	N.D.
BDCAA	N.D.	N.D.	0.08±0.00	N.D.	N.D.	0.06±0.00	N.D.	N.D.	0.04±0.01	N.D.	N.D.
DBAA	N.D.	N.D.	0.03±0.00	N.D.	N.D.	N.D.	0.03±0.00	N.D.	0.04±0.00	N.D.	N.D.
DBCAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
TBAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
IAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
CIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
BIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

	WWTP 6			WWTP 7			WWTP 8			WWTP 9	
Abbreviation	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**U**	**D**	**Eff**	**Lake**	**Eff**
DIAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a] Values reported as average ± standard error of duplicate measurements; N.D.: non-detect

Table A.9. Concentration of iopamidol in upstream (U), downstream (D), and wastewater treatment plant effluent (Eff) samples from 2019 and 2020 (ng/L).[a]

Year	WWTP ID	Upstream A (U A)	Upstream B (U B)	Downstream A (D A)	Downstream B (D B)	Effluent A (Eff A)	Effluent B (Eff B)
2019 [c]	**WWTP 1**	<RL (75.2)	<RL (76.8)	<RL (174)	<RL (268)	917 (228)	1,190 (235)
	WWTP 2	<RL (247)	<RL (141)	<RL (73.8)	<RL (265)	<RL (210)	<RL (341)
	WWTP 3	NM	NM	NM	NM	NM	NM
	WWTP 4	<RL (214)	<RL (199)	<RL (308)	<RL (165)	<RL (234)	<RL (237)
	WWTP 5	<RL (263)	<RL (261)	<RL (177)	<RL (172)	<RL (143)	<RL (299)
2020 [d]	**WWTP 1**	<RL (76.2)	<RL (75.3)	309 (116)	352 (130)	881 (114)	1250 (173)
	WWTP 2	103 (74.2) [e]	82.6 (75.0) [e]	415 (73.4)	482 (75.6)	520 (74.5)	644 (73.9)
	WWTP 3	164 (75.5)	206 (76.8)	2520 (78.0)	3420 (113)	3080 (78.3)	3660 (82.6)

NM: not measured; RL: reporting limit.[a] Values reported as concentration in ng/L (RL); [c] samples collected on Monday; [d] samples collected on Wednesday; [e] peak detected but did not meet quantification criteria, result reported represents the estimated maximum possible concentration

Table A.10. Concentration of iopamidol in upstream (U), downstream (D), and wastewater treatment plant effluent (Eff) samples from 2021 (ng/L).[a]

Year	WWTP ID	Upstream A (U A)	Upstream B (U B)	Downstream A (D A)	Downstream B (D B)	Effluent A (Eff A)	Effluent B (Eff B)
2021 [d]	**WWTP 1**	NM	NM	254 (75.1)	227 (68.4)	814 (63.0)	733 (63.4)
	WWTP 2	NM	NM	3,080 (74.1)	3,420 (72.9)	4,270 (71.6)	4,730 (72.1)
	WWTP 3	NM	NM	1,510 (86.1)	1,420 (79.1)	1,820 (67.6)	1,910 (73.5)
	WWTP 4	NM	NM	<RL (72.9)	<RL (73.5)	<RL (73.0)	<RL (73.7)
	WWTP 6	NM	NM	2,920 (79.3)	3,390 (79.9)	31,100 (144)	28,700 (151)
	WWTP 7	NM	NM	<RL (73.8)	<RL (74.1)	<RL (75.3)	<RL (73.5)
	WWTP 8	NM	NM	<RL (74.8)	<RL (73.6)	<RL (74.3)	<RL (73.0)
	WWTP 9	NM	NM	<RL (78.7) [b]	<RL (79.2) [b]	<RL (79.3)	<RL (78.8)

NM: not measured; RL: reporting limit.[a] Values reported as concentration in ng/L (RL) [b] lake sample; [d] samples collected on Wednesday

Table A.11. Summary of the CHO cell chronic cytotoxicity analyses of upstream (U) and downstream (D) samples.

Year	WWTP ID	Lowest Cytotoxic. Conc. Factor [a]	Mean LC_{50} Conc. Factor ± SE [b]	Mean CTI ± SE [c]	r^2 [d]	ANOVA Test [e]
2019	WWTP 1 U	100.0	350.72 ± 25.4	2.89 ± 0.73	0.99	$F_{14, 94} = 132$; $P < 0.001$
	WWTP 1 D	100.0	111.34 ± 10.4	9.61 ± 0.71	0.99	$F_{12, 95} = 64.7$; $P < 0.001$
	WWTP 2 U	8.0	18.54 ± 1.20	56.91 ± 4.73	0.97	$F_{12, 94} = 98.6$; $P < 0.001$
	WWTP 2 D	10.0	23.86 ± 1.89	45.48 ± 4.26	0.97	$F_{12, 95} = 64.7$; $P < 0.001$
	WWTP 4 U	40.0	155.33 ± 11.0	6.83 ± 0.58	0.98	$F_{11, 101} = 66.3$; $P < 0.001$
	WWTP 4 D	15.0	41.20 ± 1.64	24.64 ± 0.93	0.96	$F_{12, 88} = 24.9$; $P < 0.001$
	WWTP 5 U	60.0	110.41 ± 5.04	9.34 ± 0.62	0.99	$F_{11,101} = 121$; $P < 0.001$
	WWTP 5 D	40.0	135.91 ± 9.19	7.96 ± 0.93	0.99	$F_{11,101} = 57.4$; $P < 0.001$
2020	WWTP 1 U	40.0	151.43 ± 4.19	6.65 ± 0.18	0.99	$F_{10,122} = 278$; $P < 0.001$
	WWTP 1 D	60.0	212.43 ± 6.88	4.76 ± 0.15	0.99	$F_{12,120} = 121$; $P < 0.001$
	WWTP 2 U	40.0	100.19 ± 4.44	10.16 ± 0.41	0.99	$F_{11,122} = 301$; $P < 0.001$
	WWTP 2 D	-	-	-	-	Sample lost in shipping

Year	WWTP ID	Lowest Cytotoxic. Conc. Factor [a]	Mean LC_{50} Conc. Factor ± SE [b]	Mean CTI ± SE [c]	r^2 [d]	ANOVA Test [e]
	WWTP 3 U	80.0	148.01 ± 5.11	6.83 ± 0.23	0.98	$F_{11,120} = 161$; $P < 0.001$
	WWTP 3 D	40.0	107.97 ± 4.71	9.45 ± 0.45	0.98	$F_{12,124} = 241$; $P < 0.001$

[a] Lowest cytotoxic concentration was the lowest concentration factor that induced a statistically significant reduction in cell density as compared to the concurrent negative control. [b] The LC_{50} value is the concentration of the water sample, determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative controls. [c] The cytotoxicity index is $(LC_{50}^{-1})(10^3)$; the larger the value the greater the toxicity. [d] r^2 is the coefficient of determination for the regression analysis upon which the LC_{50} value was calculated. [e] The degrees of freedom for the between-groups and residual associated with the calculated *F*-test result and the resulting probability value. To compare the upstream versus downstream sample cytotoxicity for each location pair, the light red fill indicates that the sample was statistically more toxic than the corresponding sample pair indicated by a green fill. If neither the upstream nor the downstream sample of a pair was significantly different, the CTI values were filled with an amber color.

Table A.12. Summary of the CHO cell SCGE genotoxicity analyses of upstream (U) and downstream (D) samples.

Year	WWTP ID	Lowest Genotoxic Conc. Factor [a]	Average 50% TDNA (CF ± SE) [b]	Average GTI (CF ± SE) [c]	r^2 [d]	ANOVA Test Statistic [e]
2019	**WWTP 1 US**	4000	11040.9 ± 82.3	0.091 ± 0.001	0.96	$F_{30, 73} = 19.9$; $P \leq 0.001$
	WWTP 1 DS	2000	4322.56 ± 52.16	0.232 ± 0.003	0.93	$F_{21, 64} = 27.2$; $P \leq 0.001$
	WWTP 2 US	600	946.09 ± 17.82	1.06 ± 0.02	0.90	$F_{8, 41} = 22.3$; $P \leq 0.001$
	WWTP 2 DS	400	1629.80 ± 119.27	0.642 ± 0.040	0.98	$F_{5, 47} = 54.8$; $P \leq 0.001$
	WWTP 4 US	2700	5508.67 ± 34.37	0.182 ± 0.001	0.88	$F_{17, 66} = 17.1$; $P \leq 0.001$
	WWTP 4 DS	800	1187.93 ± 30.00	0.847 ± 0.022	0.97	$F_{9, 43} = 43.6$; $P \leq 0.001$
	WWTP 5 US	600	5513.67 ± 254.81	0.185 ± 0.007	0.88	$F_{8, 33} = 32.9$; $P \leq 0.001$
	WWTP 5 DS	1400	6157.97 ± 97.04	0.163 ± 0.002	0.91	$F_{13, 46} = 28.7$; $P \leq 0.001$
2020	**WWTP 1 US**	1000	9278.17 ± 119.35	0.108 ± 0.001	0.99	$F_{9, 42} = 12.0$; $P \leq 0.001$
	WWTP 1 DS	1000	5108.11 ± 99.28	0.197 ± 0.003	0.88	$F_{7, 45} = 24.4$; $P \leq 0.001$

Year	WWTP ID	Lowest Genotoxic Conc. Factor [a]	Average 50% TDNA (CF ± SE) [b]	Average GTI (CF ± SE) [c]	r^2 [d]	ANOVA Test Statistic [e]
2020	WWTP 2 US	2000	4046.95 ± 24.45	0.247 ± 0.001	0.96	$F_{7, 25} = 23.8$; $P \leq 0.001$
	WWTP 2 DS	–	–	–	–	Sample lost in shipping
	WWTP 3 US	1400	2323.59 ± 13.56	0.431 ± 0.003	0.88	$F_{8, 26} = 12.6$; $P \leq 0.001$
	WWTP 3 DS	1280	1842.65 ± 13.46	0.543 ± 0.004	0.97	$F_{13, 31} = 37.2$; $P \leq 0.001$

[a] Lowest genotoxic concentration was the lowest concentration factor of the MN sample that induced a statistically significant increase in the electrophoretic migration of genomic DNA from the nucleus as compared to the negative control. [b] The mean SCGE 50% Tail DNA value is the concentration of the MN sample, determined from a regression analysis of the data, that induced a DNA migration from the nuclei of 50%. The mean and the standard error (SE) were derived from multiple regression analyses using bootstrap statistics. [c] Genotoxicity index (GTI) value is the 50% Tail $DNA^{-1} \times 10^3$. [d] The r^2 is the coefficient of determination for the regression analysis of the concentration-response data upon which the 50%Tail DNA value was calculated. [e] The degrees of freedom for the between-groups and residual associated with the calculated *F*-test result and the resulting probability value. To compare the upstream versus downstream sample genotoxicity for each location pair, the light red fill indicates that the sample was statistically more toxic than the corresponding sample pair indicated by a green fill. If neither the upstream nor the downstream sample of a pair was significantly different, the GTI values were filled with an amber color.

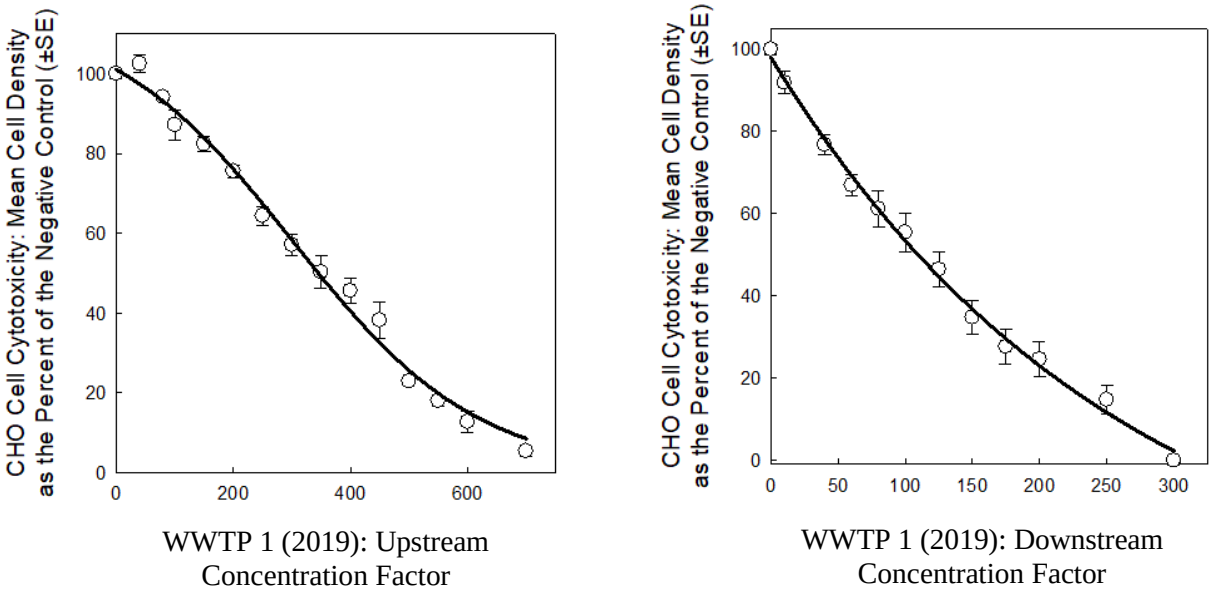


Figure A.1. CHO cell cytotoxicity concentration-response curve for upstream and downstream samples from WWTP 1 (2019).

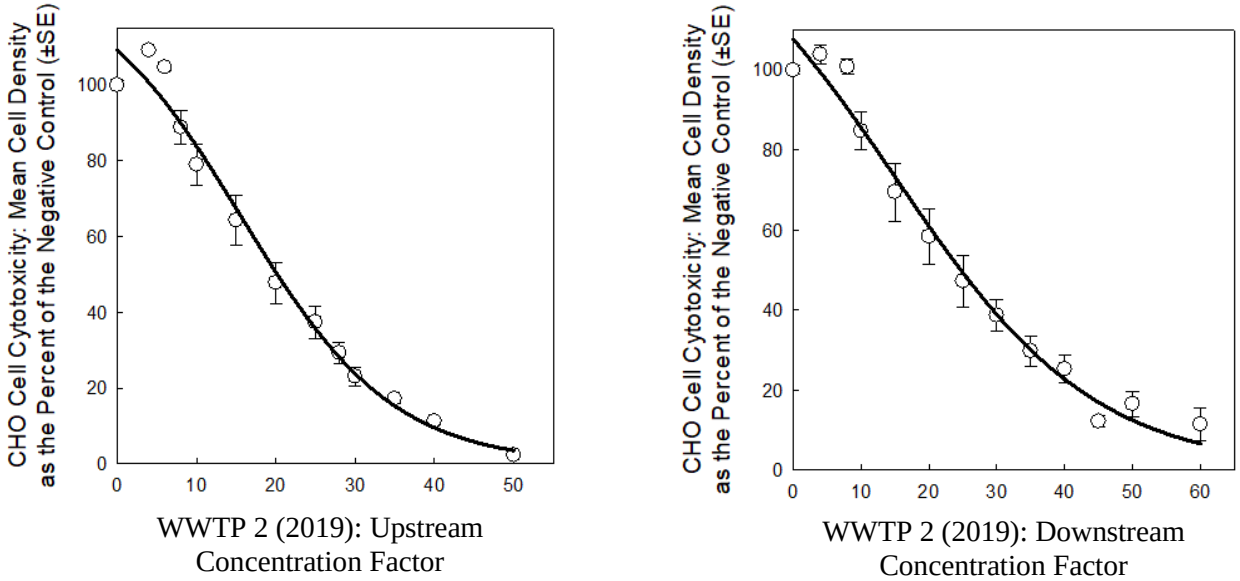


Figure A.2. CHO cell cytotoxicity concentration-response curve for upstream and downstream samples from WWTP 2 (2019).

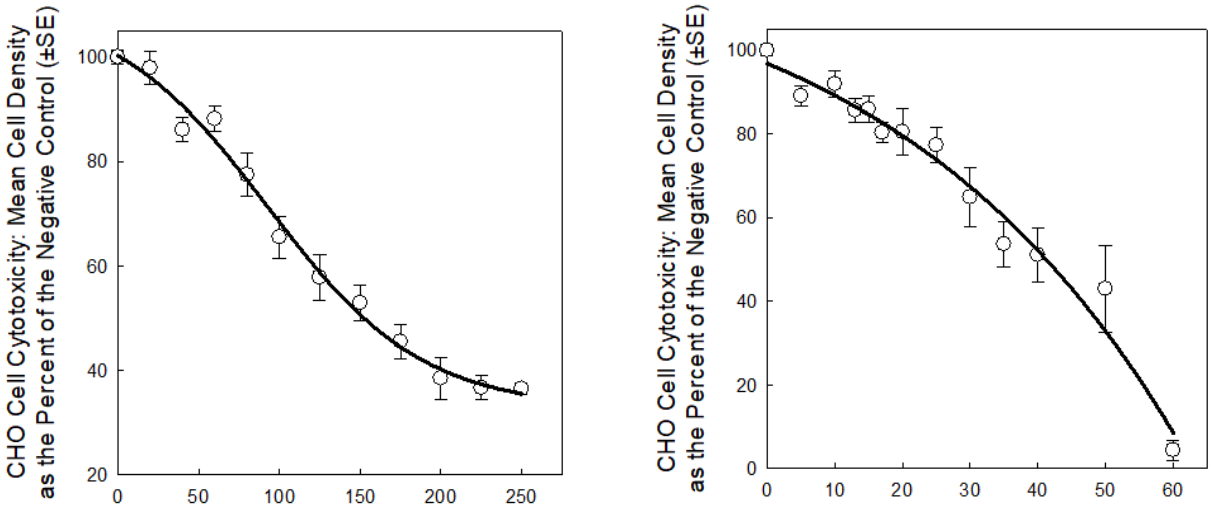


Figure A.3. CHO cell cytotoxicity concentration-response curve for upstream and downstream samples from WWTP 4 (2019).

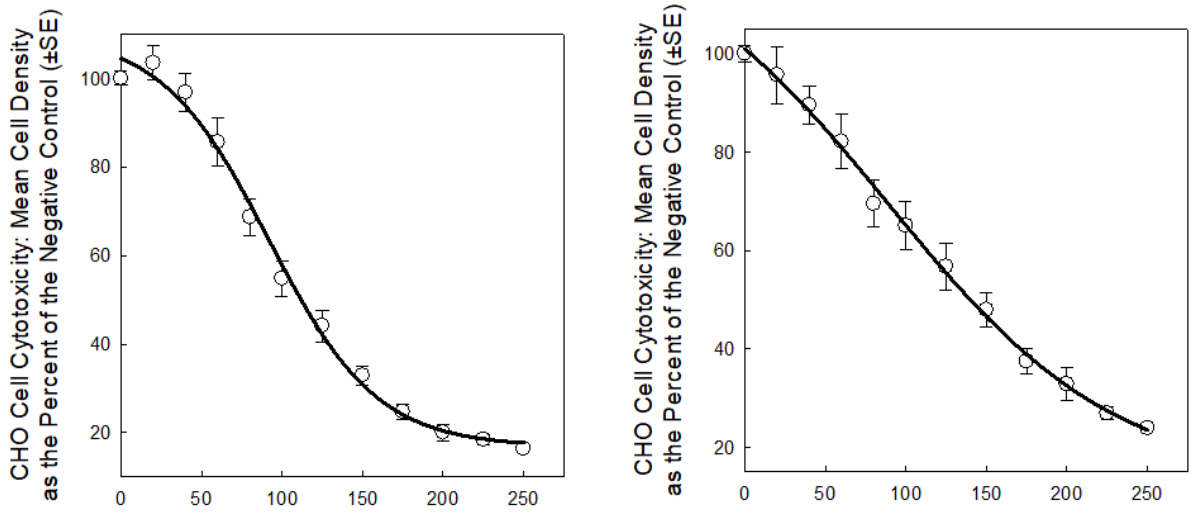


Figure A.4. CHO cell cytotoxicity concentration-response curve for upstream and downstream samples from WWTP 5 (2019).

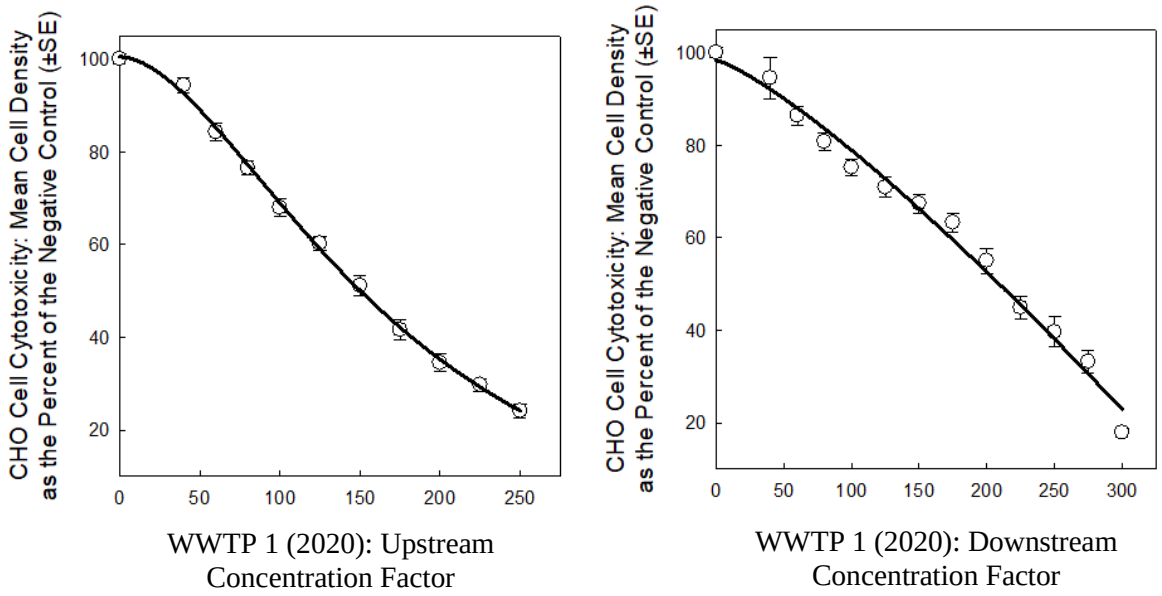


Figure A.5. CHO cell cytotoxicity concentration-response curve for upstream and downstream samples from WWTP 1 (2020).

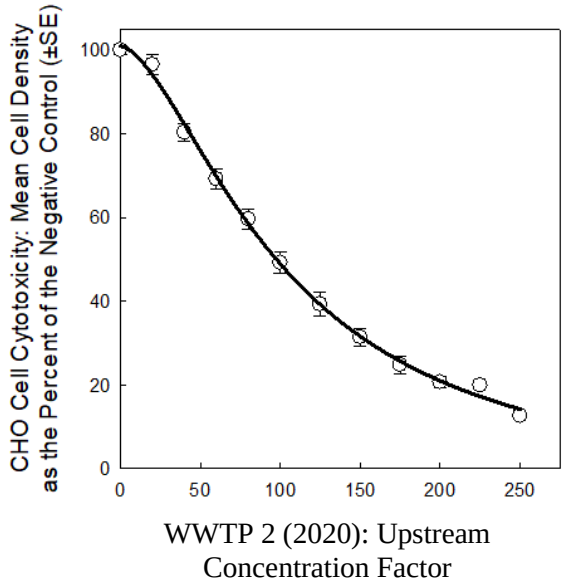


Figure A.6. CHO cell cytotoxicity concentration-response curve for upstream sample from WWTP 2 (2020).

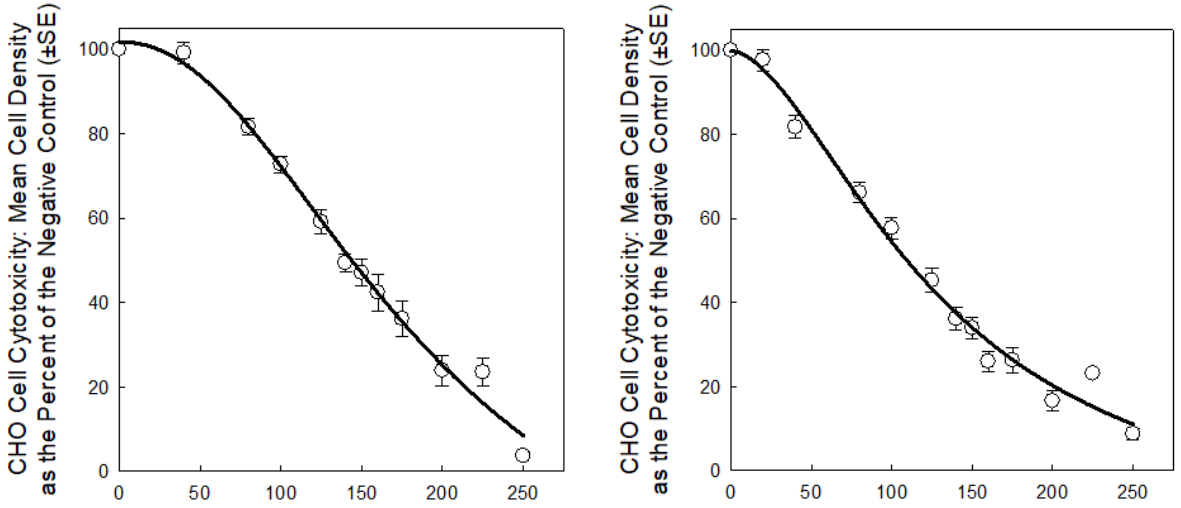


Figure A.7. CHO cell cytotoxicity concentration-response curve for upstream and downstream samples from WWTP 3 (2020).

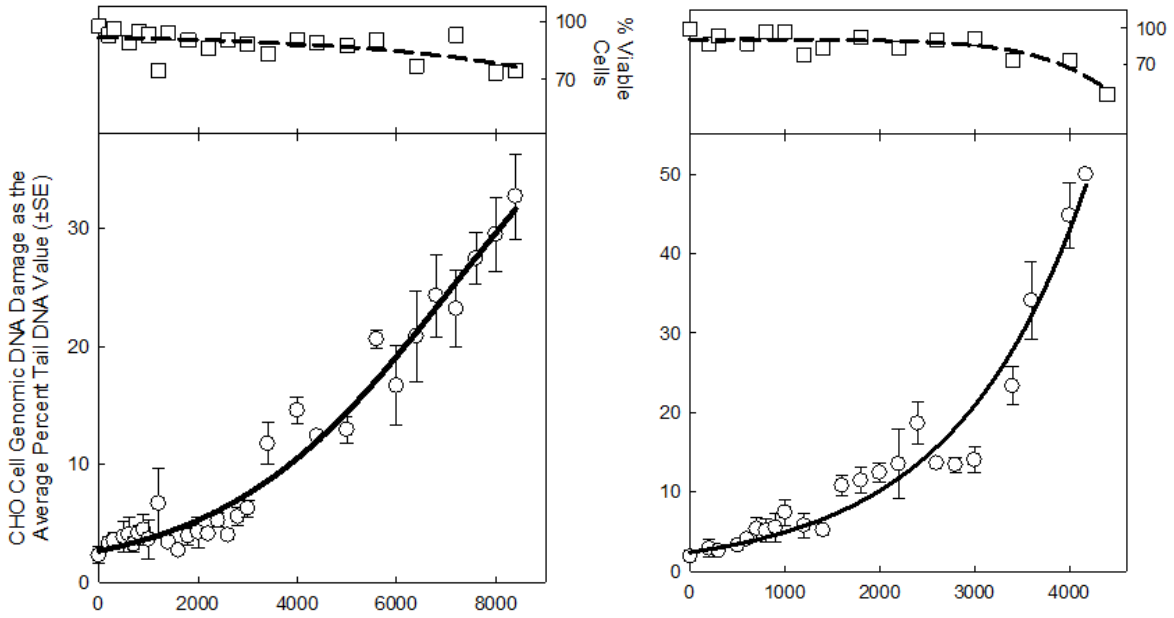


Figure A.8. CHO cell genomic DNA damage concentration-response curve (bottom panel) and acute cytotoxicity (upper panel) for WWTP 1 (2019).

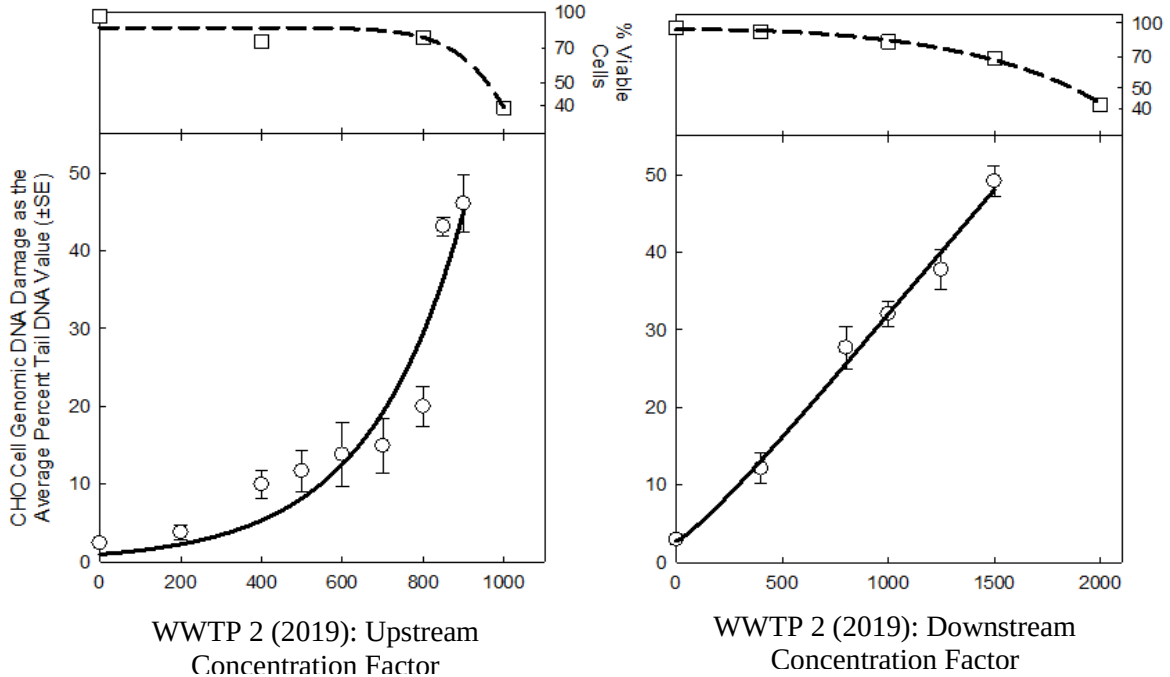


Figure A.9. CHO cell genomic DNA damage concentration-response curve (bottom panel) and acute cytotoxicity (upper panel) for WWTP 2 (2019).

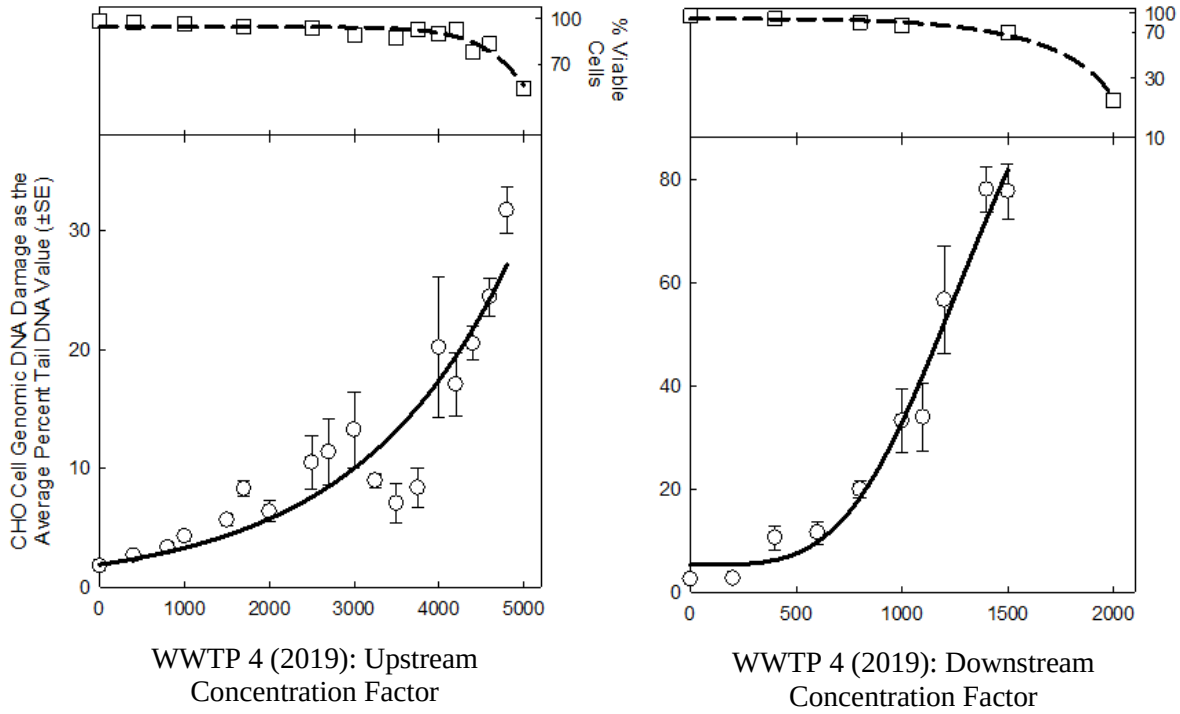
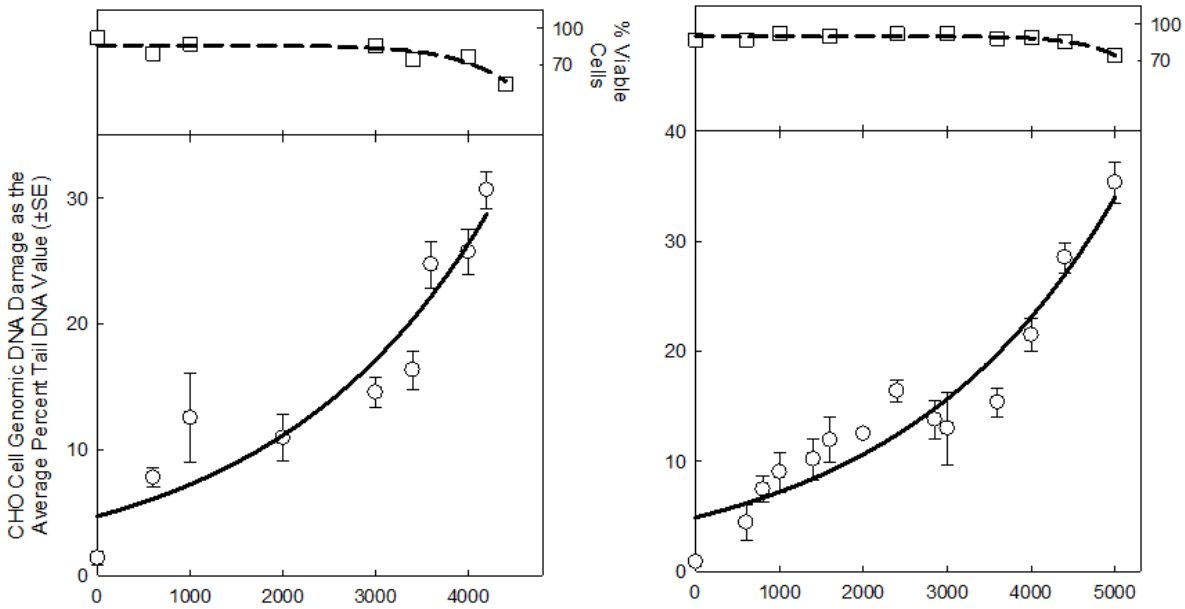


Figure A.10. CHO cell genomic DNA damage concentration-response curve (bottom panel) and acute cytotoxicity (upper panel) for WWTP 4 (2019).



WWTP 5 (2019): Upstream Concentration Factor

WWTP 5 (2019): Downstream Concentration Factor

Figure A.11. CHO cell genomic DNA damage concentration-response curve (bottom panel) and acute cytotoxicity (upper panel) for WWTP 5 (2019).

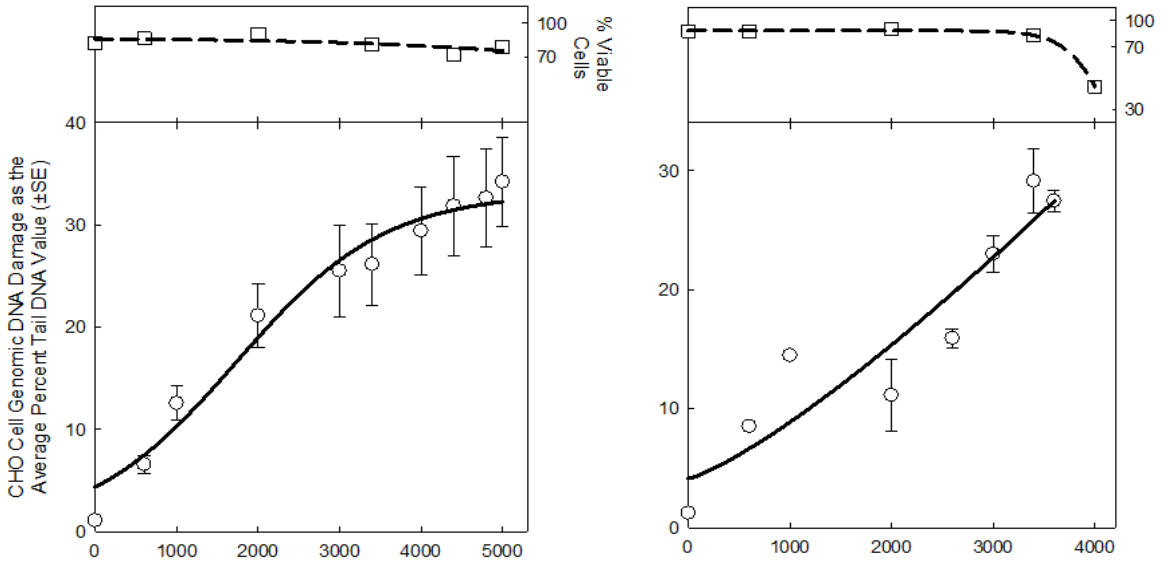


Figure A.12. CHO cell genomic DNA damage concentration-response curve (bottom panel) and acute cytotoxicity (upper panel) for WWTP 1 (2020).

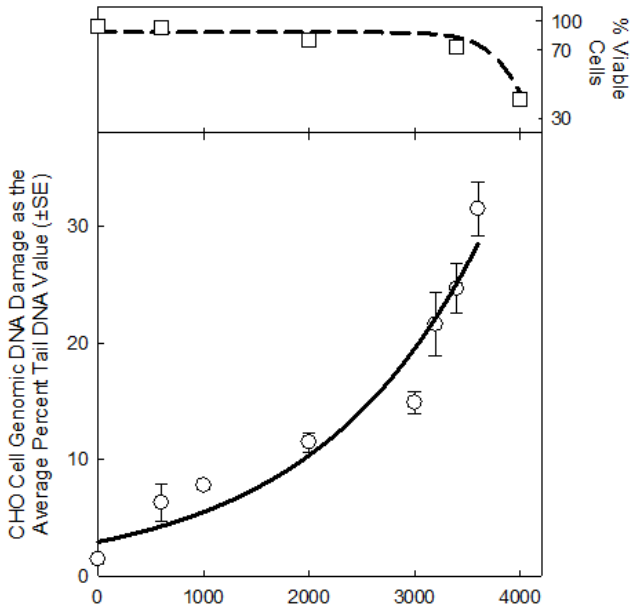


Figure A.13. CHO cell genomic DNA damage concentration-response curve (bottom panel) and acute cytotoxicity (upper panel) for WWTP 2 (2020).

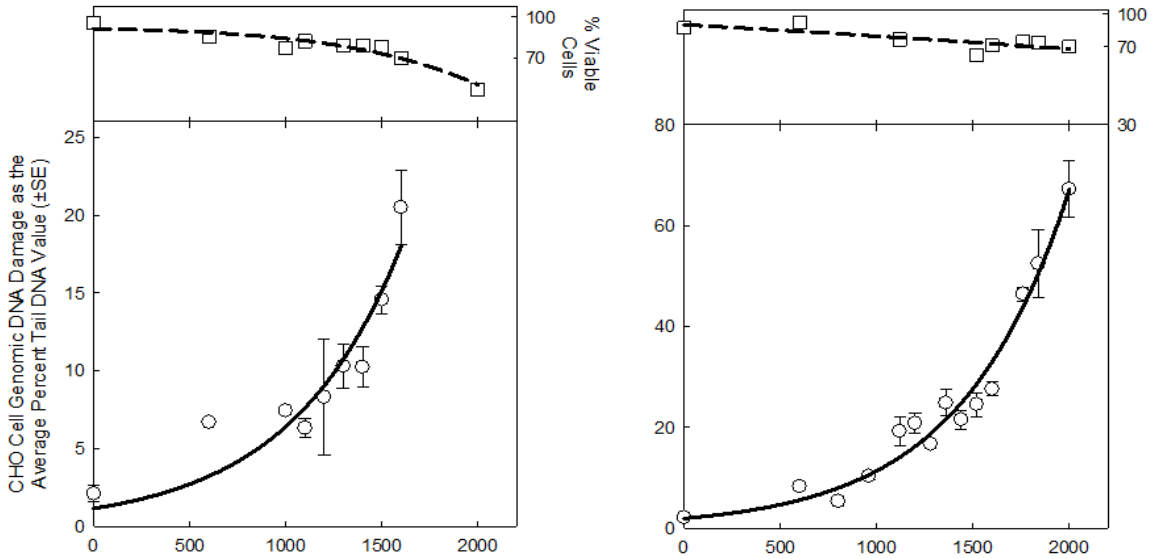


Figure A.14. CHO cell genomic DNA damage concentration-response curve (bottom panel) and acute cytotoxicity (upper panel) for WWTP 3 (2020).

APPENDIX B

SUPPORTING INFORMATION FOR CHAPTER 3

Table B.1. Specifications for salt chlorine generator for salt water pool (Saline C 11.0; Hayward; Rockville, MD).

Rated Power in DC (Amps)	Rated Pressure (psi)	Min. Water Flow Rate (gpm*)	Max. Water Flow Rate (gpm*)	Inlet/Outlet Diameter (Inches)
72	50 psi	80	250	4

*gpm = gallons per minute

Table B.2. Quantifier and qualifier ions and limits of quantification (LOQs) for DBPs quantified in this study.

DBP class	Name	Abbreviation	Quantifier ion (*m/z*)	Qualifier ion (*m/z*)	LOQ* (µg/L)
Haloacetaldehydes (HALs)	Trichloroacetaldehyde [a]	TCAL	82.0	110.9	0.1
	Bromodichloroacetaldehyde [b]	BDCAL	83.0	111.0/163.8	0.1
	Dibromochloroacetaldehyde [b]	DBCAL	128.9	127.9	0.1
	Tribromoacetaldehyde [a]	TBAL	172.8	171.8	0.1
Haloacetonitriles (HANs)	Trichloroacetonitrile [a]	TCAN	108.0	110.0	0.1
	Dichloroacetonitrile [a]	DCAN	74.0	82.0	0.1
	Chloroacetonitrile [a]	CAN	75.0	77.0	0.1
	Bromochloroacetonitrile [a]	BCAN	153.0	155.0	0.1
	Bromoacetonitrile [a]	BAN	118.9	120.9	0.1
	Dibromoacetonitrile [a]	DBAN	117.9	199.0	0.1
	Iodoacetonitrile [a]	IAN	167.0	126.9	0.1
Haloketones (HKs)	1,1-Dichloropropanone [a]	11DCP	63.0	83.0	0.1
	Chloropropanone [a]	CP	92.0	94.0	0.1
	1,1,1-Trichloropropanone [a]	111TCP	125.0	127.0	0.1
	1,1-Dibromopropanone [b]	11DBP	216.0	218.0	0.1
	1-Bromo-1,1-dichloropropanone [b]	1B11DCP	125.0	127.0	0.1
	1,3-Dichloropropanone [b]	13DCP	77.0	49.0	0.1

Haloketones (HKs)	1,1,3-Trichloropropanone [a]	113TCP	77.0	83.0	0.1
	1,1,3,3-Tetrachloropropanone [b]	1133TeCP	83.0	85.0	0.1
	1,1,3,3-Tetrabromopropanone [c]	1133TeBP	200.8	119.9	0.1
Halonitromethanes (HNMs)	Trichloronitromethane [a]	TCNM	116.9	119.0	0.1
	Dichloronitromethane [b]	DCNM	83.0	85.0	0.1
	Bromochloronitromethane [b]	BCNM	129.0	127.0	0.1
	Dibromonitromethane [b]	DBNM	172.8	171.0	0.1
Trihalomethanes (THMs)	Trichloromethane [a]	TCM	83.0	85.0	0.1
	Tribromomethane [a]	TBM	173.0	252.0	0.1
	Dibromochloromethane [a]	DBCM	129.0	127.0	0.1
	Bromodichloromethane [a]	BDCM	83.0	129.0	0.1
Iodinated-trihalomethanes (I-THMs)	Dichloroiodomethane [b]	DCIM	83.0	126.9	0.1
	Bromochloroiodomethane [b]	BCIM	128.9	126.9	0.1
	Dibromoiodomethane [b]	DBIM	172.8	299.7	0.1
	Chlorodiiodomethane [b]	CDIM	174.9	126.9	0.1
	Bromodiiodomethane [b]	BDIM	218.8	220.8	0.1
	Iodoform [a]	TIM	393.7	266.8	0.1

Haloacetamides (HAMs)	Chloroacetamide [a]	CAM	93.0	44.0	0.1
	Bromoacetamide [a]	BAM	137.0	44.0	0.1
	Dichloroacetamide [b]	DCAM	44.0	127.0	0.1
	Bromochloroacetamide [b]	BCAM	44.0	173.0	0.1
	Iodoacetamide [a]	IAM	158.0	85.0	0.1
	Trichloroacetamide [a]	TCAM	44.0	82.0	0.1
	Dibromoacetamide [d]	DBAM	44.0	217.0	0.1
	Bromodichloroacetamide [b]	BDCAM	44.0	128.0	0.1
	Chloroiodoacetamide [b]	CIAM	92.0	219.0	0.1
	Bromoiodoacetamide [b]	BIAM	136.0	138.0	0.1
	Dibromochloroacetamide [b]	DBCAM	44.0	128.0	0.1
	Tribromoacetamide [b]	TBAM	44.0	295.0	0.1
	Diiodoacetamide [b]	DIAM	184.0	311.0	0.1
Haloacetic acids (HAAs)	Chloroacetic acid [a]	CAA	108.0	77.0	0.1
	Dichloroacetic acid [a]	DCAA	83.0	85.0	0.1
	Trichloroacetic acid [a]	TCAA	119.0	117.0	0.1
	Bromoacetic acid [a]	BAA	152.0	154.0	0.1
	Dibromoacetic acid [a]	DBAA	173.0	175.0	0.1
	Tribromoacetic acid [a]	TBAA	251.0	253.0	0.1

Haloacetic acids (HAAs)	Bromochloroacetic acid [a]	BCAA	129.0	127.0	0.1
	Dibromochloroacetic acid [a]	DBCAA	207.0	209.0	0.1
	Bromodichloroacetic acid [a]	BDCAA	163.0	161.0	0.1
Iodoacetic acids (IAAs)	Iodoacetic acid [a]	IAA	200.0	73.0	0.1
	Chloroiodoacetic acid [b]	CIAA	234.0	175.0	0.1
	Bromoiodoacetic acid [b]	BIAA	151.0	278.0	0.1
	Diiodoacetic acid [b]	DIAA	326.0	199.0	0.1

[a]Sigma-Aldrich. [b]CanSyn Chem. Corp. [c]Aldlab Chemicals. [d]TCI America. * Limit of quantification

APPENDIX C

SUPPORTING INFORMATION FOR CHAPTER 4

Total organic halogen (TOX) method

Total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI) were determined using a previously published procedure with a few modifications.[58,61,137] For boiled tap water, samples were quenched with ascorbic acid in slight excess (chlorine to ascorbic acid molar ratio of 1:1.3) and adjusted to pH <2 with concentrated HNO_3. For tea, since no residual chlorine was present, no quenching was used, but samples were adjusted to pH <2 with HNO_3. Samples were processed in triplicate with a sample adsorption and combustion unit (Mitsubishi, Chigasaki, Japan; Cosa Xentaur, Yaphank, NY). A 50 mL aliquot was passed through two activated carbons (ACs) that adsorb organohalogen compounds. Then, the AC columns were washed with sodium nitrate (5 mL of 5 mg $NaNO_3^-$ per mL) to remove inorganic halides and other inorganics. Each AC 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 H2O2 solution (0.03%, prepared daily), and the gas line was washed with an additional 3 mL of H2O2 solution. Centrifuge tubes were weighed before and after collecting the off gases to determine exact volumes. Recovered aqueous solutions were analyzed for chloride, bromide, and iodide with a Dionex 1600 ion chromatograph (Dionex, Sunnyvale, CA) as described elsewhere.[61,137]

APPENDIX D

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Do DBPs swim in salt water pools? Comparison of 60 DBPs formed by electrochemically generated chlorine vs. conventional chlorine

Author: Caroline O. Granger,Susan D. Richardson
Publication: Journal of Environmental Sciences
Publisher: Elsevier
Date: Available online 14 May 2022

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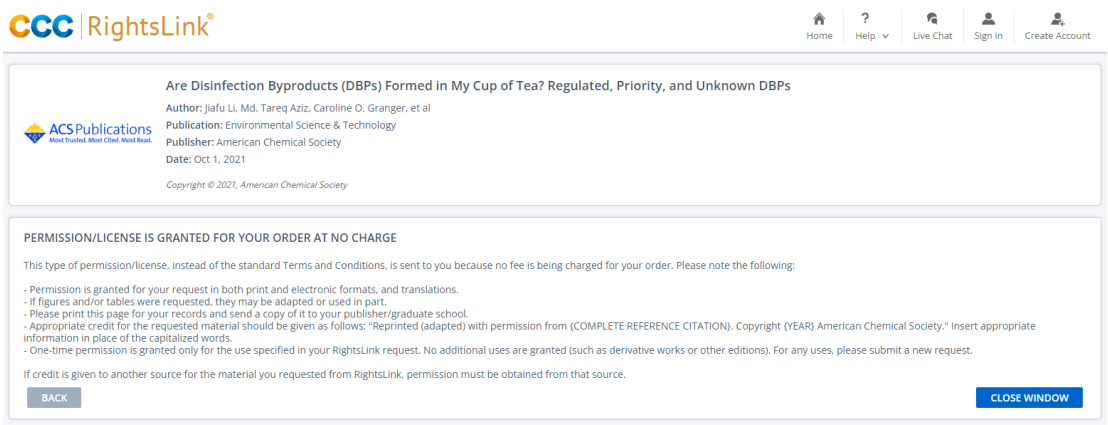

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Are Disinfection Byproducts (DBPs) Formed in My Cup of Tea? Regulated, Priority, and Unknown DBPs

Author: Jiafu Li, Md. Tareq Aziz, Caroline O. Granger, et al
Publication: Environmental Science & Technology
Publisher: American Chemical Society
Date: Oct 1, 2021

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APPENDIX E

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Do DBPs swim in salt water pools? Comparison of 60 DBPs formed by electrochemically generated chlorine vs. conventional chlorine ☆

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ABSTRACT

Disinfectants are added to swimming pools to kill harmful pathogens. Although liquid chlorine (sodium hypochlorite) is the most commonly used disinfectant, alternative disinfection techniques like electrochemically generated mixed oxidants or electrochemically generated chlorine, often referred to as salt water pools, are growing in popularity. However, these disinfectants react with natural organic matter and anthropogenic contaminants introduced to the pool water by swimmers to form disinfection byproducts (DBPs). DBPs have been linked to several adverse health effects, such as bladder cancer, adverse birth outcomes, and asthma. In this study, we quantified 60 DBPs using gas chromatography-mass spectrometry and assessed the calculated cytotoxicity and genotoxicity of an indoor community swimming pool before and after switching to a salt water pool with electrochemically generated chlorine. Interestingly, the total DBPs increased by 15% upon implementation of the salt water pool, but the calculated cytotoxicity and genotoxicity decreased by 45% and 15%, respectively. Predominant DBP classes formed were haloacetic acids, with trichloroacetic acid and dichloroacetic acid contributing 57% of the average total DBPs formed. Haloacetonitriles, haloacetic acids, and haloacetaldehydes were the primary drivers of calculated cytotoxicity, and haloacetic acids were the primary driver of calculated genotoxicity. Diiodoacetic acid, a highly toxic iodinated DBP, is reported for the first time in swimming pool water. Bromide impurities in sodium chloride used to electrochemically generate chlorine led to a 73% increase in brominated DBPs, primarily driven by bromochloroacetic acid. This study presents the most extensive DBP study to-date for salt water pools.

Introduction

In the United States, swimming is the fourth most popular recreational activity (U.S. Census Bureau, 2012). To limit swimmers' exposure to harmful viruses, bacteria, fungi, and algae, swimming pools are treated with disinfectants like chlorine, bromine, ultraviolet radiation (UV), or ozone (World Health Organization, 2006). However, these disinfectants react with natural organic matter (NOM) and anthropogenic contaminants

☆ This manuscript is dedicated to Prof. Michael Plewa, whose innovative and pioneering research has been a catalyst for improving the safety of drinking water and pools.

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introduced to the pool water by swimmers to form disinfection byproducts (DBPs) (Kim et al., 2002; LaKind et al., 2010; Daiber et al., 2016; Allen et al., 2021). Several epidemiologic studies have linked DBPs to bladder cancer, birth defects, miscarriage, and respiratory issues such as asthma (Villanueva et al., 2007a; Cardador and Gallego, 2011; Villanueva and Font-Ribera, 2012; Fornander et al., 2013; Parrat et al., 2012; Wright et al., 2017; Bove et al., 2002; Font-Ribera et al., 2019; Richardson et al., 2007; Bernard et al., 2006). Furthermore, studies have shown that dermal exposure to halogenated DBPs is an important exposure route to consider in swimming pool studies, due to the permeability of some DBPs across the skin (Xu et al, 2002; Xiao et al., 2012).

Studies quantifying DBPs in swimming pools have primarily focused on trihalomethanes (THMs) and haloacetic acids (HAAs). However, with more than 700 DBPs identified to date, many of which are more cytotoxic, genotoxic, or carcinogenic than THMs and HAAs, there is a clear need for the expansion of the classes of DBPs quantified in swimming pools. In recent years, brominated and iodinated DBPs have become of particular interest due to their elevated levels of toxicity when compared to chlorinated DBPs (Richardson et al., 2007; Wagner et al., 2017; Yang et al., 2014). Additionally, nitrogenous DBPs (N-DBPs) are generally more toxic than carbonaceous DBPs (C-DBPs) (Plewa et al., 2008). Recent studies completed in the United States and Australia have expanded on the DBPs quantified in pools using a variety of disinfection techniques to include priority, unregulated DBPs such as iodinated trihalomethanes (I-THMs), iodoacetic acids (IAAs), haloacetaldehydes (HALs), haloketones (HKs), haloacetamides (HAMs), haloacetonitriles (HANs), and halonitromethanes (HNMs) (Allen et al., 2021; Carter et al., 2019). Despite chlorine being the most commonly used disinfectant in swimming pools around the world, alternative disinfection techniques are becoming more common. One such alternative disinfection technique is electrochemically generated chlorine (or salt water pools) which works by passing an electric current through a concentrated salt solution (sodium chloride) to produce hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) as the primary oxidants (U.S. Army Center for Health Promotion and Preventive Medicine, 2006). Previous studies have shown that when compared to pools disinfected with chlorine, salt water pools had lower levels of HAAs (dichloroacetic acid and trichloroacetic acid) and trichloroacetaldehyde, but higher levels of Br-DBPs, likely due to bromide impurities in the sodium chloride (Lee et al., 2009, 2010). This increase in bromide is an important factor to monitor because previous studies show that dichloroacetic acid and trichloroacetic acid do not significantly contribute to the cytotoxicity or genotoxicity of pool waters (Yeh et al., 2014). However, there is currently no comprehensive DBP or toxicity data available that provides a direct comparison between conventional chlorine and salt water pools. To address this, a study of 60 DBPs was conducted at an indoor community pool in South Carolina while the pool was disinfecting with conventional liquid chlorine (sodium hypochlorite) and then after the implementation of electrochemically generated chlorine technology (salt water pools). Using DBP data collected over three sampling events, the calculated cytotoxicity and genotoxicity associated with each disinfection type was determined to better understand the impact each disinfection technology has on overall calculated toxicity, as well as the drivers of toxicity for each disinfection technique.

1. Materials and methods

1.1. Swimming pool sampling

Swimming pool samples were collected from an indoor community pool in South Carolina with an estimated total volume of 263,300 liters. This pool was chosen to study due to its consistent bather load (approximately 24 swimmers/day) throughout the year. The first sampling event occurred in May of 2021 when the swimming pool was using sodium hypochlorite (conventional chlorine) to disinfect the swimming pool. Two additional pool samples (November 2021 and January 2022) were collected after the implementation of an electrochemically generated chlorine system (Hayward Saline C 11.0 Commercial Salt Chlorine Generator; Rockville, MD) with a flow rate of 150 gallons per minute and a power supply rated to supply 72 amps. Additional details about the electrochemically generated chlorine system can be found in Table S1.

Samples were collected in 1 L amber glass bottles, quenched with ammonium chloride, acidified to pH 3.5-4 with 1 M sulfuric acid (for sample preservation), and filled headspace free. Samples were then shipped overnight on ice to the University of South Carolina and extracted immediately upon arrival. Further sample details can be found in Table 1.

1.2. Chemical and reagents

All solvents (methanol, metyl *tert*-butyl ether, acetonitrile, ethyl acetate) were of the highest purity and were purchased from Sigma-Aldrich (St. Louis, MO) or VWR International (Radnor, PA). General reagents were of ACS reagent grade and were purchased from Sigma-Aldrich and Fisher Scientific (Waltham, MA). DBP standards were purchased from CanSyn Chem. Corp. (Toronto, ON), Sigma-Aldrich, Aldlab Chemicals (Woburn, MA), and TCI America (Waltham, MA) at the highest purity. Specific vendor information can be found in Table S2. The internal standard, 1,2-dibromopropane, along with the diazomethane derivatization reagents (Diazald, CARBITOL™) were purchased from Sigma-Aldrich.

1.3. DBP analysis

Quantification of 60 DBPs was performed in triplicate as described previously (Cuthbertson et al., 2020; Allen et al., 2021, 2022; Aziz et al., 2022; Li et al., 2021). In brief, 100 mL of a sample was placed into a 125 mL amber bottle and acidified to pH < 1 with concentrated sulfuric acid. Then, 5 mL of methyl *tert*-butyl ether was added to each sample along with 30 g of sodium sulfate. Samples were then shaken for 15 min, allowed to settle for 10 min, and the top organic layer was removed and placed into a test tube. This procedure was completed 2 more times for a total extract volume of 15 mL. The organic extract was then dried using sodium sulfate and concentrated to 200 µL using a gentle stream of nitrogen. The concentrated

Table 1 – Sampling ID (date, time, disinfectant technology used), water quality parameters (pH, residual Cl), estimated bather load, THM levels, HAA levels, and total DBPs.

Sample ID	Sample Collection Time	Disinfectant	pH	Residual Chlorine (mg/L)	Exercise Class	THMs(μg/L)	HAAs(μg/L)	Total DBPs(μg/L)
May sample (5/17/2021)	1:00 PM	12% liquid sodium hypochlorite	7.4	6.1	No, typical bather load	777	1066	2541
November sample (11/18/2021)	12:30 PM	Electrochemically generated chlorine*	7.5	3.2	No, typical bather load	322	1798	2613
January sample (1/12/2022)	1:10 PM	Electrochemically generated chlorine*	7.3	2.0	Yes, 8-10 participants	260	2425	3251

* salt water pool.

extract was spiked with 4 μL of an internal standard (1,2-dibromopropane) and split into two equal aliquots. The first aliquot was used to analyze for 4 trihalomethanes (THMs), 9 haloketones (HKs), 4 haloacetaldehyde (HALs), 4 halonitromethanes (HNMs), 7 haloacetonitriles (HANs), 13 haloacetamides (HAMs), and 6 iodinated trihalomethanes (I-THMs).

The second aliquot was derivatized using diazomethane for the analysis of 9 haloacetic acids (HAAs) and 4 iodoacetic acids (IAAs). Diazomethane derivatization converts carboxylic acids to methyl esters for analysis by gas chromatography (GC)-mass spectrometry (MS). The diazomethane derivatization was conducted as described by the U.S. Environmental Protection Agency Standard Operating Procedure (Richardson et al., 2009). In brief, 0.367 g of Diazald and 1.0 mL of CARBITOL™ were placed inside the inner tube of a diazomethane generator. Then, 3.0 mL of methyl *tert*-butyl ether was placed in the outer tube of the generator, and the entire generator was placed in ice. Once on ice, 1.5 mL of 37% potassium hydroxide was added slowly (dropwise) to the inner tube and allowed to react for 1 hr. After 1 hr, 50 μL of diazomethane (dissolved in methyl *tert*-butyl ether) was spiked into a 100 μL organic extract aliquot and allowed to react for 30 min. After 30 min, the reaction was quenched with 10 mg of silica gel and transferred to new vials before analysis.

1.4. GC-MS analysis

Both extracts were analyzed using a gas chromatograph-mass spectrometer (Agilent 7890 GC, 5977A mass spectrometer, Agilent Technologies, Santa Clara, CA) with electron ionization (EI) at 70 eV in selection ion monitoring (SIM) mode. Sample extracts (1.0 μL) were injected into a multimode inlet (MMI) in pulsed splitless mode. Analytes were chromatographically separated using a Restek Rtx-200 column (30 m x 0.25 mm x 0.25 μm film thickness; Restek Corporation, Bellefonte, PA). This column provides improved separation and detection limits for iodo-THMs and haloacetamides, which tend to tail and give lower responses using a DB-5 column (Cuthbertson et al., 2020). The GC temperature program for the analysis of the 4 THMs, 9 HKs, 4 HALs, 4 HNMs, 7 HANs, 13 HAMs, 6 I-THMs was as follows: initial temperature of 35 °C for 5 min, increased to 220 °C at 9 °C/min, ramped at 20 °C/min to 280 °C, and held for 15 min. The GC temperature program for the analysis of the 9 HAAs and 4 IAAs was as follows: initial temperature held at 35 °C for 5 min, increased to 280 °C at 9 °C/min, and held for 15 min. Both methods held the transfer line at 280 °C and source temperature at 200 °C. Quantifier and qualifier ions along with limits of quantification (LOQ) for each DBP are listed in Table S2.

1.5. Bromide and iodide measurements

To quantify the amount of bromide and iodide present in the sodium chloride used for salt water pools, a solid sodium chloride sample used at this community pool was collected and dissolved in ultrapure water for analysis. Bromide and iodide were quantified via a Dionex Integrion high performance ion chromatography (HPIC) system (Sunnyvale, CA) with an IonPac AS20 guard column and an IonPac AS20 analytical column. The system included a 500 μL sample loop and 50 mM NaOH as the eluent. An external calibration curve was prepared in ultrapure water (1, 5, 10, 20, and 30 μg/L) using sodium bromide and sodium iodide. The limits of quantification (LOQs) for both bromide and iodide are 1.0 μg/L.

1.6. "TIC-Tox": calculated cytotoxicity and genotoxicity

"TIC-Tox" is a metric previously used in several studies to calculate cytotoxicity and genotoxicity of water samples and predict the drivers of toxicity (Smith et al., 2010; Allard et al., 2015; Plewa et al., 2017; Cuthbertson et al., 2019; Carter et al., 2019; Allen et al., 2021; Aziz et al., 2022). In brief, "TIC-Tox" calculates cytotoxicity and genotoxicity by multiplying the molar concentration of each individual DBP by their corresponding cytotoxicity or genotoxicity index values for Chinese hamster ovary (CHO) cells reported in literature (Wagner and Plewa, 2017; Plewa et al., 2017). Each product is then multiplied by a normalization factor (10^6) and summed together (Eqs. 1 and 2).

$$\text{total calculated cytotoxicity} = \sum \left([\text{DBP}] \times LC_{50}{}^{-1} \times 10^6 \right) \quad (1)$$

$$\text{total calculated genotoxicity} = \sum \left([\text{DBP}] \times 50\%TDNA^{-1} \times 10^6 \right) \quad (2)$$

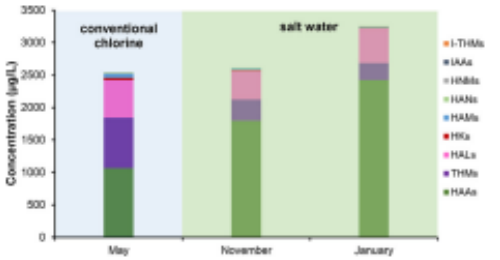

Fig. 1 – Total concentration of DBPs by class in conventional chlorine and salt water pool samples.

where LC_{50}^{-1} is the inverse of the lethal concentration at 50% in molarity (M) and $TDNA^{-1}$ is the inverse of the 50% tail DNA measurement in molarity (M). "TIC-Tox" assumes that the toxicity of individual DBPs is additive, an assumption shown to be accurate in a recently published study (Lau et al., 2020). Note that haloketones (HKs) are not included in "TIC-Tox" calculations because there are no cytotoxicity or genotoxicity index values currently available in the literature. Additional details about "TIC-Tox" and the determination of cytotoxicity and genotoxicity index values can be found in previous studies (Wagner and Plewa, 2017; Plewa et al., 2017; Faribairn et al., 1995; Tice et al., 2000; Wagner et al., 2009; Rundell et al., 2003).

2. Results and discussion

2.1. Overall findings

Of the 60 DBPs measured in this study, 68% were detected at least once. Table 2 shows the 60 DBPs quantified during each sampling event. The dominant DBP classes quantified were haloacetic acids (HAAs), followed by haloacetaldehydes (HALs), and trihalomethanes (THMs) (Fig. 1). HAAs, which accounted for 63% of the average total DBPs present, are known to accumulate in pools due to their lack of volatility (Carter et al., 2017; Simard et al., 2013; Daiber et al., 2016; Allen et al., 2021). Total HAA concentrations ranged from 1066 to 2425 µg/L and were dominated by Cl-HAAs (sum of chloroacetic acid, dichloroaceitc acid, and trichloroacetic acid). Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) were the dominant HAAs detected with an average concentration of 1332 and 277 µg/L, respectively. This finding matches well with previously published data from our lab in which another indoor pool had DCAA and TCAA at levels as high as 1230 and 275 µg/L, respectively (Allen et al., 2021). Of the haloacetaldehydes quantified in this study, trichloroacetaldehyde (TCAL) was the most commonly detected, and accounted for 19% of the average total DBPs formed. Trichloromethane (TCM) was the second most abundant DBP quantified with an average concentration of 447 µg/L. Interestingly, the January salt water pool sample contained). the highest level of total DBPs (3251 µg/L) despite having the lowest residual chlorine measured (Table 1). Prior to the January sampling, an exercise class was offered, thus the bather load was higher compared to the other two sampling events (Table 1). An increase in turbulence in the pool prior to sampling resulted in lower THMs levels (260 µg/L) but higher levels of non-volatile DBPs, particularly HAAs (2425 µg/L). This finding matches well with previous studies that noted as the turbulence in the swimming pool increases, so does the THM concentration in air samples collected at indoor pools (Aggazzotti et al., 1998; Catto et al., 2012), thus, decreasing THM levels in the water.

Iodinated trihalomethanes (I-THMs), iodoacetic acids (IAAs), and halonitromethanes (HNMs) were present at the lowest levels. On average, these classes represented <1% of the average DBPs present in all pool samples. Trichloronitromethane (TCNM) was the most frequently detected HNM in pool samples, with average levels ranging from 2.1 µg/L in the salt water pool samples to 4.6 µg/L in the conventional chlorinated pool sample. The only I-THM detected in this study was bromodiiodomethane (BDIM), which was found in the conventional chlorinated pool sample at 0.5 µg/L. Iodoacetic acid (IAA) was also detected in the conventional chlorinated pool at 0.2 µg/L and diiodoacetic acids (DIAA) and chloroiodoacetic acid (CIAA) were detected in the January salt water pool sample, both at 0.3 µg/L. This is the first report of DIAA in swimming pool water and is a significant finding due to its elevated level of toxicity. For example, DIAA is 1.8x more cytotoxic than DBAA. Ion chromatography analysis of the salt used in the salt water pool revealed that iodide was not present as an impurity, suggesting that the presence of the I-DBPs was the result of iodide in disinfected source water used to fill the pool.

2.2. Conventional chlorine vs. salt water: C-DBPs

2.2.1. Haloacetic acids (HAAs)

Upon implementation of an electrochemically generated chlorine system, there was a 15% increase in average total DBPs compared to the conventionally chlorinated pool sample. This overall increase in HAAs was driven by the January sample which had the lowest residual chlorine but the highest bather load prior to sampling. This increase in DBP formation was driven by dichloroacetic acid and trichloroacetic acid, which saw a 124% and 25% increase, respectively. Dichloroacetic acid accounted for 69% (730 µg/L) of the HAAs formed in the conventional chlorine pool and 77% (1633 µg/L) of the HAAs present in the salt water pool, a 124% increase. Trichloroacetic acid accounted for 22% (238 µg/L) of HAAs present in the conventional chlorine pool sample and 14% (297 µg/L) in salt water pool samples, a 25% increase. A previous study noted a similar increasing trend when comparing levels of dichloroacetic acid (196% increase) and trichloroacetic (229% increase) in conventional chlorine and salt water pools (Yeh et al., 2014).

2.2.2. Haloacetaldehydes (HALs)

The concentration of trichloroacetaldehyde (TCAL) was 580 µg/L in the conventional chlorine pool and 490 µg/L on average in the salt water pool, a 16% decrease. Lee et al. (2010) also noted a decrease (40%) in the formation of trichloroacetaldehyde between conventional chlorine and salt water pools. This decrease in trichloroacetaldehyde is an

Table 2 – DBPs quantified in conventional chlorine or salt water pools (μg/L).[a, *]

DBP class	Name	Abbreviation	Conventional chlorine	Salt water	
			May	November	January
HALs	Trichloroacetaldehyde	TCAL	580 ± 15.0	448 ± 10.7	532 ± 58.3
	Bromodichloroacetaldehyde	BDCAL	1.0 ± 0.0	ND	4.8 ± 0.2
	Dibromochloroacetaldehyde	DBCAL	0.2 ± 0.0	ND	0.1 ± 0.0
	Tribromoacetaldehyde	TBAL	ND	ND	0.1 ± 0.0
HANs	Trichloroacetonitrile	TCAN	0.6 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
	Dichloroacetonitrile	DCAN	5.2 ± 0.3	5.4 ± 0.2	4.2 ± 0.4
	Chloroacetonitrile	CAN	6.3 ± 0.1	2.6 ± 0.2	1.4 ± 0.0
	Bromochloroacetonitrile	BCAN	0.2 ± 0.0	0.3 ± 0.0	0.5 ± 0.0
	Bromoacetonitrile	BAN	3.1 ± 0.0	ND	ND
	Dibromoacetonitrile	DBAN	0.2 ± 0.0	0.1 ± 0.0	0.4 ± 0.0
	Iodoacetonitrile	IAN	ND	ND	ND
	1,1-Dichloropropanone	11DCP	2.8 ± 0.1	ND	ND
HKs	Chloropropanone	CP	18.0 ± 1.0	4.6 ± 0.2	3.5 ± 0.4
HKs	1,1,1-Trichloropropanone	111TCP	8.2 ± 0.3	1.0 ± 0.0	0.9 ± 0.0
	1,1-Dibromopropanone	11DBP	ND	ND	ND
	1-Bromo-1,1-dichloropropanone	1B11DCP	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
	1,3-Dichloropropanone	13DCP	2.8 ± 0.0	0.7 ± 0.1	0.7 ± 0.1
	1,1,3-Trichloropropanone	113TCP	1.6 ± 0.1	0.2 ± 0.0	ND
	1,1,3,3-Tetrachloropropanone	1133TeCP	0.8 ± 0.0	ND	ND
	1,1,3,3-Tetrabromopropanone	1133TeBP	ND	3.1 ± 0.3	1.7 ± 0.4
HNMs	Trichloronitromethane	TCNM	4.6 ± 0.1	2.4 ± 0.1	1.7 ± 0.1
	Dichloronitromethane	DCNM	0.4 ± 0.0	ND	ND
	Bromochloronitromethane	BCNM	0.2 ± 0.0	ND	ND
	Dibromonitromethane	DBNM	ND	ND	ND
THMs	Trichloromethane	TCM	764 ± 14.8	318 ± 29.0	257 ± 38.1
	Tribromomethane	TBM	0.3 ± 0.0	ND	ND
	Dibromochloromethane	DBCM	0.9 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
	Bromodichloromethane	BDCM	11.8 ± 0.1	3.5 ± 0.1	3.0 ± 0.1
	Dichloroiodomethane	DCIM	ND	ND	ND
I-THMs	Bromochloroiodomethane	BCIM	ND	ND	ND
I-THMs	Dibromoiodomethane	DBIM	ND	ND	ND
	Chlorodiiodomethane	CDIM	ND	ND	ND
	Bromodiiodomethane	BDIM	0.5 ± 0.0	ND	ND
	Iodoform	TIM	ND	ND	ND
HAMs	Chloroacetamide	CAM	ND	ND	ND
	Bromoacetamide	BAM	ND	ND	ND
	Dichloroacetamide	DCAM	21.5 ± 0.9	ND	3.4 ± 0.2
	Bromochloroacetamide	BCAM	ND	ND	ND
	Iodoacetamide	IAM	ND	ND	ND
	Trichloroacetamide	TCAM	37.0 ± 1.8	23.1 ± 0.8	9.0 ± 0.7
	Dibromoacetamide	DBAM	ND	ND	ND
	Bromodichloroacetamide	BDCAM	1.5 ± 0.1	1.9 ± 0.0	0.6 ± 0.0
	Chloroiodoacetamide	CIAM	ND	ND	ND
	Bromoiodoacetamide	BIAM	ND	ND	ND
	Dibromochloroacetamide	DBCAM	ND	ND	ND
	Tribromoacetamide	TBAM	ND	ND	ND
	Diiodoacetamide	DIAM	ND	ND	ND
	Chloroacetic acid	CAA	67.5 ± 6.8	120 ± 5.6	90.9 ± 5.5
HAAs	Bromoacetic acid	BAA	1.2 ± 0.0	ND	ND
HAAs	Dichloroacetic acid	DCAA	730 ± 113	1298 ± 150	1969 ± 32.5
	Trichloroacetic acid	TCAA	238 ± 23.4	317 ± 17.6	277 ± 24.6
	Bromochloroacetic acid	BCAA	14.9 ± 0.7	40.7 ± 3.6	69.1 ± 2.7
	Bromodichloroacetic acid	BDCAA	7.0 ± 0.2	9.0 ± 0.3	6.5 ± 0.4*
	Dibromoacetic acid	DBAA	2.8 ± 0.1	7.5 ± 0.5	12.0 ± 0.7*
	Dibromochloroacetic acid	DBCAA	3.9 ± 0.1	4.2 ± 0.0	0.5 ± 0.0*
	Tribromoacetic acid	TBAA	ND	1.6 ± 0.0	0.2 ± 0.0*
IAAs	Iodoacetic acid	IAA	0.2 ± 0.0	ND	ND
	Chloroiodoacetic acid	CIAA	ND	ND	0.3 ± 0.1*
	Bromoiodoacetic acid	BIAA	ND	ND	ND
	Diiodoacetic acid	DIAA	ND	ND	0.3 ± 0.0*

[a] Values reported as average ± standard deviation of triplicate measurements.
* Values reported as average ± standard error of duplicate measurements; ND = not detected.

important finding when considering previous studies have cited trichloroacetaldehyde as the primary driver of calculated chronic cytotoxicity in pools (Carter et al., 2019).

2.2.3. *Trihalomethanes (THMs)*

Trichloromethane concentrations in the conventional chlorine and salt water pool samples were 764 µg/L and 288 µg/L, respectively. Notably, 764 µg/L of trichloromethane in the conventional chlorinated indoor pool is the second highest reported level of trichloromethane in the literature, second to only 980 µg/L reported in a study conducted by Lahl et al. (1981). Elevated levels of trichloromethane in indoor pools underlines the importance of having adequate ventilation in indoor pools to decrease swimmers' exposure to volatile DBPs via inhalation (Villanueva et al., 2007b), especially when there is an increase in bather load like during the January sampling.

2.2.4. *Haloketones (HKs)*

The average concentration of haloketones (HKs) decreased by 76% in salt water pool samples (8.4 µg/L) compared to the conventional chlorine pool sample (34.5 µg/L). Of the 8 HKs detected in one or more pool samples, 7 of them decreased in concentration, ranging from a 10% decrease to a 100% decrease, with the exception of 1,1,3,3-tetrabromopropanone (1133TeBP), which was not detected in conventional chlorinated waters, but had an average concentration of 2.4 µg/L in salt water pool samples. The formation of 1133TeBP indicates the presence of a bromide impurity in the salt used in the salt water pool, which would lead to the formation of Br-DBPs. Further discussion of bromide levels and resulting Br-DBPs can be found in Section 2.4 (Br-DBPs in pool samples).

2.3. *Conventional chlorine vs. salt water: N-DBPs*

2.3.1. *Haloacetamides (HAMs)*

This study presents the first report of the quantification of two haloacetamides (dichloroacetamide and bromodichloroacetamide) in a salt water pool. Of the 13 HAMs quantified in this study, only 3 were detected above the limit of quantification. Of those, trichloroacetamide (TCAM) was quantified at the highest level, with an average concentration of 23.0 µg/L, followed by dichloroacetamide (DCAM) at 8.3 µg/L and bromodichloroacetamide (BDCAM) at 1.3 µg/L. For trichloroacetamide and dichloroacetamide, maximum concentrations occurred in the conventional chlorinated pool water at 37.0 µg/L and 21.5 µg/L, respectively. The maximum concentration of bromodichloroacetamide occurred in the November salt water pool sampling event at 1.9 µg/L. On average, salt water pool samples showed a decrease in trichloroacetamide (57%), dichloroacetamide (92%), and bromodichloroacetamide (17%) when compared to the conventional chlorinated pool sample.

2.3.2. *Haloacetonitriles (HANs)*

Dichloroacetonitirle (DCAN) and chloroacetonitrile (CAN) were present at the highest level of all HANs quantified in this study, with an average concentration of 4.9 µg/L and 3.4 µg/L, respectively. HANs in salt water pool samples consistently decreased when compared to conventional chlorine pool samples, with the exception of bromochloroacetonitrile (BCAN) and dibromoacetonitrile (DBAN), likely due to the presence of bromide in the salt used in the salt water pool.

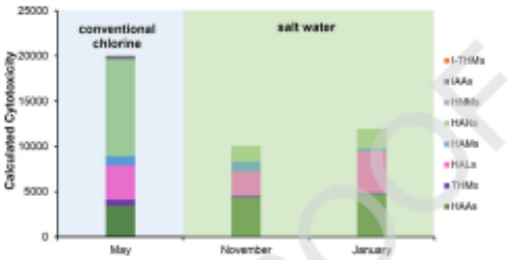

Fig. 2 – Calculated cytotoxicity of DBPs by class in conventional chlorine and salt water pool samples. Note that cytotoxicity data for haloketones (HKs) are currently not available in literature.

2.4. *Br-DBPs in pool samples*

Brominated DBPs (Br-DBPs) are of interest due to their elevated levels of toxicity compared to their chlorinated analogues (Richardson et al., 2007; Wagner et al., 2017). Previous salt water pool studies, which measured a smaller number of DBPs, have attributed the formation of Br-THMs, Br-HAAs, and Br-HANs to bromide present in sodium chloride and emphasized the importance of using high purity sodium chloride (Beech et al., 1980; Whitaker et al., 2003; Lee et al., 2010, 2009). Ion chromatography analysis of the sodium chloride used in the salt water pool in this study revealed that the salt contained approximately 0.05% bromide. Assuming the salinity of salt water pools are typically maintained around 3,000 to 5,000 mg/L, at 4,000 mg/L salinity, a 0.05% bromide impurity will contribute approximately 118 µg/L of bromide to the pool. This impurity is a significant contribution to the bromide levels in this pool, considering that the tap water in the city where the community pool is located only contained 22 µg/L of bromide. Overall, the switch to a salt water pool led to a 73% increase in Br-DBPs, primarily driven by bromochloroacetic acid (BCAA), which saw a 268% increase to an average of 54.9 µg/L.

2.5. *Calculated cytotoxicity*

Calculated cytotoxicity decreased by 45% after implementation of the salt water pool system. Overall, the calculated cytotoxicity in pool samples was driven by HANs, HAAs, and HALs, which accounted for 34%, 30%, and 26%, respectively, of the average calculated cytotoxicity in conventional chlorinated and salt water pool samples combined (Fig. 2). In pool waters disinfected with conventional chlorine, the calculated cytotoxicity was driven by HANs (53%), followed by HALs (19%) and HAAs (17%). The 45% decreasein calculated cytotoxicity of salt water pools was primarily driven by HANs. Although the concentration of BCAN and DBAN increased in salt water pools, the concentration of BAN increased in conventional chlorine pool samples compared to salt water pool samples (3.1 µg/Land ND, respectively). Therefore, despite the overall increase in Br-DBPs upon

implementing the electrochemically generated chlorine system, an increase in the formation of BAN (contributing 0.1% of the total DBPs formed) in conventional chlorine pools resulted in a substantial increase in calculated cytotoxicity and accounted for 40% of the calculated cytotoxicity in the conventional chlorine sample.

Trichloroacetaldehyde, despite being the least cytotoxic haloacetaldehyde quantified, contributed 22% of the average calculated cytotoxicity of all pool samples. In the conventional chlorinated pool sample, trichloroacetaldehyde accounted for 17% of the total calculated cytotoxicity and 26% in the salt water pool samples. Although to a lesser degree, this finding is consistent with a previous study in which trichloroacetaldehyde was cited as a significant driver of calculated cytotoxicity in swimming pools (Carter et al., 2019). Unlike HANs and HALs, HAAs did not have a clear driver of cytotoxicity in conventional chlorinated pool samples, but was driven by several HAAs like chloroacetic acid (4%), bromoacetic acid (5%), dichloroacetic acid (4%), and trichloroacetic acid (3%).

In salt water pools, the calculated cytotoxicity was driven by HAAs (41%), HALs (33%), and HANs (17%). On average, dichloroacetic acid only contributed 16% to the calculated cytotoxicity for HAAs despite contributing 77% of the HAAs detected. Chloroacetic acid, which contributed 13% of the calculated cytotoxicity of the HAAs, only accounted for 5% of the average HAAs formed. Trichloroacetaldehyde contributed to 26% of the total calculated cytotoxicity but only 17% of the average total DBPs in salt water pool samples. Dichloroacetonitrile (7%) and dibromoacetonitrile (4%) were the primary HANs contributing to calculated cytotoxicity in salt water pool samples. The cases described above further showcase the importance of utilizing "TIC-Tox" to determine drivers of calculated cytotoxicity rather than inferring toxicity based on total DBP concentrations.

All classes of DBPs saw a decrease in calculated cytotoxicity when comparing the conventional chlorine pool to the salt water pool, with the exception of HAAs that saw an increase of 31%. When compared to conventional chlorinated pool samples, Br-HAAs and Br/Cl-HAAs were major contributors to the increase in calculated cytotoxicity. The calculated cytotoxicity contributed by bromochloroacetic acid and dibromoacetic acid saw a 268% and 250% increase, respectively, in the salt water pool. Interestingly, bromoacetic acid was not detected in salt water pool samples but was present at low levels (1.2 μg/L) in the conventional chlorinated pool sample and contributed 5% of the total calculated cytotoxicity of that sample. Chloroacetic acid, dichloroacetic acid, and trichloroacetic acid also contributed substantially to the calculated cytotoxicity (56%, 124%, and 25%, respectively) in the salt water pool, likely due to the increase in bather load prior to the January sampling event (Table 1).

2.6. Calculated genotoxicity

Calculated genotoxicity decreased by 15% upon implementation of a salt water system. Primary drivers of calculated genotoxicity were HAAs, which accounted for 80% of the average calculated genotoxicity in all pool samples. The calculated genotoxicity of the conventional chlorine pool samples was driven by a combination of HAAs (62%) and HANs (23%).

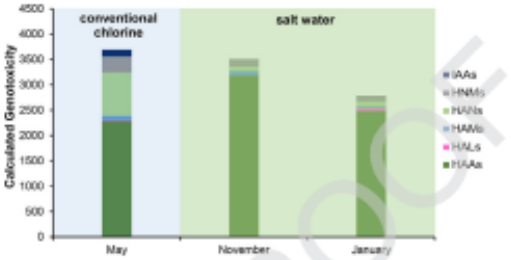

Fig. 3 – Calculated genotoxicity of DBPs by class in conventional chlorine and salt water pool samples. Note that genotoxicity data for HKs are currently not available in literature. I-THMs and THMs are not shown in this figure due to their presence at low or non-detect levels and/or their low genotoxicity values reported in literature.

Chloroacetic acid (47%) and bromoacetic acid (14%) were the main contributors to calculated genotoxicity (Fig. 3), despite only contributing 3% and <1% of the total DBPs in the conventional chlorine pool sample, respectively. Like with calculated cytotoxicity, bromoacetonitrile (18%) was also the primary driver of calculated genotoxicity in the conventional chlorine pool sample, despite contributing <1% of the total DBPs formed. Chloroacetic acid (73%) was the calculated genotoxicity driver in salt water pools samples, despite only contributing 4% of the total DBPs formed.

All classes of DBPs saw a decrease in calculated genotoxicity in salt water pool samples when compared to the conventional chlorinated pool, with the exception of HALs and HAAs, which saw an increase of 89% and 24%, respectively. IAAs (99%), HANs (89%), and HNMs (59%) saw the largest percent reduction in calculated genotoxicity, but were only responsible for 7% of the total calculated genotoxicity in the salt water pool. Bromochloroacetic acid and dibromoacetic acid saw the largest increase in calculated genotoxicity, with a 268% and 250% increase, respectively. However, this increase in Br-HAAs only contributed 4% of the total genotoxicity of the pool samples. Furthermore, bromoacetic acid was not detected in salt water pool samples, but was present at low levels (1.2 μg/L) in the conventional chlorine pool sample, contributing 14% of the total calculated genotoxicity of that sample.

3. Conclusions

This study provides an extensive analysis of 60 DBPs in the same indoor community pool treated with either conventional chlorine or electrochemically generated chlorine (salt water). Of the 60 DBPs measured, 68% were detected at least once, with dominant DBP classes including HAAs, HALs, and THMs, with average concentrations of 1763 μg/L, 522 μg/L, and 453 μg/L, respectively. DBP levels were consistent with previous studies that reported these 3 classes, with the exception of trichloromethane, which was present at 764 μg/L in the conventional chlorine pool sample, likely due to a high residual chlorine (6.1 mg/L). This finding highlights the importance of maintaining a lower residual

chlorine (1.0 to 2.0 mg/L) and ensuring adequate ventilation in indoor pools to decrease swimmers' exposure to volatile DBPs. The switch from conventional chlorine to a salt water system saw a 15% increase in the average total DBPs present, driven by trichloroacetic acid and dichloroacetic acid. The overall increase in total DBPs in the salt water pool samples was driven by the January sample which was collected after an exercise class and contained 28% and 24% more total DBPs compared to the conventional chlorine sample and the November salt water pool sample, respectively.

However, the implementation of a salt water system led to a 45% and 15% decrease in calculated cytotoxicity and genotoxicity, respectively. Calculated cytotoxicity values for both conventional chlorine and salt water pool samples were driven by HALs, HANs, and HAAs. This decrease in calculated cytotoxicity and genotoxicity further indicates that maintaining a low residual chlorine is also just as important as limiting the bather load. Further, our calculated cytotoxicity findings match well with a previous drinking water study that demonstrated a statistically significant correlation between the concentration of N-DBPs and cytotoxicity (Allen et al., 2021). Therefore, limiting the formation of N-DBPs by reducing the amount of nitrogen sources like sweat and urine (Li and Blatchley, 2007; Yeh et al., 2014; Shah and Mitch, 2012) in swimming pools will be crucial in reducing the overall toxicity of swimming pools.

I-THMs, HNMs, HAMs, and THMs contributed only 9% on average to the total calculated cytotoxicity of all three pool samples. IAAs, despite their elevated levels of toxicity, only contributed 1% of the calculated cytotoxicity, due to their presence at low or non-detect levels. Trichloroacetaldehyde was the primary driver of calculated cytotoxicity, contributing 23% of the calculated cytotoxicity on average.

Calculated genotoxicity values for both conventional chlorine and salt water pool samples were driven by HNMs, HANs, and HAAs, with chloroacetic acid contributing 73% on average, despite only accounting for 3% of the average total DBPs. HAMs, HALs, and I-THMs were not significant contributors to calculated genotoxicity due to their presence at low or non-detect levels. Further, despite their high levels, THMs are not genotoxic in CHO cells (Wagner and Plewa, 2017), so they did not contribute to the calculated genotoxicity for these pool samples.

Ion chromatography analysis of the sodium chloride used in the salt water pool system revealed a 0.05% bromide impurity. Based on the average salinity required for salt water pools, this 0.05% impurity results in an increase of bromide levels by more than 100 µg/L. As a result, the concentration of Br-DBPs and Br/Cl-DBPs increased from 49.9 µg/L to 86.1 µg/L, a 73% increase. This increase in Br-DBPs was primarily driven by bromochloroacetic acid, which increased by 268% to 54.9 µg/L, but did not substantially contribute to the calculated genotoxicity.

This study provides important insights for pools utilizing conventional chlorine vs electrochemically generated chlorine (salt water). Overall, the change from a conventional chlorinated pool to a salt water pool system reduced the calculated cytotoxicity and genotoxicity despite the presence of a bromide impurity and the increase in bather load prior to the second (January) salt water sample. Due to the increasing popularity of salt water pools, future work focusing on controlled lab reactions and measurement of whole water toxicity of salt water pools using a variety of sodium chloride salts would be beneficial to better understand the impact bromide impurities may have on the toxicity of the pool water. Additionally, future research studying a larger number of both indoor and outdoor pools (utilizing both salt water and conventional chlorine) will aid in a more robust understanding of the factors that drive toxicity in each treatment technique.

Uncited references

Carter and Joll, 2017, Fairbairn et al., 1995, Richardson, 2009, Richardson and Plewa, 2020, Wagner and Plewa, 2009, Zwiener et al., 2007

Declaration of Competing Interest

No conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.04.044.

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Article

Are Disinfection Byproducts (DBPs) Formed in My Cup of Tea? Regulated, Priority, and Unknown DBPs

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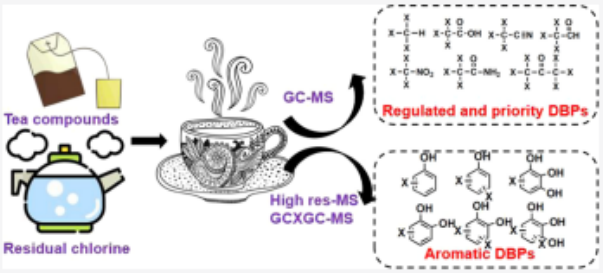

ABSTRACT: Globally, tea is the second most consumed nonalcoholic beverage next to drinking water and is an important pathway of disinfection byproduct (DBP) exposure. When boiled tap water is used to brew tea, residual chlorine can produce DBPs by the reaction of chlorine with tea compounds. In this study, 60 regulated and priority DBPs were measured in Twinings green tea, Earl Grey tea, and Lipton tea that was brewed using tap water or simulated tap water (nanopure water with chlorine). In many cases, measured DBP levels in tea were lower than in the tap water itself due to volatilization and sorption onto tea leaves. DBPs formed by the reaction of residual chlorine with tea precursors contributed ~12% of total DBPs in real tap water brewed tea, with the remaining 88% introduced by the tap water itself. Of that 12%, dichloroacetic acid, trichloroacetic acid, and chloroform were the only contributing DBPs. Total organic halogen in tea nearly doubled relative to tap water, with 96% of the halogenated DBPs unknown. Much of this unknown total organic halogen (TOX) may be high-molecular-weight haloaromatic compounds, formed by the reaction of chlorine with polyphenols present in tea leaves. The identification of 15 haloaromatic DBPs using gas chromatography−high-resolution mass spectrometry indicates that this may be the case. Further studies on the identity and formation of these aromatic DBPs should be conducted since haloaromatic DBPs can have significant toxicity.

KEYWORDS: *DBPs, tea, TOX, high-resolution mass spectrometry, nontarget analysis*

INTRODUCTION

Globally, tea is the second most consumed nonalcoholic beverage next to drinking water.[1,2] World production of tea was ~4.8 million tons in 2012, and per capita consumption is ~100 g/year.[1,3] Popular types of tea include green and black tea, both of which use leaves from the *Camellia sinesis* plant.[4] Green tea is not fermented (oxidized), while black tea has undergone fermentation (oxidation). In East Asian countries, tea is generally brewed using boiled water and loose tea leaves, and in Western countries, tea bags are popular.[5] Both green tea and black tea contain approximately 500 compounds, including polyphenols, amino acids, caffeine, pigments, esters, polysaccharides, vitamins, minerals, and aromatic substances.[4,6,7] Some of these compounds have functional groups that can react with chlorine to form disinfection byproducts (DBPs). For example, chlorine is well known to undergo electrophilic aromatic substitution reactions with phenols to form DBPs.[8,9]

To protect drinking water safety, disinfection is widely used to control waterborne pathogens.[10] Although disinfection is important, one downside is DBP formation.[11,12] Epidemiologic studies show that bladder cancer, colorectal cancer, and adverse birth outcomes are associated with DBPs in drinking water.[13−29] Eleven DBPs are currently regulated in the United States,[30] and DBPs are also regulated in other countries.[12] Thus, DBPs are a global concern.

While chloramine and ozone are often used for disinfection, chlorine is still the most commonly used chemical disinfectant

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for drinking water.[31] To control the regrowth of microorganisms, residual chlorine is typically maintained in drinking water distribution systems.[5,32] In the United States and China, up to 4 mg/L residual chlorine is allowed and up to 0.5 mg/L is generally maintained in the United Kingdom.[5,30,33,34] Boiling water in a kettle removes only 5−19% of the chlorine residual.[5] Therefore, a significant chlorine residual remains, which can form DBPs when this water is used to make tea. Bond et al. quantified six DBPs (trichloromethane (TCM), trichloronitromethane (TCNM), dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), 1,1-dichloropropanone (1,1-DCP), and 1,1,1-trichloropropanone (1,1,1-TCP)) in tea and coffee created using boiled simulated tap water, and their formation potentials were 14.7−62.4, ≤0.26, ≤1.04, ≤0.49, and ≤1.32 μg/mg DOC, respectively.[5] Fakour et al. measured four regulated trihalomethanes (THMs) in tea brewed using tap water, and up to 82.5 μg/L THMs were measured in boiled tap water brewed tea.[35]

To date, more than 700 DBPs have been identified in drinking water, and many are cytotoxic, genotoxic, mutagenic, or carcinogenic.[10,11] Among these compounds, iodo-THMs (I-THMs), iodoacetic acids (IAAs), haloacetonitriles (HANs), haloacetaldehydes (HALs), halonitromethanes (HNMs), haloacetamides (HAMs), and haloketones (HKs) are considered as priority DBPs, owing to their detection frequency, concentrations, and toxicities.[36−38] Compared to regulated THMs and haloacetic acids (HAAs), most of these unregulated priority DBPs are much more cytotoxic and genotoxic.[11,36−39] However, there is little information on the occurrence and formation of DBPs in tea, with only one paper reporting one class of DBPs (THMs) in tea brewed using real tap water.[35]

DBPs in tea can come from two sources: from the tap water used to brew the tea (DBPs already formed) and from the reaction of residual chlorine in tap water with tea precursors. In this study, we conducted a comprehensive study to quantify 60 regulated and priority DBPs and total organic halogen (TOX) in three popular green and black teas in the United States. Unknown DBPs were also identified using gas chromatography−high-resolution mass spectrometry.

MATERIALS AND METHODS

Chemicals and Reagents. Dichloroacetonitrile, bromochloroacetonitrile, dibromoacetonitrile, trichloroacetonitrile, trichloronitromethane, 1,1-dichloropropanone, 1,1,1-trichloropropanone, and trichloroacetaldehyde were purchased from AccuStandard as individual standards in acetone (New Haven, CT). All other DBP standards (Table S1) were purchased or custom synthesized from Sigma-Aldrich (St. Louis, MO), CanSyn Chem. Corp. (Toronto, Ontario), TCI America (Boston, MA), and Aldlab (Boston, MA) at the highest purity. Methyl *tert*-butyl ether (MTBE), ethyl acetate, acetonitrile, and methanol were purchased from Sigma-Aldrich and Honeywell International (Muskegon, MI) at the highest purity. Nanopure water (18.2 MΩ) was used to perform experiments involving simulated tap water. Twinings green tea, Twinings Earl Grey (black) tea, and Lipton (black) tea (all in tea bags, 2.0, 2.0, and 4.1 g, respectively) were purchased from a local supermarket. Earl Grey and Lipton teas were both fermented black teas. Finished water (10 L) was collected from a local drinking water plant that uses chlorine for disinfection in September 2020 (residual chlorine of 1.4 mg/L).

Experimental Design. Four groups of experiments were conducted. The water used for brewing the tea was boiled tap water with a boiling time of 1 min. Group 1 was a control experiment, in which 250 mL of boiled nanopure water was used to brew each tea bag. Group 2 experiments brewed tea with boiled nanopure water containing 1.0 mg/L chlorine (before boiling; hereafter denoted as "simulated tap water"). Group 3 was another controlled experiment with boiled real tap water without tea. Group 4 was a realistic situation where boiled real tap water was used to brew tea. Tea was brewed in a glass beaker, then cooled at room temperature (combined time for brewing and cooling of 30 min, Figure S1), and 500 mL was used to quantify regulated and priority DBPs and TOX. Experiments were conducted and analyzed in triplicate for regulated and priority DBPs and TOX.

For the analysis of unknown DBPs, a higher residual chlorine level (4 mg/L, before boiling) was used to allow somewhat higher levels of DBPs to enable their detection by gas chromatography (GC)-full-scan mass spectrometry, and to simulate real tap water with the maximum level of residual chlorine allowed by regulation.[40] In addition, 200 μg/L sodium bromide as bromide and 20 μg/L sodium iodide as iodide were added to the simulated tap water (nanopure water with chlorine) to mimic real source waters that can have high bromide and iodide.[40−43] In these cited studies, bromide in Rolla, MO tap water was 10.1 μg/L,[42] and iodide in Hong Kong tap water was 0.1−0.4 μg/L.[43] Tea was brewed in 1 and 2 L beakers (1 L of water per tea bag) and then cooled at room temperature (combined brew and cooling time of 30 min). Control experiments were conducted using boiled nanopure water. Experiments for unknown (nontarget) DBP identification were conducted in duplicate.

Sorption of DBPs onto Tea Leaves. To test the potential for sorption of DBPs onto tea leaves, two groups of experiments were conducted (one experimental group and one control group). For the experimental group, 250 mL of boiled nanopure water was used to brew each tea bag. Once the tea was cooled to room temperature (30 min), a 5.0 μg/L DBP mixture was added to the tea and allowed to sorb for 30 min. For the control group, a 5.0 μg/L DBP mixture was added to the room temperature-cooled boiled nanopure water (no tea) and allowed to sorb for 30 min.

Quantitative Analysis of 60 DBPs. Sixty DBPs were measured, including 4 HALs, 7 HANs, 9 HKs, 4 HNMs, 4 THMs, 6 I-THMs, 9 HAAs, 4 IAAs, and 13 HAMs (Table S1). Extraction and derivatization methods were adapted from previously published methods from our laboratory.[44−46] In brief, 100 mL of water or brewed tea was pH adjusted to a pH < 1 using concentrated H_2SO_4. Then, 30 g of sodium sulfate and 10 mL of MTBE (for the first extraction) were added to 125 mL amber bottle and the samples were shaken for 15 min. The bottles were then allowed to settle for 10 min and the organic layer (MTBE) was removed and placed into a glass test tube. Two more liquid−liquid extractions were performed with 5 mL of MTBE added each time for a total organic extract volume of 20 mL per sample. Extracts were then passed through a sodium sulfate column to remove excess water and concentrated to 200 μL under nitrogen and spiked with 25 μL of methanol to help dissolve organic matter, and transferred to GC vials. Afterward, 4 μL of 1,2-dibromopropane was added to sample extracts as an internal standard. Samples were then divided into two separate GC vials (one 100 μL and one 125 μL). One set of samples (125 μL vial) was analyzed for THMs, HKs, HALs, HNMs, HAMs, and HANs; the other set (100 μL vial) was used for the analysis of HAAs and IAAs.

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Diazomethane derivatization was used for the analysis of HAAs and IAAs. Diazomethane was freshly generated according to a US EPA Standard Operating Procedure.[44−47] An Aldrich diazomethane generator apparatus (St. Louis, MO) was used. In brief, approximately 0.367 g of Diazald and 1.0 mL of CARBITOL (Sigma-Aldrich, St. Louis, MO) were added to the inner piece of the generator, and 3.0 mL of MTBE were added to the outer piece. The apparatus was assembled and placed in ice, and 1.5 mL of 37% potassium hydroxide was injected dropwise (very slowly) through the septum into the inner portion of the generator. After reacting for 1 h, the diazomethane (dissolved in MTBE in the outer tube) was transferred to a glass conical vial. A volume of 50 μL diazomethane solution was added to each 100 μL extract for HAA/IAA analysis. After 30 min of reaction, excess diazomethane was quenched with approximately 10 mg of silica gel. Derivatized extracts were transferred to new vials to remove solid silica from the samples for instrumental analysis.

Quantification of DBPs in water was done using an internal calibration as described in Cuthbertson et al.,[45] and standard addition was used for quantification in brewed tea samples (due to the complexity of the matrix). For THMs, I-THMs, HAAs, IAAs, HKs, HALs, HNMs, HAMs, and HANs, samples were analyzed using an Agilent 7890 gas chromatograph-5977A mass spectrometer (GC-MS) with electron ionization, as described in Cuthbertson et al.[45] Sample extracts (1.0 μL) were injected into a multimode inlet in pulsed splitless mode using a Restek Rtx-200 column (30 m × 0.25 mm × 0.25 μm; Restek Corporation, Bellefonte, PA). This column allowed sharper peaks and optimal separation of DBPs. The GC temperature program was as follows: initial temperature of 35 °C held for 2 min, increased to 160 °C at 9 °C/min, then increased to 180 °C at 5 °C/min and finally increased to 280 °C at 22 °C/min held for 20 min. DBPs were quantified by selected ion monitoring mode (SIM). Retention times and selected ions are listed in Table S1.

Quantitative and Semiquantitative Analysis of Halophenols. Nine halophenols (DBP-1, DBP-2, DBP-3, DBP-4, DBP-5, DBP-6, DBP-7, DBP-8, and DBP-9) were quantified using matrix-matched calibration curves created in Twinings green tea, Earl Grey tea, and Lipton tea that was brewed with nanopure water. Samples were prepared using simulated tap water (4.0 mg/L chlorine before boiling, 200 μg/L sodium bromide as bromide, and 20 μg/L sodium iodide as iodide) with 1 tea bag per 250 mL of nanopure water. Samples were then cooled to room temperature, 100 mL for each replicate sample was transferred to a 125 mL amber bottle, and extracted using the procedure described in the Quantitative Analysis of 60 DBPs section. Samples were analyzed using the Agilent 7890 GC-MS instrument described above with electron ionization. Sample extracts (1.0 μL) were injected into a multimode inlet in pulsed splitless mode using a Restek Rtx-200 column; 30 m × 0.25 mm × 0.25 μm. The GC temperature program was as follows: initial temperature of 35 °C held for 5 min, increased to 220 °C at 9 °C/min, then increased to 280 °C at 20 °C/min and held for 10 min. The transfer line was held at 280 °C, and the ion source temperature was 200 °C. Halophenols were quantified using SIM mode. Retention times and selected ions are shown in Table S2; an example matrix-matched calibration curve is shown in Figure S13.

Semiquantitative concentrations of DBP-1, DBP-2, DBP-3, DBP-4, DBP-5, DBP-6, DBP-7, DBP-8, and DBP-9 in XAD samples were also calculated using their corresponding standards at 10 mg/L. For DBP-10, DBP-11, DBP-12, DBP-13, DBP-14, and DBP-15, due to lack of corresponding standards, their concentrations were calculated using a one-point calculation curve of 4-chlorophenol (4CP; 10 mg/L) to obtain a 4CP-equivalent concentration (Table S11).

Total Organic Halogen (TOX). Total organic chlorine (TOCl), bromine (TOBr), and iodine (TOI) were analyzed using a Mitsubishi TOX analyzer (Mitsubishi Chemical Analytech, Chigasaki, Japan; Cosa Xentaur, Yaphank). Procedures were based on published papers, with a few modifications described in Text S1.[31,44,45] Briefly, acidified samples (pH < 2) were adsorbed onto activated carbon, washed with sodium nitrate, and combusted at 1000 °C in the presence of oxygen and argon as the carrier gas. Combusted gases were collected in an aqueous solution containing 0.03% hydrogen peroxide, which was analyzed for chloride, bromide, and iodide using a Dionex 1600 ion chromatograph (Dionex, Sunnyvale, CA).

Nontarget Identification of Unknown DBPs in Tea. For the nontarget analysis of unknowns, samples were extracted using precleaned XAD resins as described in our previous research.[48] Briefly, 5.0 L of tea was acidified to pH < 1 using concentrated H_2SO_4. A glass column was packed with two kinds of XAD resins (XAD-2 and XAD-8, 9 mL each). XAD resins were preconditioned with 25 mL of nanopure water, 12.5 mL of 0.1 M HCl, 12.5 mL of 0.1 M NaOH, 25 mL of 0.1 M HCl, and 25 mL of nanopure water, in sequence. Afterward, aqueous samples were passed through the XAD resins and allowed to drain completely. Adsorbed compounds (i.e., DBPs) were eluted with 70 mL of ethyl acetate. The eluent was collected, residual water was removed using a separatory funnel, further dried with Na_2SO_4, and concentrated to 200 μL with high-purity nitrogen.[48]

A LECO Pegasus GC-HRT time-of-flight (TOF) high-resolution mass spectrometer (GC-HRT-TOF-HRMS; 50 000 resolution; LECO Corp., St. Joseph, MI) was used for these nontarget analyses with electron ionization at 70 eV in full-scan mode (m/z 33−530). Procedures were similar to a previously published paper from our lab.[48] A LECO Pegasus BT 4D GC × GC-TOF-MS (with 2 decimal place accuracy) was also used in GC × GC (2D) mode to improve separations of DBPs in the complex tea extract matrices and in single-GC (1D) mode for increased sensitivity of low abundant compounds that did not have enough ion statistics using the high-resolution TOF mass spectrometer. Extracts (1.5 μL) were injected into a multimode inlet in pulsed spitless mode. For analyses in single-GC mode, the type of column (Restek Rxi-5ms GC column; 30 m × 0.25 mm ID × 0.25 μm) and temperature program were the same as described above for regulated and priority DBPs. For analyses in GC × GC mode, the primary column was the same as above, and the secondary column was a Restek Rxi-17Sil MS column (0.48 m × 0.1 mm ID × 0.1 μm), with both columns run with the same temperature program (35 °C, held for 4 min, ramped to 280 °C at 9 °C/min, and held for 20 min). The transfer line was held at 250 °C, the ion source temperature was 250 °C, and electron ionization (70 eV) was used in full-scan mode (m/z 33 to 600).

The numbers and types of halogens were determined using characteristic isotopic patterns, and high-resolution MS was used to determine empirical formulas for the molecular ions and fragment ions. Based on the fragmentation patterns and

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Environ. Sci. Technol. 2021, 55, 12994−13004

molecular formulas, possible structures were proposed. Library database searching (NIST) was also utilized.

Quality Assurance and Quality Control. To ensure data quality, a set of quality control experiments was conducted for tap water and tea, including procedural blanks, solvent blanks, and matrix-spiked samples. For procedural blanks, nanopure water and boiled nanopure water brewed tea were used to determine background concentrations of DBPs. No DBPs were detected in the nanopure water procedural blanks. For tea, some THMs, HAAs, and HAMs were detected in procedural blank samples and were subtracted from the concentrations of samples. Fakour et al. also observed THMs in blank samples of Oolong tea, green tea, and black tea, at levels of 0.1, 0.6, and 2.3 $\mu g/L$, respectively, indicating that DBPs can be present in tea bags.[35] For standard addition, each tea sample was spiked with 60 DBPs at two separate levels (2 and 5 $\mu g/L$); recoveries for most DBPs were within acceptable levels (70−130%). All tea and tap water samples were analyzed in triplicate, and the relative standard deviations of most DBPs were <30%. LOQs for the 60 DBPs ranged from 0.01−2.0 $\mu g/L$ (Table S3).

RESULTS AND DISCUSSION

Quantitative Measurements for 60 DBPs and TOX. *Overall Findings.* The DOC values in nanopure water brewed green tea, Earl Grey tea, and Lipton tea were 425, 389, and 1216 mg/L, respectively. Note that Lipton tea bags contained about twice the mass of tea leaves as the other two. Means of total concentrations of the 60 DBPs measured in real tap water brewed tea were 25.7, 24.4, and 18.2 $\mu g/L$ for green tea, Earl Grey tea, and Lipton tea, respectively. It was somewhat surprising to see lowest DBP levels formed in Lipton tea, when it had the highest DOC and highest mass of tea leaves in its bag. An ANOVA test was conducted with these concentrations against the boiled tap water control ($F_{3,8} = 26.1$; $P \leq 0.001$; with power $(1\text{-}\beta)$ of >0.8). Using a Holm-Sidak Multiple Comparison test, the mean summed DBP concentrations for each of the teas were significantly lower ($P \leq 0.01$) than in the boiled tap water control (mean of 32.2 $\mu g/L$) (Table S4). We believe the lower levels in brewed tea are due to sorption of DBPs onto the tea leaves, as described below. Specifically, as shown in Table 1, the total I-THMs, HAAs, IAAs, HALs, HKs, HNMs, and HANs in the three teas were similar. However, for THMs and HAMs, concentrations in Twinings green tea and Twinings Earl Grey were higher than those in Lipton tea. A previous study showed that concentrations of THMs and HAMs in unfermented (green) teas were higher than fermented (black) tea because their adsorption increased as tea fermentation increased.[2]

Because chlorine residuals in the boiled tap water (0.7 mg/L), simulated tap water (0.8 mg/L), and tap water brewed tea (0.7 mg/L) are very close (by design) after boiling, some important comparisons can be made (Table S6). First, from our control experiments of tea brewed with simulated tap water (nanopure water with chlorine added), it is clear that, of the 60 DBPs quantified, only THMs (chloroform) and HAAs (dichloroacetic acid and trichloroacetic acid) are formed directly from tea precursors (Table 1). The other quantified DBPs were introduced by the tap water itself, with natural organic matter in the source water serving as their main precursors. In addition, when comparing tap water brewed tea to the corresponding boiled tap water controls, it is evident that only THMs show a notable increase in the formation in brewed tea. For example, THMs are 3.3 $\mu g/L$ in the boiled tap

Table 1. Concentrations of Regulated and Priority DBPs in Unboiled and Boiled Tap Water, and Simulated and Real Tap Water Brewed Tea ($\mu g/L$)[a]

water/tea		residual chlorine (mg/L)	THMs	I-THMs	HAAs	IAAs	HALs	HKs	HNMs	HANs	HAMs	total DBPs
unboiled tap water		1.3 ± 0.0	20.2 ± 2.5	1.3 ± 0.1	11.0 ± 1.1	0.2 ± 0.0	11.5 ± 1.0	13.4 ± 1.1	0.3 ± 0.0	1.1 ± 0.1	9.1 ± 1.0	68.1 ± 2.7
boiled tap water		0.7 ± 0.0	3.3 ± 0.2	0.3 ± 0.3	14.0 ± 2.9	0.2 ± 0.0	1.4 ± 0.5	4.1 ± 0.1	0.05 ± 0.0	0.3 ± 0.0	8.6 ± 0.6	32.2 ± 3.2
tea (simulated tap water)[b]	green tea	0.8 ± 0.0	0.6 ± 0.3	ND	4.0 ± 0.7	ND	ND	ND	ND	ND	ND	4.5 ± 0.8
	Earl Grey	0.8 ± 0.0	0.4 ± 0.4	ND	2.3 ± 0.1	ND	ND	ND	ND	ND	ND	2.6 ± 0.4
	Lipton tea	0.8 ± 0.0	0.2 ± 0.2	ND	1.5 ± 0.3	ND	ND	ND	ND	ND	ND	1.7 ± 0.3
tea (real tap water)	green tea	0.7 ± 0.0	7.4 ± 0.5	0.3 ± 0.06	13.5 ± 0.7	0.12 ± 0.01	0.09 ± 0.0	1.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	2.9 ± 0.6	25.7 ± 1.1
	Earl Grey	0.7 ± 0.0	5.7 ± 0.4	0.4 ± 0.1	14.8 ± 1.0	0.12 ± 0.01	0.08 ± 0.0	1.6 ± 0.1	0.05 ± 0.1	0.09 ± 0.0	1.6 ± 0.4	24.4 ± 0.9
	Lipton tea	0.7 ± 0.0	4.4 ± 0.3	0.3 ± 0.0	12.5 ± 1.7	0.05 ± 0.04	0.07 ± 0.0	0.7 ± 0.2	0.08 ± 0.0	0.2 ± 0.0	ND	18.2 ± 1.7

[a]Means of triplicate measurements and standard deviations shown. ND: nondetect. [b]Nanopure water with 1.0 ppm chlorine added.

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Environ. Sci. Technol. 2021, 55, 12994−13004

water, but they increase to 7.4, 5.7, and 4.1 μg/L levels in green tea, Earl Grey tea, and Lipton tea, respectively, brewed with the same tap water. The detailed reasons are proposed in the following paragraphs.

THMs and HAAs. HAAs were found at higher levels than THMs in the three tap water brewed teas (mean concentrations of 13.3 μg/L vs 5.8 μg/L, for total HAAs and THMs, respectively), due to their lower volatility (Table 1 and Figure 1). While they were also present in the tap water used

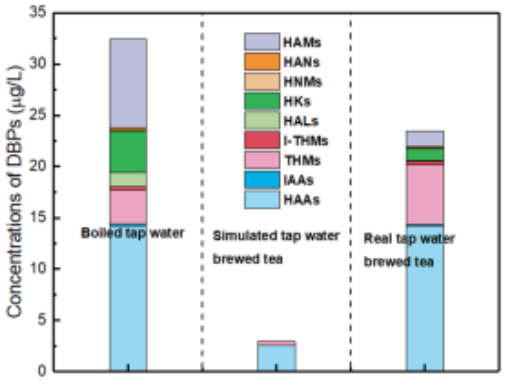

Figure 1. Concentrations of regulated and priority DBPs in boiled tap water, simulated tap water brewed tea (1 ppm Cl_2), and real tap water brewed tea (measured in triplicate). Both simulated and real tap water were boiled before brewing tea; "tap water brewed tea" represents the averages across the three types of tea.

to brew the tea, brewed tea and boiled tap water levels were nearly identical (e.g., 12.5−14.8 μg/L vs 14.0 μg/L, respectively) despite the tea clearly serving as a precursor in simulated tap water brewed tea (Table 1 and Figure 1). Due to higher boiling points (>200 °C) and lower Henry's law constants (<10^{-8}) (Table S7), HAAs are less volatilized during the cooling process at room temperature. We suspected that this phenomenon might be due to adsorption of HAAs onto tea leaves during the brewing. Sorption experiments confirmed this hypothesis. After comparing measured concentrations of DBPs spiked into the tea brewed in nanopure water vs the control without tea, substantially lower levels were observed in the spiked tea samples, demonstrating sorption of DBPs onto tea leaves. Sorption percentages of 0−61 and 6−44% were observed for individual HAAs and total HAAs, respectively (Figure S5 and Tables S8 and S9).

The composition (speciation) of HAAs in tea was similar to boiled tap water (Figure S2). Dichloro-, bromochloro-, chloro-, and dibromoacetic acid were dominant contributors to total HAAs in tea with mean concentrations of 6.9, 3.3, 1.1, and 1.1 μg/L, respectively. The sum of these four HAAs accounted for 90% of the total HAAs. Trichloro-, bromo-, and bromodichloroacetic acids were also detected in tea at relatively lower concentrations (0.7, 0.4, and 0.1 μg/L, respectively). Dibromochloroacetic acid and tribromoacetic acid were not detectable in either boiled tap water or tea, likely due to their lower concentrations in tap water, and lower concentrations of bromide in the source waters. In addition, previous studies have shown that brominated trihaloacetic acids are unstable at high temperatures,[49−51] and dibromochloroacetic acid and tribromoacetic acid are easily converted to THMs during boiling.[50]

Due to their higher volatility, ~50% of the THMs are volatilized during 1 min boiling.[52] However, a significant amount remained in the cooled boiled tap water (3.3 μg/L) (Table 1). In tea brewed in tap water, chloroform was the dominant THM, with a mean concentration of 2.6 μg/L (Table S5), contributing 44% of total THMs. Chloroform was also the dominant THM in boiled tap water and is an important product in the reaction of residual chlorine with tea precursors (0.6 μg/L). Bromodichloromethane and dibromochloromethane were also predominant in tea, with means of 1.8 and 1.3 μg/L, respectively. Bromoform had the lowest concentration (0.1 μg/L) of THMs, likely due to lower concentrations in boiled tap water and low bromide present in source waters. No bromoform formed in reactions of residual chlorine and tea precursors (with no bromide added). While the composition of THMs in tea was similar to that in boiled real tap water, THM concentrations in tea were higher (Figure S2).

This suggests that tea may reduce the volatilization of THMs during the cooling process at room temperature (considering that lower concentrations of chloroform were formed in simulated tap water brewed tea). The total concentration of THMs in tap water was 20.2 μg/L. Since about 50% of the THMs will be volatilized within 1 min boiling, residual THMs in boiled tap water should be about 10 μg/L before cooling.[52] Due to the higher predicted adsorption coefficient (K_{oc} > 100, Table S7), THMs would be expected to adsorb somewhat onto tea leaves during the brewing process, which could reduce their volatilization during cooling.[2] Sorption experiments we conducted confirmed this hypothesis. Adsorption of individual and total THMs during brewing the three types of tea were 13−68 and 26−66%, respectively (Figure S5 and Tables S8 and S9). Only one paper has reported the concentration of THMs in boiled tap water brewed tea.[35] This study reported much higher THM levels in tap water (with 0.95 mg/L Cl_2) brewed teas at 76.9 μg/L. Our levels were much closer to those in the Bond et al. study, which used simulated tap water with 1 mg/L chlorine to brew tea.[5]

Unregulated Priority DBPs. HAMs were also important contributors to total DBPs in tea, with concentrations of 2.9 and 1.6 μg/L in Twinings green tea and Twinings Earl Grey tea, respectively. They were not detected in Lipton tea or in simulated tap water brewed tea (Table 1 and Figure S3). HAM levels in real tap water brewed tea were much lower than in cooled boiled tap water, which may be due to adsorption onto tea leaves (considering that HAMs have lower Henry's law constants (<10^{-8}) and volatility compared to other DBPs besides HAAs; Table S7). An earlier study and present study indicate that >40% of HAMs will adsorb onto tea leaves during brewing, due to their relatively high predicted adsorption coefficients (e.g., predicted K_{oc} values of 26.1 and 96 for dichloroacetamide and dibromoacetamide, respectively) (Figure S5 and Tables S7−S9).[2] Because the green tea and two black teas in our study underwent different levels of fermentation during processing, their components will have different chemical structures, which can result in differences in adsorption properties.[2] It is also possible that HAMs degrade by hydrolysis to form the corresponding HAAs;[53] however, corresponding increases in HAAs were not seen. Dichloroacetamide and bromochloroacetamide were the two HAMs detected in tea, at concentrations of 1.2 and 0.3 μg/L,

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Environ. Sci. Technol. 2021, 55, 12994−13004

respectively. They were also the dominant HAMs in boiled tap water.

HKs were detected in real tap water brewed tea at a mean of 1.2 μg/L. No HKs were detected in simulated tap water brewed tea. HKs in real tap water brewed tea were much lower than in boiled tap water (Table 1 and Figure S3), which suggests some sorption during brewing. 1,1-Dichloropropanone was the dominant haloketone DBP in tea, with a mean of 0.5 μg/L, followed by 1,1,3,3-tetrachloropropanone (0.4 μg/L), chloropropanone (0.2 μg/L), 1,3-dichloropropanone (0.1 μg/L), and 1,1,1-trichloropropanone (0.05 μg/L). The composition of HKs in boiled real tap water and real tap water brewed tea was similar. In boiled real tap water, 1,1,1-trichloropropanone, 1,1-dichloropropanone, 1-bromo-1,1-dichloropropanone, and chloropropanone were the dominant species with means of 1.4, 1.1, 0.6, and 0.6 μg/L, respectively, but in real tap water brewed tea, these concentrations were 0.05, 0.5, nondetect, and 0.2 μg/L, respectively, possibly due to sorption. Sorption experiments confirmed this, with measured adsorption of individual and total HKs of 10−100% and 30−71%, respectively, in the three types of brewed tea (Figure S5 and Tables S8 and S9).

I-THMs, HANs, IAAs, HNMs, and HALs were also detected in real tap water brewed tea at relatively low concentrations (means of 0.3, 0.1, 0.1, 0.09, and 0.08 μg/L, respectively) (Table 1, Figure S2 and Figure S4). While their concentrations were low, most of these have much higher cytotoxicity and genotoxicity compared to regulated THMs and HAAs.[10,11,31] None of these DBPs were detected in tea brewed with simulated tap water, suggesting that they came from the boiled tap water itself. While I-THMs—dichloroiodo-, bromochloroiodo-, dibromoiodo-, and chlorodiiodomethane—were detected in tap water brewed tea, only dichloroiodomethane was detected in boiled tap water. Dichloroacetonitrile and dibromoacetonitrile were the two dominant HANs in both boiled tap water and tap water brewed tea. Their concentrations in tea were significantly lower than in tap water ($P < 0.05$), possibly due to adsorption onto the tea leaves during brewing owning to their relatively high predicted adsorption coefficient (e.g., predicted K_{oc} values of 77.1 and 169 for dichloroacetonitrile and dibromoacetonitrile, respectively; Table S7). Sorption experiments confirmed this, with measured adsorption for total I-THMs, HANs, HNMs, and HALs of 30−43, 41−72, 42−68, and 20−56%, respectively, in the three types of brewed tea (Figure S5 and Tables S8 and S9).

Iodoacetic acid and chloroiodoacetic acid were detected in tap water brewed tea at means of 20 and 100 ng/L, respectively, but in boiled tap water, their concentrations were 10 and 190 ng/L, respectively. Iodoacetic acid concentrations in tap water brewed tea were somewhat higher than in boiled tap water, but chloroiodoacetic acid was lower. Because IAAs might be impacted by both adsorption (Figure S5) and hydrolysis during brewing, a more detailed study is needed to explore this phenomenon. Trichloronitromethane and dichloronitromethane were detected in both boiled tap water and tap water brewed tea, but concentrations were not statistically different in water vs tea. Of the four HALs measured, only trichloroacetaldehyde was detected in boiled tap water, with a mean of 1.4 μg/L, and only bromodichloroacetaldehyde was detected in real tap water brewed tea at 0.08 μg/L, which is near its LOQ of 0.05 μg/L. During brewing and cooling of tea, HALs are likely impacted by volatilization, hydrolysis, and adsorption (Figure S5). Therefore, like IAAs, a more detailed study is needed to explore this phenomenon.

Role of Tea in DBP Formation and Total Organic Halogen. Tea is rich in polyphenols,[40] which contain activated benzene rings that can readily react with chlorine,[54−57] producing halogenated (poly)phenols and other compounds that might be stable end products under the conditions used for brewing tea. As a result, higher-molecular-weight DBPs might be more dominant than the 60 lower-molecular-weight DBPs quantified in this study. To test this hypothesis, total organic chlorine, bromine, and iodine (TOCl, TOBr, and TOI, respectively) were measured in boiled simulated and real tap water brewed tea. TOI was not detected in any of the samples above the LOQ (5 μg/L). Results revealed that the 60 regulated and priority DBPs only account for 3.7 and 17.3% of the total organic halogen (TOX) in boiled simulated and real tap water brewed tea, respectively (Figure 2 and Table S10). This confirms that most of TOX in tea was due to unknown halogenated DBPs.

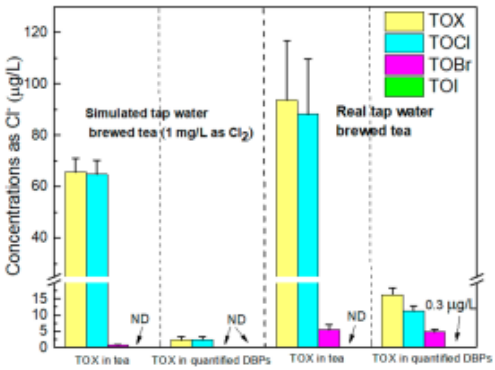

Figure 2. Total organic halogen in tea (ND = not detectable). "Tap water brewed tea" represents the averages across the three types of tea.

In addition, the TOX values in boiled tap water, real tap water brewed tea, and simulated tap water brewed tea were 55, 94, and 66 μg/L (as Cl^-), respectively. TOX in real tap water brewed tea has two sources. The first is TOX in boiled tap water, from DBPs already formed in tap water. The other is TOX from the reaction of residual chlorine in boiled tap water with tea precursors. Since residual chlorine in the simulated tap water is close to that in the real tap water (0.8 mg/L vs 0.7 mg/L), TOX in simulated tap water brewed tea can be used to represent the TOX, which was formed by the reaction of residual chlorine in boiled tap water with tea precursors. Therefore, the theoretical TOX in real tap water brewed tea should be the sum of TOX in boiled tap water and simulated tap water brewed tea (55 + 66 = 121 μg/L). However, the measured TOX in real tap water brewed tea was somewhat lower than this (94 μg/L vs 121 μg/L). This phenomenon might be explained by the slightly higher residual chlorine level in the simulated tap water (0.8 mg/L) vs the real tap water (0.7 mg/L). In boiled tap water, real tap water brewed tea, and simulated tap water brewed tea, TOCl was the dominant contributor to TOX with an average contribution of 94%. In a

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Environ. Sci. Technol. 2021, 55, 12994−13004

Table 2. Unknown DBPs Identified in Simulated Tap Water Brewed Tea[a]

DBP	formula	retention time (min)	theoretical m/z			observed m/z			Δ (ppm)			structure
			M	M+2	M+4	M	M+2	M+4	M	M+2	M+4	
DBP-1	C_6H_5ClO	8.85	128.0023	129.9994	–	128.0024	129.9995	–	0.78	0.77	–	
DBP-2	C_6H_5ClO	10.43	128.0023	129.9994	–	128.0024	129.9995	–	0.78	0.77	–	
DBP-3	$C_6H_4Cl_2O$	12.71	161.9634	163.9604	165.9575	161.9635	163.9605	165.9576	0.62	0.61	0.60	
DBP-4	$C_6H_4Cl_2O$	14.28	161.9634	163.9604	165.9575	161.9635	163.9605	165.9578	0.62	0.61	1.81	
DBP-5	C_6H_5BrO	11.91	171.9518	173.9498	–	171.9519	173.9495	–	0.58	-1.72	–	
DBP-6	C_6H_5BrO	15.70	171.9518	173.9498	–	171.9522	173.9498	–	1.74	0	–	
DBP-7	C_6H_4ClBrO	15.66	205.9129	207.9106	209.9080	205.9130	207.9107	209.9080	0.49	0.48	0	
DBP-8	C_6H_4ClBrO	15.66	205.9129	207.9106	209.9080	205.9130	207.9107	209.9080	0.49	0.48	0	
DBP-9	C_6H_4ClBrO	15.66	205.9129	207.9106	209.9080	205.9130	207.9107	209.9080	0.49	0.48	0	
DBP-10	$C_6H_5ClO_2$	13.28	143.9973	145.9943	–	143.9974	145.9944	–	0.69	0.68	–	
DBP-11	$C_6H_5ClO_2$	14.07	143.9973	145.9943	–	143.9974	145.9946	–	0.69	2.05	–	
DBP-12	$C_6H_5ClO_3$	17.19	159.9922	161.9892	–	159.9923	161.9894	–	0.63	1.23	–	
DBP-13	$C_6H_5ClO_3$	20.29	159.9922	161.9892	–	159.9922	161.9893	–	0	0.62	–	
DBP-14	$C_6H_4Cl_2O_3$	20.10	193.9532	195.9503	197.9477	193.9534	195.9504	197.9475	1.03	0.51	-1.01	
DBP-15	$C_6H_4Cl_2O_3$	20.49	193.9532	195.9503	197.9477	193.9533	195.9503	197.9474	0.52	0	-1.52	

[a]Unknown DBPs were detected in all tea samples; however, DBP-3 was not detected in brewed green tea. DBP-7/8/9 showed one peak in GC-HRT-TOF-HRMS but showed three isomers using GC × GC-TOF-MS. The isomers of DBP-1/2, DBP-3/4, DBP-5/6, and DBP-7/8/9 were confirmed by the retention time and mass spectra of standard in GC × GC-TOF-MS.

previously published study on simulated tap water treated tea, 164–196 μg/L (as Cl^-) of TOX was generated when instant tea reacted with 4 mg/L chlorine for 24 h.[40] These higher TOX levels are likely due to a higher chlorine level and longer contact time compared to our present study.

Nontarget Identification of Unknown DBPs. For the identification of unknown tea DBPs, simulated tap water (containing only bromide, iodide, and chlorine) was used as the water source to investigate DBPs formed directly from tea precursors only. Fifteen unknown DBPs were identified using GC with medium and high-resolution MS, including halogenated phenols, halogenated dihydroxybenzenes, and halogenated trihydroxybenzenes, which are reported here for the first time as tea DBPs. All of these DBPs are haloaromatic compounds, which can have tens to hundreds of times higher toxicity than THMs and HAAs.[58] For example, the toxicity of 2,4,6-tribromophenol is about 125 times higher than bromoacetic acid when measured in *Tetraselmis marina* algae.[59] GC × GC was helpful in separating some DBPs from other coeluting compounds in the complex tea extracts. For example, GC × GC allowed complete separation of 2,6-dichlorophenol from a coeluting unknown compound (Figure S6).

Six haloaromatic DBPs (DBP-1, DBP-2, DBP-3, DBP-4, DBP-10, and DBP-11) had full-scan mass spectral matches in the NIST library, and could be tentatively assigned to 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, and two monochloro-hydroxyphenols (Table 2). High-resolution data for the molecular ions, as well as isotopic patterns and fragment ions, supported these structural assignments. For example, molecular ions for DBP-1 and DBP-2 (Table 2), identified as 2-chlorophenol and 4-chlorophenol, respectively, had high-resolution accurate masses that corresponded to a molecular formula of C_6H_5ClO (observed m/z 128.0024; theoretical m/z 128.0023), indicating a chlorophenol structure (Table 2 and Figure S7). The relative isotopic abundance of m/z 128 to m/z 130 was 3:1, indicative of a compound containing 1 chlorine atom (Figure S7), and fragment ions m/z 92 and 64 could be assigned to $[M\text{-}HCl]^+$ and $[M\text{-}Cl\text{-}CHO]^+$, respectively, further supporting an assignment of a chlorophenol structure. Of the 3 possible chlorophenol isomers (2-, 3-, and 4-chlorophenol),

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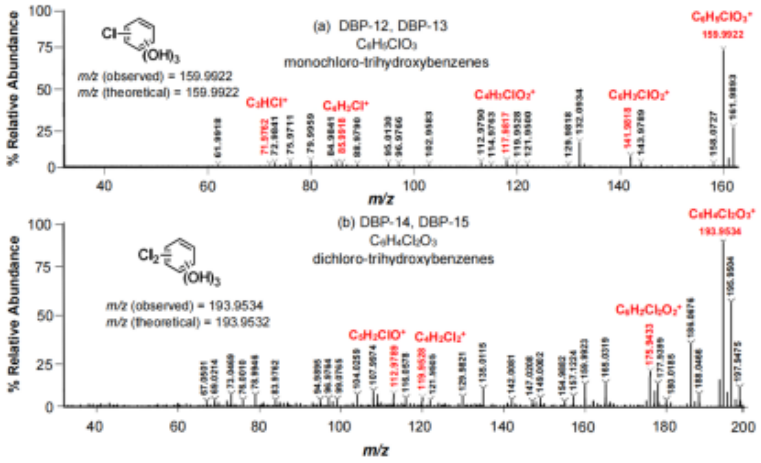

Figure 3. High-resolution EI mass spectra of unknown DBPs in tea.

ortho and *para* substitution is favored in chlorination reactions;[60] in fact, these were the 2 isomers confirmed by the analysis of authentic standards (2-chloro- and 4-chlorophenol), which showed a good match of their GC retention times (RTs) and full-scan mass spectra with the unknown DBPs (Figure S7). Structures for DBP-3 and DBP-4 were confirmed in a similar manner (Table 2 and Figure S8). Structures of DBP-10 and DBP-11 were not confirmed, due to large number (six) of possible isomers and difficulty in obtaining some of the standards.

Five other haloaromatic DBPs (DBP-5, DBP-6, DBP-7, DBP-8, and DBP-9) were present in the NIST library database (as 2-bromophenol, 4-bromophenol, 2-bromo-4-chloro-phenol, 2-chloro-4-bromophenol, and 2-bromo-6-chlorophenol, respectively), but due to matrix interferences in the tea extracts (that could not be sufficiently background subtracted), good library matches were not obtained (Figures S9–S11). However, high-resolution data was used to propose the molecular ions, and authentic standards were obtained to confirm the structures. For example, molecular ions for DBP-5 and DBP-6 (Table 2), identified as 2-bromophenol and 4-bromophenol, respectively, had high-resolution accurate masses that corresponded to a molecular formula of C_6H_5BrO (observed m/z 171.9519; theoretical m/z 171.9518), indicating a bromophenol structure (Table 2 and Figure S11). Like chlorophenols, *ortho* and *para* substitution is favored, and the mass spectra and the RTs of DBP-5 and DBP-6 matched with authentic standards of 2-bromo- and 4-bromophenol. Structures for DBP-7, DBP-8, and DBP-9 were confirmed in a similar manner (Table 2 and Figures S11 and S12). While halogenated phenols have also been observed as DBPs in drinking water,[61,62] this is the first time they have been shown to form with tea precursors.

Four haloaromatic DBPs (DBP-12, DBP-13, DBP-14, and DBP-15) were not present in the NIST library database, and their structures were proposed as described below. DBP-12 and DBP-13 at RT 17.19 and 20.29 min, respectively, have the same molecular ion at m/z 160/162, with an isotopic abundance ratio of 3:1, indicating that these compounds contain 1 chlorine atom (Figure 3a). Their accurate masses and isotopic abundance ratios matched well with those of monochloro-trihydroxybenzenes (theoretical and observed m/z of 159.9922 and 159.9922, respectively; Δ = 0.0 ppm). Fragment ions m/z 141.9818/143.9789, 117.9817, 85.9918, and 71.9762 are due to $[M\text{-}H_2O]^+$, $[M\text{-}C(OH)CH]^+$, $[M\text{-}C_2H_2O_3]^+$, and $[M\text{-}C_3H_4O_3]^+$, respectively. DBP-14 and DBP-15 at RT 20.10 and 20.49 min have the same molecular ion at m/z 194/196/198, and an isotopic abundance ratio of 9:6:1, indicating that these compounds contain 2 chlorine atoms (Figure 3b). A molecular formula of $C_6H_4Cl_2O_3$ was indicated by the accurate mass of m/z 193.9534 (theoretical m/z of 193.9532; Δ = 1.03 ppm). These compounds are proposed to be dichloro-trihydroxybenzenes. Accurate mass data indicate that fragment ions m/z 175.9433/177.9399, 119.9528, and 112.9789 are due to $[M\text{-}H_2O]^+$, $[M\text{-}C_2H_2O_3]^+$, and $[M\text{-}Cl\text{-}CHOOH]^+$, respectively. This is the first time halogenated trihydroxybenzenes have been reported as DBPs in both drinking water and tea.

Quantitation of Halophenols in Tea. To estimate the concentrations of the new halophenols identified in simulated tap water brewed tea, a quantitative approach was taken by using matrix-matched calibration for each type of tea. Of the 9 halophenols measured in Twinings green tea, Earl Grey tea, and Lipton tea (DBP-1 through DBP-9), DBP-4 was the only halophenol quantified above the LOQ (0.05 μg/L) at a concentration of 0.2 μg/L in Lipton tea. While the other halophenols were positively identified in the tea samples using XAD resin extraction, the quantitative method does not afford the huge concentration factor of XAD extraction (500× vs 25 000×) for a 100 mL vs a 5000 mL sample. We believe the concentrations for these other haloaromatic DBPs fall in the 1–50 ng/L range (Table S11). Further method optimization could be done in the future to potentially lower the LOQs to allow their lower levels to be determined.

■ IMPLICATIONS

Tea is the second most globally consumed nonalcoholic beverage next to drinking water, accounting for a large portion of the daily consumption of tap water. This study demonstrates that a wide variety of regulated and priority DBPs also widely

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Environ. Sci. Technol. 2021, 55, 12994–13004

occur in tea brewed with tap water. However, in many cases, measured DBP levels in tea were lower than in the tap water itself due to volatilization and sorption. This is because chlorine reactions with tea formed few of the 60 measured DBPs (mostly HAAs), while many DBPs already in the tap water were removed during brewing.

However, the known DBPs may not be the most important. Notably, the TOX in tea nearly doubled relative to tap water, with only ~4% represented by known, quantified DBPs. The ~96% unknown TOX could be due to unidentified DBPs that have some associated toxicity. It is possible that much of this unknown TOX are high-molecular-weight haloaromatic compounds, formed by the reaction of chlorine with polyphenols present in tea leaves. In fact, the identification of several haloaromatic DBPs indicates this may be the case. Haloaromatics are also recognized as DBP intermediates that can react further to form lower-molecular-weight THMs/HAAs, etc. over time.[63] Given the short chlorine contact times involved (to mimic realistic tea brewing conditions), haloaromatic intermediates would be expected as DBPs. Further studies on the identity and formation of these aromatic DBPs should be conducted since haloaromatic DBPs can have significant toxicity.[58,59]

To lower DBP/TOX levels, chlorine-free bottled water could be used as a substitute for tap water when brewing tea. Recent studies also indicate that adding ascorbate or lemon slices (rich in vitamin C) before boiling could reduce the toxicity of DBPs in chlorinated tap water.[64,65] This might be another simple way to mitigate DBP risks when preparing hot tea.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c03419.

Instrumental parameters, mass spectral and chromatographic data, detailed concentration data for DBPs, Henry's law constants and predicted adsorption coefficients (K_{oc}) of DBPs, and description of the total organic halogen procedure (PDF)

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Notes

The authors declare no competing financial interest.

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Microseira wollei and *Phormidium* algae more than doubles DBP concentrations and calculated toxicity in drinking water

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Lyngbya

ABSTRACT

Warm weather and excess nutrients from agricultural runoff trigger harmful algal blooms, which can affect drinking water safety due to the presence of algal toxins and the formation of disinfection by-products (DBPs) during drinking water treatment. In this study, 66 priority, unregulated and regulated DBPs were quantified in chlorinated controlled laboratory reactions of harmful algae *Microseira wollei* (formerly known as *Lyngbya wollei*) and *Phormidium* using gas chromatography (GC)-mass spectrometry (MS). Live algae samples collected from algae-impacted lakes in South Carolina were chlorinated in both ultrapure water and real source waters containing natural organic matter. DBPs were also measured in finished water from a real drinking water plant impacted by a *Microseira* bloom. Results show that the presence of *Microseira* and *Phormidium* more than doubles total concentrations of DBPs formed by chlorination, with levels up to 586 μg/L formed in natural lake waters. Toxic nitrogen-containing DBPs also more than doubled in concentration, with levels up to 36.1, 3.6, and 37.9 μg/L for haloacetamides, halonitromethanes, and haloacetonitriles, respectively. In ultrapure water, DBPs also formed up to 314 μg/L when algae was chlorinated, demonstrating their ability to serve as direct precursors for these DBPs. When environmentally relevant levels of bromide and iodide were added to chlorination reactions, total DBPs increased 144, 51, and 24% for drinking water reservoir, Lake Marion and Lake Wateree *Microseira* respectively and 29% for *Phormidium*. Iodo-DBPs, bromochloroiodomethane, chloroiodoacetic acid, bromoiodoacetic acid, and diiodoacetic acid were observed in finished water from a drinking water plant impacted by *Microseira*, and bromochloroiodomethane and dibromoiodomethane were observed in chlorinated ultrapure water containing algae, bromide, and iodide. Notably, total calculated cytotoxicity tripled in *Microseira*-impacted waters and doubled for *Phormidium*-impacted waters. Calculated genotoxicity doubled for *Microseira*-impacted waters and more than doubled in *Phormidium*-impacted waters. Haloacetonitriles were major drivers of calculated cytotoxicity in algae-impacted waters, while haloacetic acids were major drivers of calculated genotoxicity in algae-impacted waters. These results provide the most extensive assessment of DBPs formed from chlorination of algae-impacted waters and highlight potential impacts to drinking water and human health. Results from this study are particularly applicable to drinking water treatment plants that employ pre-chlorination, which can cause the release of algal organic matter (AOM) precursors to form DBPs.

1. Introduction

Harmful algal blooms (HABs) are a major concern worldwide for public health and safety. In 2019 alone, 242 HAB events were reported by 14 states in the U.S. (CDC, 2019), resulting in 63 cases of human illness and 364 cases of animal illness. Of the 364 animal illness cases reported, 56% died as a result of their exposure. In 2002, a teenager from Wisconsin died after suffering from HAB related illness after swimming in an *Anabaena flos-aquae* impacted pond known to produce neurotoxic anatoxin-a (also known as "very fast death factor") (Behm, 2003). HABs are not only a concern for human exposure during recreational use of impacted waters such as swimming or fishing, but they have also been shown to impact drinking water safety. Drinking water treatment plants in the U.S. rely heavily on surface waters, and about 75% of reported HAB events occur in fresh waters (CDC, 2019). In 2014, Toledo, Ohio's water supply was shut down due to a harmful *Microcystis*

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aeruginosa bloom in Lake Erie, affecting 400,000 residents (EOS, 2021).

Microseira wollei is also of major concern for lakes around the world, including Lake Wateree and Lake Marion in South Carolina (SC) and several drinking water treatment plants that use these waters as their source for drinking water. *Microseira wollei* is a filamentous, benthic, and perennial algae which produces toxic saxitoxin analogues known as *Microseira wollei* toxins (Carmichael et al., 1997; Foss et al., 2012; Onodera et al., 1997; Smith et al., 2019; Yin et al., 1997). *Phormidium*, which is another filamentous harmful algae known to produce the acute neurotoxin anatoxin-a (Gugger et al., 2005; Teneva et al., 2005; Wood et al., 2007; Borges et al., 2015), was also found in Lake Wateree. These algal species release intracellular organic matter (IOM) and extracellular organic matter (EOM) into the water column, which includes carbohydrates, proteins, amino acids, aliphatic amines, and peptides (Fang et al., 2010; Baroni et al., 2020). Algal organic matter (AOM) also contains more organic nitrogen and hydrophilic carbon material than natural organic matter (NOM) (Widrig et al., 1996; Nguyen et al., 2005; Her et al., 2004; Ma et al., 2006), which mostly contains humic substances with more aromatic carbon and low nitrogen content. As a result, algal organic matter has a lower specific UV absorbance (SUVA) and higher heterogeneity compared to NOM.

Neither coagulation nor pre-oxidation mediated coagulation processes can remove IOM and EOM in drinking water treatment (Ma et al., 2006; Richardson et al., 2007). When algae-impacted source water is disinfected, IOM and EOM can react with the disinfectant (e.g., Cl_2, NH_2Cl, O_3, or UV) and produce toxic carbonaceous and nitrogenous DBPs (C-DBPs and N-DBPs) (Richardson et al., 2007, 2011). DBPs have been linked to adverse human health effects, such as bladder cancer, colorectal cancer, and birth defects (Richardson et al., 2007; Cantor et al., 2010; Nieuwenhuijsen et al., 2000; Savitz et al., 2005; Villanueva et al., 2004, 2007; Waller et al., 1998; Costet et al., 2011; Rahman et al., 2007). Currently, only 11 DBPs are regulated by the U.S. Environmental Protection Agency (EPA) (US EPA, 2006) despite the identification of more than 700 DBPs to date (Richardson et al., 2007; Richardson and Plewa, 2020; Richardson, 2011). Many of these priority, unregulated DBPs are cytotoxic, genotoxic, carcinogenic, or mutagenic, and most are more toxic than the DBPs currently regulated (Richardson et al., 2007; Richardson and Plewa, 2020).

Previous research has reported DBP formation from AOM, EOM, and sometimes intact algal cells, including material from *Microcystis aeruginosa, Oscillatoria sp., Microseira sp., Scenedesmus quadricauda, Nitzschia palea, Dolichospermum circinale, Cylindrospermopsis raciborskii,* and *Chlorella vulgaris* (Fang et al., 2010; Liu et al., 2022; Wert and Rosario-Ortiz, 2013; Zhou et al., 2015; Bond et al., 2011, 2012; Hoehn, 1980; Hong et al., 2008; Huang et al., 2009; Zhang et al., 2017; Li et al., 2020). A new study by Liu also shows preferential halogenation of AOM by iodine (over chlorine and bromine) when iodide is present in chloraminated waters (Liu et al., 2022).

However, only a relatively small number of DBPs have been investigated to-date (up to 33), and a comprehensive understanding of the broad range of DBPs is missing. In addition, DBPs from *Phormidium* have not previously been investigated. Therefore, the goal of this research was to investigate the formation of 66 priority and regulated DBPs following the chlorination of live algae *Microseira wollei* and *Phormidium*, which impact not only drinking water sources in South Carolina, but also many regions throughout the U.S. and the world. These results provide a more complete assessment of DBPs formed from algae during chlorination. Chlorine was chosen because it is the most common disinfectant in the U.S. (Cuthbertson et al., 2020). The 66 priority and regulated DBPs quantified include 10 trihalomethanes (THMs), 13 haloacetic acids (HAAs), 4 haloaldehydes (HALs), 9 haloketones (HKs), 13 haloacetamides (HAMs), 7 halonitromethanes (HNMs), and 10 haloacetonitriles (HANs). Among them, the N-DBPs (HAMs, HNMs, and HANs) and iodo-DBPs (iodo-THMs and iodoacetic acids) are considered high priority DBPs due to their significantly higher toxicity (Richardson et al., 2007; Krasner et al., 2006), and they were recently reported as important drivers of toxicity in U.S drinking waters (Allen et al. 2022).

2. Materials and methods

2.1. Chemicals and reagents

Individual standards of dichloroacetonitrile, trichloroacetonitrile, 1,1,1-trichloropropanone, dibromoacetonitrile, 1,1-dichloropropanone, trichloronitromethane, trichloroacetaldehyde, and bromochloroacetonitrile, four THMs (trichloromethane, bromodichloromethane, dibromochloromethane, and tribromomethane) and nine HAAs (chloroacetic acid, dichloroacetic acid, trichloroacetic acid, bromoacetic acid, dibromoacetic acid, tribromoacetic acid, bromochloroacetic acid, dibromochloroacetic acid, and bromodichloroacetic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other DBP standards were either purchased from Sigma-Aldrich (St. Louis, MO), TCI America (Boston, MA), and Aldlab (Boston, MA) or synthesized by CanSyn Chem. Corp. (Toronto, Ontario). A complete list is given in Table S1, Supporting Information. All solvents (methyl *tert*-butyl ether (MTBE), ethyl acetate, acetonitrile, and methanol) were purchased from Sigma-Aldrich and Honeywell International (Muskegon, MI) at their highest purity (GC-MS grade). Barnstead ultrapure water (18.2 MΩ) was used to perform controlled laboratory reactions with live algae. Monobasic potassium phosphate, dibasic potassium phosphate, sodium hydroxide, sulfuric acid, hydrochloric acid, and sodium hypochlorite solution (5.65-6%) were purchased from Fisher Scientific (Pittsburg, PA). Sodium bromide and sodium iodide standards were purchased from Sigma-Aldrich.

2.2. Sampling

Live *Microseira wollei* grab samples (surface floating mat) were collected from Lake Wateree, Lake Marion, and a small drinking water reservoir (DWR) in South Carolina (SC). Live *Phormidium* samples were collected from Lake Wateree, SC. Samples were collected in 500 mL polytetrafluoroethylene (PTFE) bottles and stored on ice (0°C) in a cooler during transportation. Live algae samples were cleaned immediately upon arrival and refrigerated. Raw lake water samples were also collected from the sites where algae were collected. Finished (chlorinated) drinking water (2 L) was collected from a drinking water plant in a small city in SC whose water reservoir was experiencing a *Microseira* bloom in September 2020 (residual chlorine of 1.3 mg/L). Raw water samples were filtered with 5 μm filters (vacuum filtration) and stored at 4 °C until extractions. Residual chlorine in finished drinking water samples was quenched immediately with ammonium chloride (1:1.3 molar ratio of $Cl:NH_2Cl$) and extracted to quantify DBPs.

2.3. Experimental design

Live algae samples were carefully separated (physical separation) to remove dirt and small aquatic animals. Once cleaned, the algae samples were then washed with their corresponding source water several times to remove any remaining dirt and then pressed by hand to remove extraneous water. Each type of algae was chlorinated in 250 mL amber bottles (headspace free) in ultrapure water and source water at pH 7 (1 mM phosphate buffer) at a ratio of approximately 15 mg/L cellular mass to 4.0-25.0 mg/L free chlorine (standard sodium hypochlorite solution) based on chlorine demand experiments to achieve 1-2 mg/L residual chlorine after 24 hr reaction (Table S6). The active cellular mass of algae was calculated to contain 15 mg/L of *Microseira wollei*. To determine the amount of algae to add to each reactor, the percent of cell bound water and unreactive sheath mass was subtracted from the mass of the pressed wet algae. The same amount of *Phormidium* was reacted in order to allow for a direct comparison between the two algae species. The calculation described above is given in the Supporting Information (Text S1). The concentration of algae used (15 mg/L) for reactions was chosen because

it is similar to high DOC levels observed in drinking water source waters in the U.S. (Richardson et al., 2008). Samples were also chlorinated following the addition of 500 μg/L bromide and 100 μg/L iodide to represent elevated levels of these salts that can be found in surface waters (Richardson et al., 2008). Bromide and iodide are of interest because they can form HOBr and HOI in the presence of chlorine, which can then react with natural organic matter and other contaminants (e.g., toxins or algal organic matter) to form brominated and iodinated DBPs, which are more toxic than chlorine containing DBPs (Richardson et al., 2007; Wagner and Plewa, 2017). The residual chlorine was then quenched after 24 hr using ammonium chloride and the samples were then filtered using 5 μm filters to remove reacted algae. The reactions were carried out at room temperature (25°C) and in duplicate with chlorinated ultrapure water was used for blank measurements.

2.4. Extraction and sample preparation

To explore the potential for DBP formation, 100 mL (filtered with 5 μm filters) from each reactor (in duplicate) was extracted and analyzed to quantify 66 regulated and priority, unregulated DBPs, including 10 THMs (trichloromethane, bromodichloromethane, dibromochloromethane, tribromomethane, dichloroiodomethane, bromochloroiodomethane, dibromoiodomethane, chlorodiiodomethane, bromodiio domethane, and triiodomethane), 13 HAAs (chloroacetic acid, bromoacetic acid, dichloroacetic acid, bromochloroacetic acid, dibromoacetic acid, trichloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid, tribromoacetic acid, iodoacetic acid, chloroiodoacetic acid, bromoiodoacetic acid, and diiodoacetic acid), 4 HALs (trichloroacetaldehyde, bromodichloroacetaldehyde, dibromochloroac etaldehyde, and tribromoacetaldehyde), 9 HKs (chloropropanone, 1,1-dichloropropanone, 1,1-dibromopropanone, 1,3-dichloropropanone, 1,1,1-trichloropropanone, 1,1,3-trichloropropanone, 1-bromo-1,1-dichloropropanone, 1,1,3,3-tetrachloropropanone, and 1,1,3,3-tetrabromopropanone), 13 HAMs (chloroacetamide, bromoacetamide, iodoacetamide, dichloroacetamide, bromochloroacetamide, dibromoacetamide, chloroiodoacetamide, bromoiodoacetamide, diiodoacetamide, trichloroacetamide, bromodichloroacetamide, dibromoch loroacetamide, and tribromoacetamide), 7 HNMs (dichloronitromethane, bromochloronitromethane, dibromonitromethane, trichloronitromethane, bromodichloronitromethane, dibromochlor onitromethane, and tribromonitromethane), and 10 HANs (chloroacetonitrile, bromoacetonitrile, iodoacetonitrile, dichloroacetonitrile, bromochloroacetonitrile, dibromoacetonitrile, trichloroacetonitrile, bromodichloroacetonitrile, dibromochloro
acetonitrile, and tribromoacetonitrile) (Table S1).

Liquid-liquid extraction (LLE) followed by analysis using GC-MS was conducted following previously methods published from our laboratory (Cuthbertson et al., 2020, 2019; Allen et al., 2017, 2022). Briefly, 100 mL samples were transferred into a 125 mL amber bottle, pH adjusted to <1 using concentrated sulfuric acid, and spiked with 30 g of Na_2SO_4 and 5 mL of methyl *tert*-butyl ether (MTBE). Samples were then shaken for 15 min using a mechanical shaker then allowed to settle to 10 min before the organic layer was removed and collected into a glass test tube using glass Pasteur pipettes. The process of adding MTBE was repeated two more times for a total organic extract volume of approximately 15 mL. The organic layer was then passed through an anhydrous Na_2SO_4 column to remove any excess water and concentrated to 200 μL using a gentle stream of nitrogen. Four μL of 30 mg/L 1,2-dibromopropane was added to the concentrated extract as an internal standard.

The sample was then divided into 2 aliquots (100 μL each)—one aliquot was used to quantify THMs, HALs, HKs, HAMs, HNMs, and HANs, and the other aliquot was derivatized using diazomethane for the quantification of HAAs and IAAs. Briefly, 0.0367 g of Diazald and 1 mL of diethylene glycol monoethyl ether (Carbitol) were placed into the inner portion of a Sigma Aldrich diazomethane-generator apparatus. Then, 3 mL of MTBE was added to the outer portion of the apparatus and both portions were assembled. Then, the apparatus was place in ice and 1.5 mL of 37% KOH was added into the inner part slowly using a syringe to pierce the septum. After 1 hr, 50 μL of the diazomethane (dissolved in MTBE) was spiked into 100 μL of the extracted samples and allowed to react for 30 min and quenched using silica gel. Derivatized samples were then transferred into new GC vials and analyzed using GC-MS.

2.5. GC-MS analysis

Internal calibration was used to quantify 66 DBPs as described by Cuthbertson et al. (2020). For the analysis of THMs, HALs, HKs, HAMs, HNMs, and HANs, instrumental analysis was performed on a single quadrupole GC mass spectrometer (Agilent 7890 GC, 5977A mass spectrometer, Agilent Technologies, Santa Clara, CA) with electron ionization (EI) in selected ion monitoring (SIM) mode. Briefly, 1.0 μL of sample was injected into a multimode inlet (MMI) in pulsed splitless mode and the separation performed using a Rtx-200 column (30 m × 0.25 mm × 0.25 μm; Restek Corporation, Bellefonte, PA). The GC temperature program was as follows: 35°C for 5 min, increased to 200°C at 9°C/min, then ramped to 280°C and held for 20 min. The source temperature was 200°C, quadrupole temperature was 150°C, and an ionization energy of 70 eV was used. For bromodichloroacetonitrile, bromodichloronitromethane, dibromochloroacetonitrile, dibromochloronitromethane, tribromoacetonitrile, and tribromonitromethane, the same GC program was used with the exception of the inlet and transfer line temperatures, which were kept at 125 and 250°C, respectively, due to their thermal instability.

For the analysis of HAAs and IAAs, samples were analyzed using both a Thermo Trace 1310 GC coupled to a TSQ 9000 triple quadrupole mass spectrometer (Thermo Fisher Scientific Corporation, Waltham, MA) and an Agilent 7890 GC coupled to a 5977A mass spectrometer. The GC temperature program was as follows: 35°C for 2 min, increased to 280°C (rate 9°C/min), and held for 20 min. The transfer line and source temperature were 280 and 200°C, respectively and ionization energy was 70 eV. Selected ions and limits of quantification (LOQs) for the 66 DBPs quantified are listed in Table S1.

2.6. Water quality parameters

Water quality parameters, including pH, UV absorbance, dissolved organic carbon (DOC), specific UV absorbance (SUVA), chloride, bromide, and iodide are listed in Table 1. To determine the DOC content in the raw water samples, they were first filtered through nylon syringe filters (0.45 μm) to remove insoluble particles prior to instrumental analysis. A calibration curve was made (1-20 mg/L) using monobasic potassium phthalate (Sigma-Aldrich, USA) as carbon. Samples were then analyzed using a Shimadzu Total Organic Carbon analyzer (Kyoto, Japan) and were blank (ultrapure water) subtracted. Specific ultraviolet

Table 1
Water quality parameters of algae-impacted source waters.

Location	pH	UV_{254} (abs/cm)	DOC (mg/L)	SUVA (L/mg-M)	Cl^- (μg/L)	Br^- (μg/L)	I^- (μg/L)
DWR (*Microseira* impacted)	6.9	0.118	0.9	13.1	332	1.6	ND
Lake Marion (*Microseira* impacted)	6.9	0.365	7.7	4.7	651	3.9	ND
Lake Wateree (*Microseira* impacted)	6.9	0.168	1.9	8.8	444	2.1	ND
Lake Wateree (*Phormidium* impacted)	7.0	0.145	1.2	12.1	709	3.0	<1

ND: not detected.

absorbance (SUVA) was calculated by dividing the UV_{254} values (cm^{-1}) by DOC (mg/L) and then multiplied by 100 cm/M. UV absorbance at 254 nm was measured using a SpectraMax M5 UV spectrometer (Molecular Devices, San Jose, CA). Bromide (Br^-) and iodide (I^-) were measured in raw water samples using a Dionex Integrion High Performance Ion Chromatography (HPIC) system (Dionex Corporation, Sunnyvale, CA) with a 500 µL sample loop. Separation was performed using an IonPac AS20 analytical column, which was attached to an IonPac AS20 guard column with 50 mM NaOH as the eluent. External calibration was used with standards prepared in ultrapure water (1-750 µg/L) using sodium chloride (as Cl^-), sodium bromide (as Br^-), and sodium iodide (as I^-). The limits of quantification (LOQs) for all three are 1.0 µg/L.

2.7. Calculated toxicity

Calculated toxicity associated with DBPs in each sample was based on the "TIC-Tox" method Plewa et al., 2017), where molar concentrations of each DBP are multiplied by their reported cyto- or genotoxicity index values in Chinese hamster ovary cells (Wagner and Plewa, 2017) and summed together (Eq. (1) and (2)). Haloketones are not included because no cytotoxicity and genotoxicity data are available for them. THMs are not genotoxic except chlorodiiodomethane.

$$\text{Total calculated cytotoxicity} = \sum\left([DBP] * LC_{50}^{-1} * 10^6\right) \quad (1)$$

$$\text{Total calculated genotoxicity} = \sum\left([DBP] * 50\%TDNA^{-1} * 10^6\right) \quad (2)$$

[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.

3. Results and discussion

3.1. Chlorination of algae

Upon chlorination of *Microseira*, the green cells (intracellular material) inside the outer sheaths (Fig. 1) reacted with chlorine, but the outer sheaths remained intact after 24 hr reaction with chlorine (Fig. 2). In contrast, both the intracellular material and the outer sheaths of *Phormidium* visibly reacted with chlorine (Fig. 3). Both *Microseira* and *Phormidium* have polysaccharide sheaths (Burkholder, 2009 and Hoiczyk, 1998). A comprehensive listing of individual DBP concentrations measured in all waters can be found in Tables S2–S5.

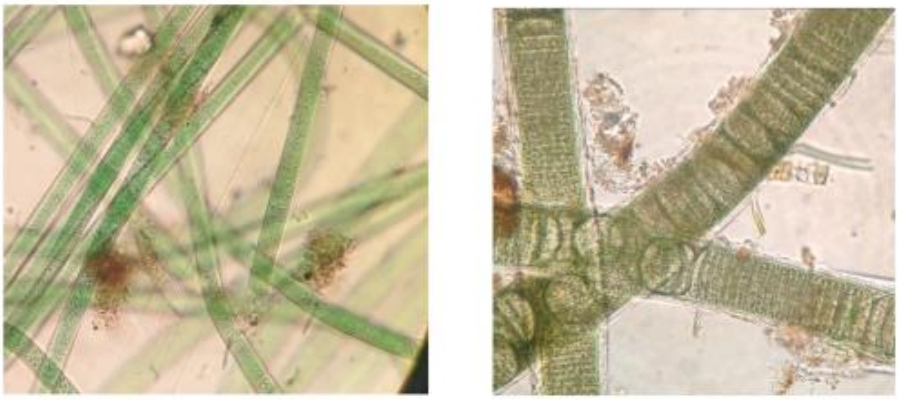
3.2. Phormidium DBPs

Several DBPs were observed in chlorination reactions of *Phormidium* in ultrapure water (Table 2 and Table S2), demonstrating that the algal organic matter contained in *Phormidium* can directly contribute to DBP formation and can impact chlorinated drinking water. Individual DBP levels ranged from 0.1-70 µg/L, with trichloromethane, dichloroacetic acid, trichloroacetic acid, dichloroacetamide, dichloroacetonitrile, and trichloroacetaldehyde forming at the highest levels, up to 314 µg/L. Dichloroacetamide (19.5 µg/L) and dichloroacetonitrile (17.9 µg/L) are among the more toxic N-DBPs. When bromide and iodide were added to reactions (forming HOBr), total DBPs increased from 314 to 406 µg/L (Fig. 4) when compared to algae in ultrapure water with chlorine. Brominated DBPs include dibromochloromethane (39.8 µg/L), bromodichloromethane (41.7 µg/L), bromodichloroacetic acid (28.7 µg/L), and dibromochloroacetic acid (24.7 µg/L), which are more cytotoxic and genotoxic than their chlorinated analogues (Richardson et al., 2007; Wagner and Plewa, 2017). No iodinated DBPs were observed from chlorinated *Phomidium* samples, likely due to iodide reacting quickly with HOCl to form iodate (IO_3^-), a sink for iodide (Richardson et al., 2008; Bichsel and Gunten, 1999).

The impact of *Phormidium* on drinking water is even more apparent when comparing DBPs formed in natural lake waters to those with added *Phormidium*. The presence of *Phormidium* more than doubled the levels of DBPs upon chlorination, with total DBPs increasing from 254 µg/L in chlorinated lake water to 586 µg/L in chlorinated lake water containing *Phormidium*. Thus, while NOM is often recognized as a primary precursor to DBP formation, it is evident that algal organic matter is also a significant precursor to DBP formation.

3.3. Microseira wollei DBPs

Higher levels of DBPs were also observed in chlorinated reactions in Lake Wateree *Microseira* samples with algae present (452 µg/L) when compared to chlorinated source water (135 µg/L) (Table 2 and Fig. 4) which is 3.3x higher. When comparing the different *Microseira* chlorination reactions in ultrapure water, *Microseira wollei* collected from the



Phormidium filaments *Microseira* filaments

Fig. 1. Microscopic pictures of *Microseira* and *Phormidium* filaments (400 times magnified) using an Olympus BH-2 microscope (Olympus Corporation, Tokyo, Japan).

Fig. 2. Pictures of *Microseira wollei* before and after 24 hr chlorination.

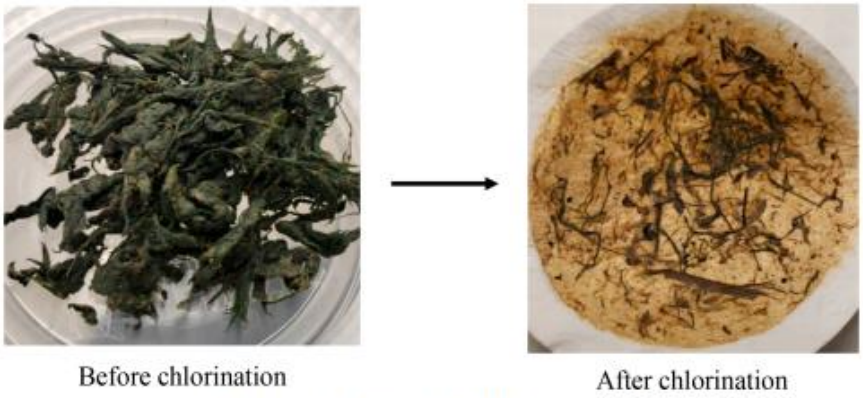


Fig. 3. Pictures of *Phormidium* before and after 24 hr chlorination.

DWR formed lower levels of DBPs (66.7 μg/L). The *Microseira* in this reservoir was believed to be at an early stage of growth, which may have rendered it less reactive than the more mature forms, which produced 146 and 167 μg/L total DBPs for *Microseira* collected from Lake Marion and Lake Wateree, respectively. Fig. 2 presents a visual comparison of *Microseira* from these three different locations (before and after chlorination), which indicates that *Microseira* from the DWR was somewhat different than the others (light green and viscous).

DBPs formed from the DWR, Lake Marion, and Lake Wateree samples are provided in Table S3–S5. A variety of DBPs formed, ranging individually from <0.1 to 34.5, <0.1 to 172, and 0.1 to 140 μg/L for DWR, Lake Marion, and Lake Wateree *Microseira* samples, respectively. HAAs were the most abundant class of DBPs formed at all three sampling locations, with an average total value of 122 μg/L (Table 2, Fig. 4). In every chlorinated sample containing algae in ultrapure water, total DBPs were higher when compared to chlorinated lake water (for DWR, 2.4x; for Lake Wateree *Microseira*, 1.2x; for Lake Wateree *Phormidium*, 1.2x), with the exception of Lake Marion raw water (Fig. 4). In chlorinated Lake Marion water, trichloroacetaldehyde (87.2 μg/L) and trichloroacetic acid (172 μg/L) were formed at the highest levels, at a total of 422

Table 2
Formation of DBPs (μg/L) from controlled lab reactions of *Microseira* and *Phormidium* and from real drinking water.

Chlorinated Sample	C-DBPs				N-DBPs			Total DBPs
	THMs	HAAs	HALs	HKs	HAMs	HNMs	HANs	
Real drinking water	3.5	16.8	0.5	1.2	0.2	2.9	3.0	28.1
Lake Marion raw water	18.5	287.9	83.5	14.7	6.9	0.5	10.3	422.3
Lake Wateree raw water	6.6	82.9	14.6	10.2	8.3	3.6	8.9	135.1
* Lake Wateree raw water	84.8	120.8	20.1	15.8	3.6	1.5	7.1	253.7
[a] DWR *Microseira*	0.0	57.6	4.9	1.8	1.0	0.0	1.4	66.7
[a] Lake Marion *Microseira*	0.0	48.5	69.0	6.8	11.8	0.1	10.2	146.4
[a] Lake Wateree *Microseira*	0.1	58.8	68.3	3.5	18.9	0.4	17.0	167.0
[a] Lake Wateree *Phormidium*	51.7	141.5	63.6	8.6	25.9	1.6	21.4	314.3
[b] DWR *Microseira*	54.1	99.2	4.9	1.9	1.1	0.6	1.2	163.0
[b] Lake Marion *Microseira*	64.3	86.1	21.9	12.3	13.5	0.4	22.2	220.7
[b] Lake Wateree *Microseira*	46.2	92.1	20.9	2.2	21.0	3.6	20.2	206.2
[b] Lake Wateree *Phormidium*	131.9	151	58.8	9.3	28.1	2.6	23.9	405.6
Lake Wateree *Microseira* in lake water	6.4	239.9	121.8	6.7	36.1	3.2	37.9	452.0
Lake Wateree *Phormidium* in lake water	189.5	230	82.2	20.8	33.9	3.3	25.9	585.6

*Phormidium impacted.
[a] in ultrapure water
[b] in ultrapure water (500 μg/L Br^- and 100 μg/L I^- added)

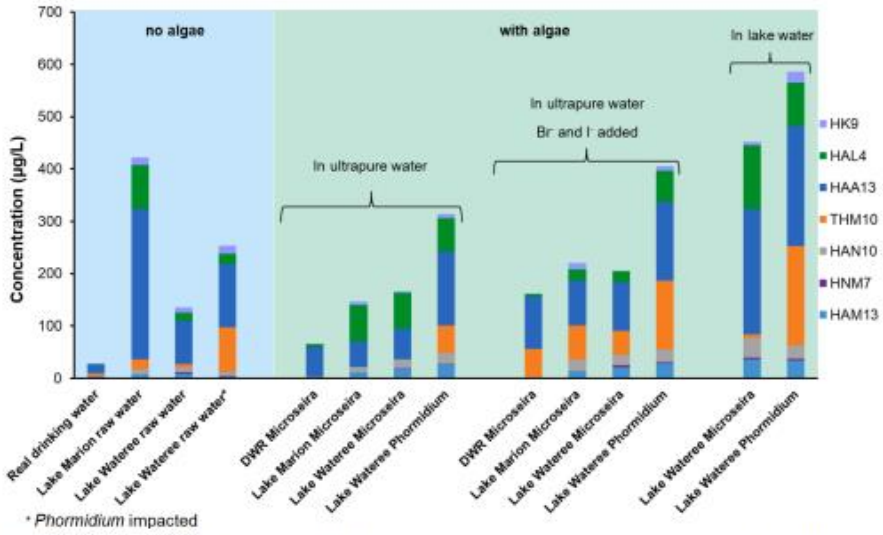


Fig. 4. Total DBPs: Trihalomethanes (THMs), haloacetic acids (HAAs), haloaldehydes (HALs), haloketones (HKs), haloacetonitriles (HANs), halonitromethanes (HNMs), and haloacetamide (HAMs) in chlorinated samples. Numbers following DBP classes represent the number of DBPs measured in each class.

μg/L. As with *Phormidium*, total DBPs were higher for every sample spiked with bromide and iodide vs. not spiked (2.4x for DWR, 1.5x for Lake Marion, and 1.2x for Lake Wateree *Microseira*) (Fig. 4).

As expected, the addition of bromide and iodide led to an increase in species variation for DBPs. No iodinated DBPs were observed in controlled lab reactions of Lake Marion and Lake Wateree samples, but four I-DBPs were observed in the real drinking water sample (bromochloroiodomethane (0.2 μg/L), chloroiodoacetic acid (0.3 μg/L), bromoiodoacetic acid (0.1 μg/L), and diiodoacetic acid (0.3 μg/L)) and two from chlorinated algae in ultrapure water when bromide and iodide ions were present (bromochloroiodomethane (0.1 μg/L) and dibromoiodomethane (0.2 μg/L)) (Table S3). The formation of I-DBPs is of concern because they generally have much higher cytotoxicity and genotoxicity when compared to brominated or chlorinated DBPs (Wagner and Plewa, 2017). A total of 30 DBPs were measured in the real drinking water sample, including the I-DBPs mentioned above. Levels ranged from <0.1 to 6.5 μg/L, with a total concentration of 28.1 μg/L for DBPs, which is significantly lower than controlled laboratory reactions, likely due to the difference in the *Microseira* in this real reservoir and lower DOC in its source water (0.9 mg/L) (Table 1, Table S3). Among them, bromodichloromethane (2.9 μg/L), dichloroacetic acid (6.2 μg/L), and trichloroacetic acid (6.5 μg/L) were formed at the highest concentrations.

3.4. N-DBPs

When comparing chlorinated algae in ultrapure water vs. lake water containing NOM and no algae, higher levels of nitrogenous DBPs (N-DBPs) formed when *Phormidium* and *Microseira* algae were present (except the DWR samples) (Fig. 4). For example, total HAMs increased from 8.3 to 18.9 μg/L and total HANs increased from 8.9 to 17.0 μg/L for chlorinated lake water vs. chlorinated *Microseira* in ultrapure water. Similarly total HAMs increased from 3.6 to 25.9 μg/L and total HANs

increased from 7.1 to 21.4 μg/L for chlorinated lake water vs. chlorinated *Phormidium* in ultrapure water (Table 2). These increases in N-DBPs are likely because algae contain more nitrogen (up to 45% of organic nitrogen can be excreted by algae) (Westerhoff and Mash, 2002) compared to natural organic matter (e.g., 1-3% in humic acid) found in surface waters (Boggs et al., 1985). Total N-DBPs in chlorination reactions containing algae in ultrapure water were 22.1, 36.3, and 48.9 μg/L for Lake Marion (*Microseira*), Lake Wateree (*Microseira*), and Lake Wateree (*Phormidium*), respectively (Fig. 4). When comparing *Phormidium* to *Microseira*, higher levels of N-DBPs were formed from *Phormidium* than *Microseira* upon chlorination, likely due to a greater release of intracellular organic matter from *Phormidium* compared to *Microseira*, as *Phormidium's* outer sheaths degrade much more readily with chlorine (Fig. 3) than those of *Microseira*, which do not degrade (Fig. 2). DWR *Microseira* samples produced lower amounts of N-DBPs compared to that from Lake Wateree and Lake Marion. Highest levels of N-DBPs formed when algae samples were chlorinated in lake water (77.2 and 63.1 μg/L for Lake Wateree *Microseira* and *Phormidium* in lake water, respectively) due to the presence of both algal organic matter and NOM. Fang et al. (2010) reported higher levels of dichloroacetonitrile and cyanogen chloride from chlorination of *Microcystis aeruginosa* cells vs. chlorinated NOM.

Total N-DBPs in the real drinking water sample was 6.1 μg/L, including chloroacetonitrile (1.2 μg/L), bromodichloronitromethane (1.3 μg/L), and dibromochloronitromethane (1.6 μg/L), which were significantly lower than the controlled laboratory reactions of algae. As mentioned earlier, N-DBPs are especially important because their cytotoxicity and genotoxicity are generally higher than DBPs that do not contain nitrogen (C-DBPs) (Plewa et al., 2008). Therefore, if algae-impacted source water is disinfected, increased algal organic matter may contribute to the formation of higher N-DBP levels, which can affect drinking water quality and safety.

3.5. Calculated cytotoxicity and genotoxicity

Total calculated cytotoxicity tripled when *Microseira* was present in chlorinated Lake Wateree source water compared to chlorinated source water without algae, and it doubled for *Phormidium* (Fig. 5, Table S7). HANs (48%) and HNMs (26%) were the main toxicity drivers for chlorinated source waters without algae, while HANs (68%) dominated calculated cytotoxicity when algae (*Microseira*) was present. In chlorinated *Phormidium*-impacted source water, HANs (45%) and HAMs (20%) were the main drivers of calculated cytotoxicity, while HANs (56%) were the main drivers when *Phormidium* was added. When bromide and iodide were present, calculated cytotoxicity of algae reacted in ultrapure water was 16x, 13x, 8x, and 12x higher for DWR *Microseira*, Lake Marion *Microseira*, Lake Wateree *Microseira*, and Lake Wateree *Phormidium*, respectively, compared to no halide addition.

Similarly, calculated genotoxicity was 2x higher in Lake Wateree source water when *Microseira* was present and 3x higher for *Phormidium* (Fig. 6, **Table S8**). In both cases, calculated genotoxicity was driven by HAAs (24 and 47%) and HNMs (54 and 23%), respectively, when source waters (no algae added) were chlorinated. When algae was added to these source waters, HAAs (56 and 59% for *Microseira* and *Phormidium*, respectively) contributed most to the calculated genotoxicity. Calculated genotoxicity values followed the same trend as cytotoxicity when bromide and iodide were added to algae reactions in ultrapure water, with 6.7x, 37.4x, 6.1x, and 8.7x higher values for DWR *Microseira*, Lake Marion *Microseira*, Lake Wateree *Microseira*, and Lake Wateree *Phormidium*, respectively, compared to no halide addition.

4. Conclusions and environmental implications

This study reports the most comprehensive study of algal DBPs to-date, with 66 DBPs measured. Results show that *Microseira* and *Phormidium* can have a profound impact on DBPs formed in chlorinated drinking water, which is especially relevant for plants that use pre-chlorination.

Key take home messages from this study include:

- Total DBP and N-DBP concentrations double when *Microseira* and *Phormidium* algae are present.
- Presence of bromide and iodide further increase the DBP levels and produce more toxic DBP species.

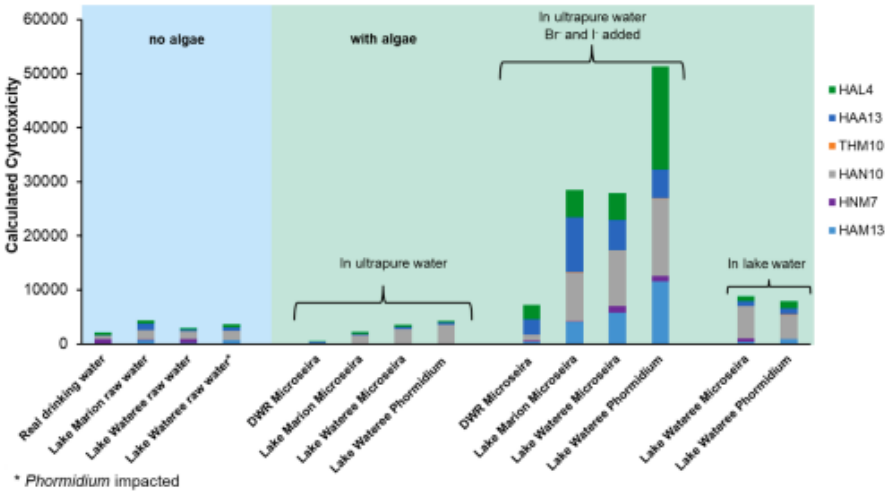


Fig. 5. Calculated cytotoxicity of chlorinated samples. Cytotoxicity data for haloketones (HKs) are not currently available in the literature. Numbers following DBP classes represent the number of DBPs measured in each class.

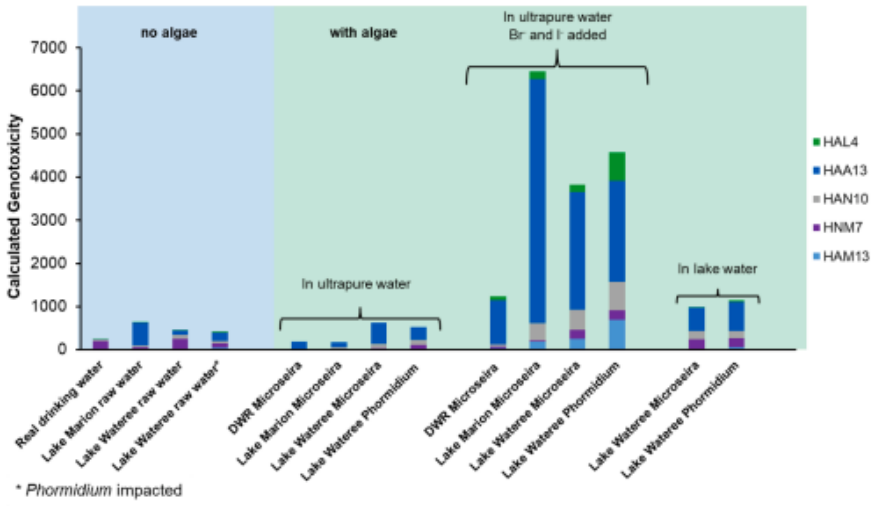


Fig. 6. Calculated genotoxicity of chlorinated samples. Genotoxicity data for haloketones (HKs) are not currently available in the literature. Numbers following DBP classes represent the number of DBPs measured in each class.

- Notably, *Microseira* and *Phormidium* result in a 2-3-fold increase in calculated cytotoxicity and a 2-fold increase in calculated genotoxicity.
- Haloacetonitriles are a major driver of calculated cytotoxicity in algae-impacted waters, while haloacetic acids are major drivers of calculated genotoxicity in algae-impacted waters.
- Most research on algae focuses on the algal toxins, including microcystins, saxitoxins, and *Microseira wollei* toxins (which are very important), but exposure to increased levels of DBPs also pose a major human health risk that has not been fully investigated.
- Three of the 14 *Phormidium*-impacted samples exceed THM regulations (80 μg/L), and 7 exceed HAA regulations (60 μg/L) in the U.S. for both *Phormidium*- and *Microseira*-impacted waters.
- Thus, increasing presence of algae in source waters may necessitate changes in drinking water treatment, including improved methods to remove algae and algal organic matter before disinfection.

In addition, it will be important to investigate DBP formation from chloramination of algae-impacted waters, as chloramine has become a popular disinfectant in the U.S. and in many other countries, and there is the possibility for increased toxic iodo-DBP formation. Finally, future studies should investigate the formation of high molecular weight DBPs and highly polar DBPs using liquid chromatography (LC)-MS that would be missed with the GC-MS methods used here.

Declaration of Competing Interest

The authors declare no competing financial interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.118316.

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How well does XAD resin extraction recover halogenated disinfection byproducts for comprehensive identification and toxicity testing?☆

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ABSTRACT

Halogenated disinfection byproducts (DBPs) are an unintended consequence of drinking water disinfection, and can have significant toxicity. XAD resins are commonly used to extract and enrich trace levels of DBPs for comprehensive, nontarget identification of DBPs and also for in vitro toxicity studies. However, XAD resin recoveries for complete classes of halogenated DBPs have not been evaluated, particularly for low, environmentally relevant levels (ng/L to low μg/L). Thus, it is not known whether levels of DBPs or the toxicity of drinking water might be underestimated. In this study, DAX-8/XAD-2 layered resins were evaluated, considering both adsorption and elution from the resins, for extracting 66 DBPs from water. Results demonstrate that among the 7 classes of DBPs investigated, trihalomethanes (THMs), including iodo-THMs, were the most efficiently adsorbed, with recovery of most THMs ranging from 50-96%, followed by halonitromethanes (40-90%). The adsorption ability of XAD resins for haloacetonitriles, haloacetamides, and haloacetaldehydes were highly dependent on the individual species. The adsorption capacity of XAD resins for haloacetic acids was lower (5-48%), even after adjusting to pH 1 before extraction. Recovery efficiency for most DBPs was comparable with their adsorption, as most were eluted effectively from XAD resins by ethyl acetate. DBP polarity and molecular weight were the two most important factors that determine their recovery. Recovery of trichloromethane, iodoacetic acid, chloro- and iodo-acetonitrile, and chloroacetamide were among the lowest, which could lead to underestimation of toxicity, particularly for iodoacetic acid and iodo-acetonitrile, which are highly toxic.

☆ This manuscript is dedicated to Prof. Michael Plewa, whose innovative and pioneering research has been a catalyst for improving the safety of drinking water.

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Introduction

To protect humans against harmful pathogens and waterborne disease, disinfection of drinking water is important (Calderon, 2000). However, disinfection byproducts (DBPs) are produced unintentionally from the reaction of disinfectants and organic matter in water, and they have been widely detected in drinking water all over the world (Richardson and Postigo, 2015; Chaukura et al., 2020). To date, halogenated DBPs have received the most attention (Hua and Reckhow, 2007; Chaukura at al., 2020), and >800 DBPs have been reported (Richardson, 2011a; Li and Mitch, 2018; Yang et al., 2019). This number will likely increase with improved detection methods (Cuthbertson et al., 2020; Richardson and Postigo, 2015; Li and Mitch, 2018). Although most DBPs in drinking water are usually present at low levels (ng/L-μg/L) (Kali et al., 2021; Allen et al., 2022; Krasner et al., 2006), millions of people consume disinfected water daily over a lifetime, making DBPs a continuous, ubiquitous exposure. DBPs have been associated with adverse health effects, including bladder cancer, colorectal cancer, miscarriage, and birth defects (Waller et al., 1998; Villanueva et al., 2004 and 2007; Savitz et al., 2005; Bove et al., 2007; Nieuwenhuijsen et al., 2009; Grellier et al., 2010; Rahman et al., 2014; Cantor et al., 2010; Costet et al., 2011; Beane-Freeman et al., 2017; Summerhayes et al., 2020). In addition, many DBPs are cytotoxic, genotoxic, mutagenic, carcinogenic, or teratogenic (Richardson et al., 2007; Wagner and Plewa, 2017; Gonsioroski et al., 2020); moreover, recent studies also reported developmental toxicity and growth inhibition (Yang et al., 2013; Gao et al., 2021; Li et al.,2016).

Since most DBPs are present at trace levels, toxicity research is contingent on the development of reliable methods to extract and enrich DBPs at sufficient concentration (Le Roux et al., 2017; Wagner and Plewa, 2017). Liquid-liquid extraction (LLE), freeze-drying, reverse osmosis, roto-evaporation, and solid phase extraction (SPE), including XAD resin adsorption, are well-documented methods for DBP extraction and concentration (Kool et al., 1981; Plewa et al., 2004a and 2004b; Richardson et al., 2008; Zhou et al., 2013; Han et al., 2018; Lau et al., 2021). Of these, macro-porous XAD resins are popular because they are simple to use and enable extraction of large quantities of water (e.g.,10-20 L) (Daignault et al., 1988; Le Roux et al., 2017). Among the XAD resins, XAD-2 and DAX-8 have gained widespread acceptance because of their chemical stability across a broad pH range and their ability to extract a wide range of DBPs (Allen et al., 2017; Daiber et al., 2016; Richardson et al., 2010; Pressman et al., 2010; Richardson et al., 2008; Krasner et al., 2006; Plewa et al., 2004a and 2004b; Richardson et al., 2003; Daignault et al., 1988). Non-polar XAD-2 resins are styrene-divinylbenzene copolymers, while polar DAX-8 resins are methyl methacrylate polymers. Their combination makes them effective for concentrating a broad range of compounds of differing polarity (Kool et al., 1981; Ringhand et al., 1987).

A protocol is published for DBP extraction, analysis, and toxicity assessment, consisting of the extraction of large volumes (>5 L) of disinfected waters by DAX-8 and XAD-2 resins in series, followed by elution with ethyl acetate (Richardson, 2011). This extraction method has been widely used for comprehensive, nontarget identification of unknown DBPs and also for the analysis of *in vitro* toxicity in drinking water, swimming pool water, and treated wastewater effluent (Allen et al., 2022; Allen et al., 2021; Liberatore et al., 2017; Allen et al., 2017; Daiber et al., 2016; Richardson et al., 2010; Plewa et al., 2011; Jeong et al., 2012; Dong et al., 2016; Le Roux et al., 2017; Pressman et al., 2010; Richardson et al., 2008; Krasner et al., 2006; Plewa et al., 2004a; Plewa et al., 2004b; Richardson et al., 2003). For *in vitro* toxicity measurements (including mutagenicity, cytotoxicity, and genotoxicity), the final 1.0 mL ethyl acetate extract is typically solvent-exchanged into dimethylsulfoxide (DMSO). Understanding the recovery of DBPs from XAD resins is important because it directly determines whether the concentrations and toxicities of these treated waters are underestimated, or potentially overestimated due to DBPs transforming during the extraction process. However, information is lacking whether DAX-8/XAD-2 resins can effectively adsorb and extract halogenated DBPs. In addition, DBPs retained on XAD resins are desorbed by eluting with an organic solvent by gravity (Richardson, 2011); recovery during this elution step is also unknown.

At present, despite the growing application of XAD resin extraction for DBP toxicity measurements, few studies have investigated their recoveries. In a study by Le Roux et al., (2017), DAX-8 resin recovery of wastewater-associated toxicity was 72%, and recovery of DAX-8/XAD-2 resins for mixed organic compounds in river water samples from China was 63% (Le Roux et al., 2017; Zhou et al., 2013). Stalter et al. (2016) reported recoveries for total organic halogen (TOX) as low as ~33% (Stalter et al., 2016). However, the origins of these losses were not determined, nor the specific DBP recoveries. More recently, Lau et al. (2021) found that recoveries of 22 (semi-)volatile DBPs by XAD resins was lower than by liquid-liquid extraction and other traditional SPE methods (Lau et al., 2021). However, except for volatility, the effects of other chemical properties on DBP recovery have not been described. In addition, the distinction between differences in adsorption and elution have not been reported.

Thus, the goals of this study were to investigate the adsorption, elution, and overall recovery efficiencies of 7 different classes (66 species) of trace individual halogenated DBPs by XAD resins. Moreover, Pearson correlation analyses between the recoveries and DBP characteristics (pK_a, log K_{ow}, and molecular weight (MW)) were made.

1. Materials and methods

1.1. *Chemicals and instruments*

The 66 halogenated DBPs were obtained from Sigma-Aldrich (St. Louis, MO), CanSyn Chem. Corp. (Toronto, ON), Aldlab Chemicals (Woburn, MA), and TCI America (Waltham, MA). These DBPs belong to 7 classes: trihalomethanes (THMs) (including iodo-THMs), haloacetic acids (HAAs) (including iodo-HAAs), haloacetonitriles (HANs), haloacetamides (HAMs), halonitromethanes (HNMs), haloacetaldehydes (HALs), and haloketones (HKs). The complete lists of these DBPs and their abbreviations can be found in supporting information (Table S1-Table S7); their structures are shown in Fig 1.

Fig. 1 – Structures of 7 DBP classes studied.

The following solvents, methanol, dichloroacetonitrile, acetonitrile, ethyl acetate, and methyl tert-butyl ether (MTBE) were purchased from Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Pittsburgh, PA), and VWR International (Radnor, PA) and were obtained at the highest level of purity. Vendor information for each DBP standard is available elsewhere (Cuthbertson et al., 2020). Amberlite XAD-2 and Supelite DAX-8 resins were obtained from Sigma-Aldrich (St. Louis, MO).

A Burrell wrist-action shaker was used for liquid-liquid extractions, and an Agilent 7890 gas chromatograph (GC) coupled to a 5977A single quadrupole mass spectrometer (MS) (Santa Clara, CA) was used for analysis of all DBPs except HAAs and iodo-HAAs. Derivatized HAAs and iodo-HAAs were analyzed using a GC-triple quadrupole-MS (GC-MS/MS) (Thermo Scientific, Waltham, MA).

1.2. Experimental procedures

Preparation of solutions: Stock solutions of 66 halogenated DBPs were prepared by dissolving individual standards in methanol or acetonitrile (Cuthbertson et al., 2020). Then, mixed sub-stock solutions were made up at 10 mg/L. Appropriate dosages of sub-stock solutions were spiked into 6 L nanopure water (18 MΩ-cm) to make initial levels of 1.0 μg/L, close to the levels of halogenated by-products found in drinking water (Krasner et al., 2006; Richardson et al., 2008; Allen et al., 2022). Exact concentrations were determined using GC-MS or GC-MS/MS.

XAD resin preparation: DAX-8 and XAD-2 resins were cleaned using sequential 24-h Soxhlet extractions with methanol, ethyl acetate, and methanol; afterwards, they were stored in methanol and refrigerated (Richardson, 2011b). Ten mL of DAX-8 and 10 mL XAD-2 resins were poured into a glass column (25 × 1.5 cm), with DAX-8 placed on top of the XAD-2 resins and glass wool at the bottom of the column and 1 inch above the resin bed to ensure that resins stay in place while passing water through the columns (Richardson, 2011b; Allen et al., 2022). The resins were conditioned prior to use by rinsing with 0.1 M HCl, 0.1 M NaOH, and finally nanopure water after packing the column (Richardson, 2011b). The cleanliness of the resins was verified by analysis of an extracted nanopure water control, ensuring a clean background by GC-MS and that the organic matter in the effluent was <0.2 mg/L.

XAD resin adsorption and elution: To convert anionic haloacetic acid DBPs to a neutral state for better adsorption, nanopure water samples (6 L) containing a mixture of 66 DBPs were adjusted to pH 1 using H_2SO_4. DBPs were concentrated 250-fold by passing 5 L of this water slowly (by gravity, about 30 mL/min) through the XAD resin columns. We prepared two columns for parallel experiments (duplicate) and for each sample obtained, we tested 3 times and calculated the average value. The ratio of water to XAD resins (250:1) was well within the recommended ratio of 770:1 (Richardson, 2011b). The remaining 1 L of water was used for determining the amount of DBPs in the influent of XAD columns. The effluent of the column was also collected for DBP analysis. The resin-adsorbed DBPs were eluted with 50 mL ethyl acetate and concentrated to 1.0 mL with nitrogen using a TurboVap (Biotage). The concentrated eluent extract was analyzed for the 66 target DBPs using GC-MS.

Liquid-liquid extraction: Influent and effluent water samples (100 mL each, pH <1) were extracted using liquid-liquid extraction (LLE) with 5 mL MTBE, repeated three times, yielding a 15 mL extract. The extract was then dried using sodium sulfate and concentrated further under nitrogen to 200 μL. This extract was separated into two equal parts for analysis of HAAs/iodo-HAAs and the other DBPs (THMs, HALs, HKs, HAMs, HANs, and HNMs), respectively (Cuthbertson et al., 2020; Allen et al., 2022).

1.3. Analytical methods

Sensitive analytical methods were used to analyze the chloro-, bromo-, and iodo-DBPs species for these 66 compounds from 7 chemical classes (Cuthbertson et al., 2020; Allen et al., 2022). Sample extracts (liquid-liquid and XAD resin extracts) were analyzed using GC-MS (Agilent 7890 GC, 5977A mass spec-

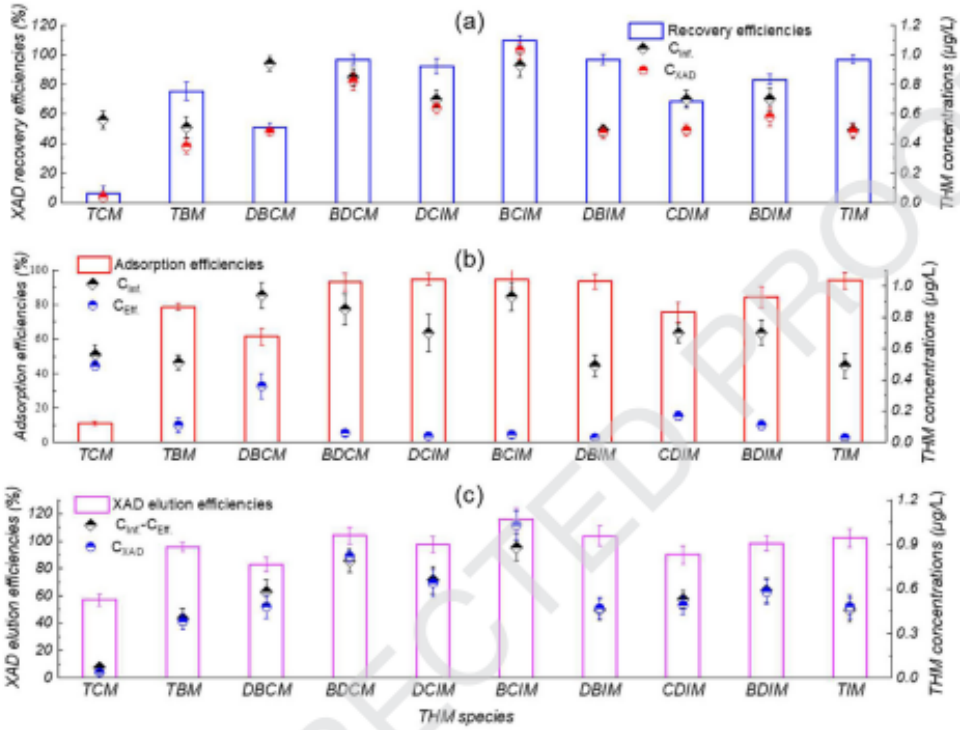

Fig. 2 – XAD recovery, adsorption and elution efficiencies of trihalomethanes (including I-THMs)

trometer, Agilent Technologies, Santa Clara, CA) with electron ionization at 70 eV in selected ion monitoring mode. Nine HAAs and 4 iodo-HAAs were derivatized using diazomethane and analyzed by GC-MS/MS with multiple reaction monitoring mode. Sample extracts (1.0 μL) were injected into a multimode inlet (MMI) in pulsed splitless mode, and separated using a Restek Rtx-200 column (30 m x 0.25 mm x 0.25 μm film thickness; Restek Corporation, Bellefonte, PA). This column provides improved separation and detection limits particularly for iodo-THMs and haloacetamides (Cuthbertson et al., 2020). The GC temperature program for all DBPs except HAAs was as follows: initial temperature of 35°C for 5 min, increased to 220°C at 9°C/min, and then ramped at 20°C/min to 280°C and held for 15 min. The GC temperature program for the analysis of the 9 HAAs and 4 iodo-HAAs was as follows: initial temperature held at 35°C for 5 min, increased to 280°C at 9°C/min, and then held for 15 min. The transfer line was held at 280°C and source temperature at 200°C for both methods.

1.4. *Recovery calculations*

Calibration curves were made using mixtures of the 66 DBPs at the following concentrations: 0.1, 0.25, 0.5, 1, and 2.5 μg/L. An additional blank sample was extracted and analyzed for each calibration curve. Taking in consideration the influence of solvent on GC-MS measurements, the quantitation of XAD eluents was done using a calibration curve where standards were spike into ethyl acetate (to match the XAD elution solvent). Moreover, DBP concentrations from the analysis of XAD resin extracts were corrected with the concentration factor of extraction and evaporation steps; DBP levels in the influent and effluent of the XAD columns were similarly corrected. The recovery, adsorption, and elution of 66 halogenated DBPs by XAD resins was calculated according to Eqns. 1-3 (below).

$$Recoverye\,f\,ficiency = \frac{C_{XAD}}{C_{Inf}} \quad (1)$$

$$Adsorptione\,f\,ficiency = \frac{C_{Inf} - C_{Eff}}{C_{Inf}} \quad (2)$$

$$Elutione\,f\,ficiency = \frac{C_{XAD}}{C_{Inf} - C_{Eff}} \quad (3)$$

Where,

- C_{XAD} is the concentration of DBPs that eluted from the XAD resins;
- C_{Inf} is the concentration of DBPs in the influent of the XAD resin column;
- C_{Eff} is the concentration of DBPs in the effluent of the XAD resin column.

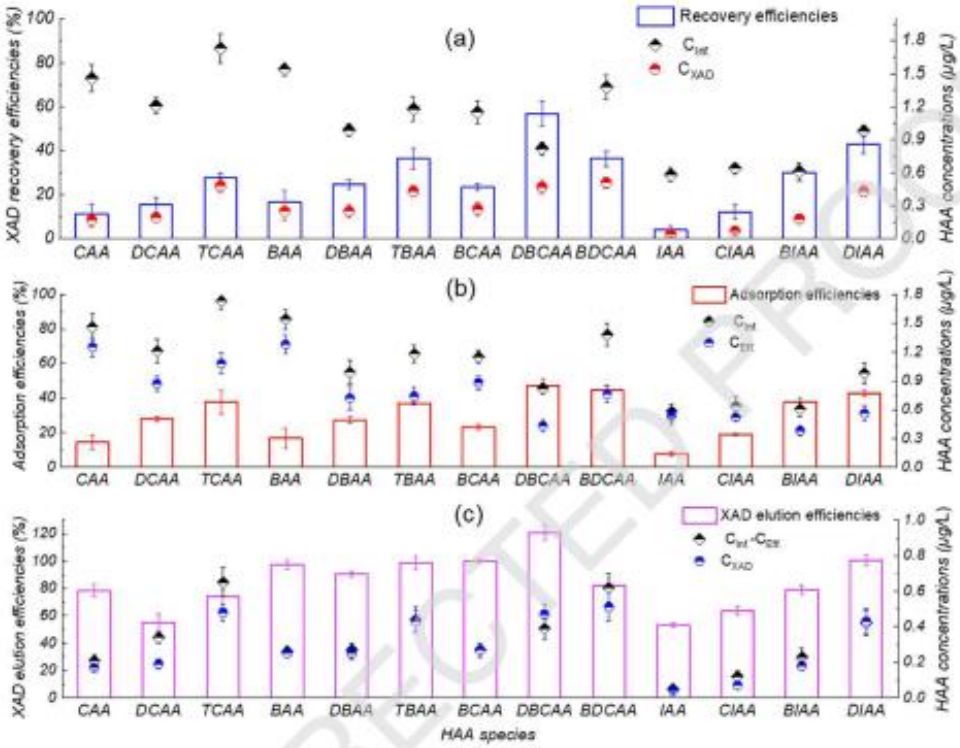

Fig. 3 – XAD recovery, adsorption, and elution efficiencies of haloacetic acids (including iodo-HAAs)

1.5. *DBP Correlation Statistics*

Regression analyses were performed to determine the Pearson product-moment (r) and the corresponding P value. An $r >$ 0.90 was considered to have a strong, positive relationship between XAD recovery and the given characteristic (pK_a, $logK_{ow}$, molecular weight), while an $r < 0.50$ was considered to have a weak, positive relationship. $P < 0.05$, <0.01, and <0.001 were considered significantly correlated, highly significantly correlated, and very highly significantly correlated, respectively.

2. Results and discussion

2.1. *Recoveries of 10 trihalomethanes (including iodo-THMs)*

THMs are typically formed at highest concentrations of all DBPs in drinking water and have been reported in water disinfected with chlorine, chloramine, ozone, and chlorine dioxide disinfection (Postigo et al., 2018; Krasner et al., 2006; Richardson, 2011). Four THMs (trichloromethane, bromodichloromethane, dibromochloromethane, and tribromomethane) are regulated in the U.S. and in many other countries (Chaukura et al., 2020; U.S. EPA, 2020; Richardson, 2021). Iodo-THMs have also been reported in chlorinated, chloraminated, and ozonated water in the presence of iodide ions (Allen et al., 2022; Ioannou et al., 2016; WHO, 2011; Richardson et al., 2008; Krasner et al., 2006), and they are favored in chloraminated waters (Bichsel and von Gunten, 1999; Richardson et al., 2008). Although iodo-THMs are not yet regulated, they are much more cytotoxic and genotoxic than regulated THMs (Wagner and Plewa, 2017; Richardson et al., 2008).

The recovery efficiencies for THMs and iodo-THMs are shown in Fig 2(a) and were calculated by the amount of DBPs eluted from the XAD column (C_{XAD} shown as red dots) divided by amount in the influent ($C_{Inf.}$ in black dots). Adsorption efficiencies shown in Fig 2 (b) were obtained from the DBP concentration difference between the influent (C_{Inf} in black dots) and effluent (C_{Eff} in blue dots) divided by $C_{Inf.}$. The elution efficiency in Fig 2 (c) is representative of a mass balance between the concentration of the XAD eluate (C_{XAD}) and that adsorbed by the column ($C_{Inf.}$-$C_{Eff.}$). The same representations are used in following figures for other DBP classes (Fig 3-Fig 8).

As shown in Fig 2, most THM species were well adsorbed by XAD resins except trichloromethane (TCM), and their recoveries were relatively high (50-96%). Moreover, the adsorption and recovery by XAD resins were similar, with XAD elutions close to 100% for all except TCM. The adsorption efficiency for TCM was <20%, and its recovery was <10%, due to low elution efficiency (<60%). This is consistent with Le Roux et al. (2017), who reported that the most hydrophobic THMs are easily re-

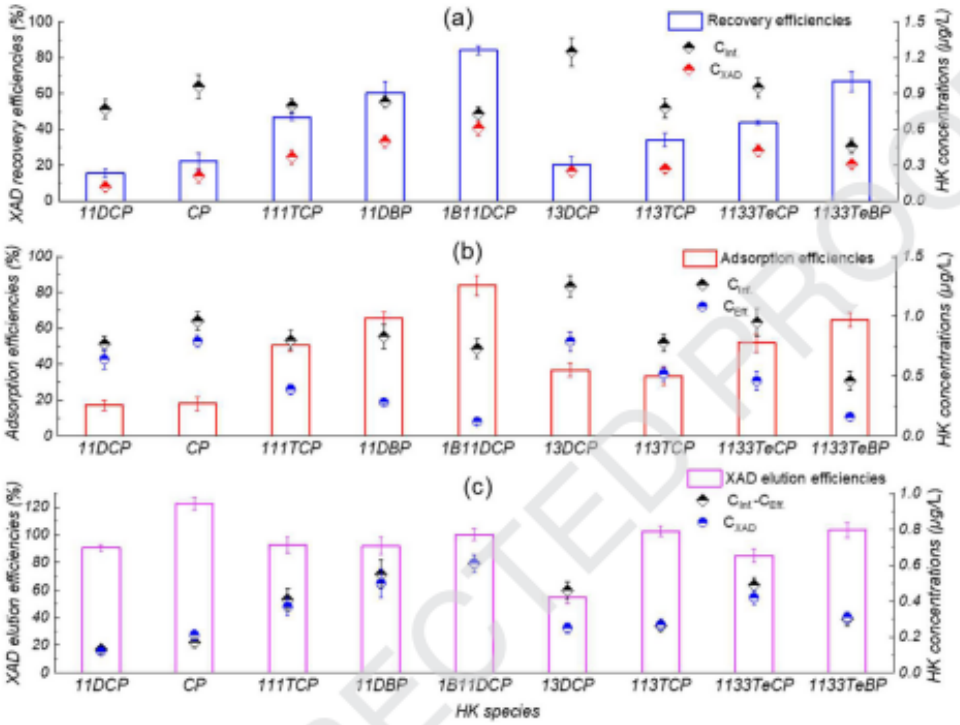

Fig. 4 – XAD recovery, adsorption, and elution efficiencies of haloketones (HKs)

tained on DAX-8 resins (Le Roux et al., 2017). Ethyl acetate (eluent) can also desorb these THMs from XAD resins effectively. Compounds in non-polar or non-ionic states are principally adsorbed by van der Waal forces, which are relatively weak, and therefore, they are easily desorbed from resins with solvents of comparably low polarity (Daignault et al., 1988). THMs were in acidic solution (pH 1), so they were in neutral, non-ionic states and could be easily desorbed from the resins with ethyl acetate. Interestingly, THM recovery by XAD resins in this paper were significantly higher than by liquid-liquid extraction (39-83%) in a previous paper from our group (Cuthbertson et al., 2020).

Thus, while TCM is typically a major DBP in drinking water, it is poorly recovered by XAD resins (Fig 2), likely due to its high volatility combined with poor adsorption. Thus, its contribution to the overall drinking water toxicity might be underestimated, but this may not be a major concern because its cytotoxicity is quite low, and it is not genotoxic (Wagner and Plewa, 2017). Our recovery results agree with a recent study by Lau et al. (2021) (Lau et al., 2021). On the other hand, iodo-THMs not only adsorbed strongly onto XAD resins, but they also eluted effectively from the resins, yielding an overall recovery of 90-116%. Thus, the toxicity of iodo-THMs would not be significantly underestimated (based on extraction and evaporation to 1.0 mL).

To explain the distinct absorption for THMs, the pH of the solution is important, as it has a large effect on the recoveries of compounds adsorbed onto XAD resins (Daignault et al., 1988). At pH 1, THMs were in a neutral state and could be easily adsorbed onto the nonionized surface of the XAD resins. This is consistent with a previous study that reported that XAD resins concentrated neutral compounds more efficiently than those that are ionized (Ringhand et al., 1987). Moreover, the recovery of THMs also relates to the types of halogen atoms (Cl, Br, I) they possess. In general, it follows the principle: Cl < Br < I. For example, three THM recoveries followed the following order: TIM (97%) > TBM (76%) > TCM (6%), which also followed the MW order.

In addition, as shown in Table S8, we found no statistically significant correlation between XAD recovery for THMs and MW or between XAD recovery and log K_{ow}, although log K_{ow} values indicate that the THMs might partition well to the XAD resins (vs. water). Moreover, the volatility of THMs (particularly TCM) also played a role in their lower recoveries (Lau et al., 2021).

2.2. *Recoveries of 13 haloacetic acids (including iodo-HAAs)*

Besides THMs, the second most prevalent DBP group is HAAs; five HAAs are regulated by the U.S. EPA (chloro-, bromo-, dichloro-, dibromo-, and trichloroacetic acid), and several are regulated in other countries (Richardson, 2021; Islam et al., 2016; U.S. EPA, 2006). Iodo-HAAs are among the most cytotoxic

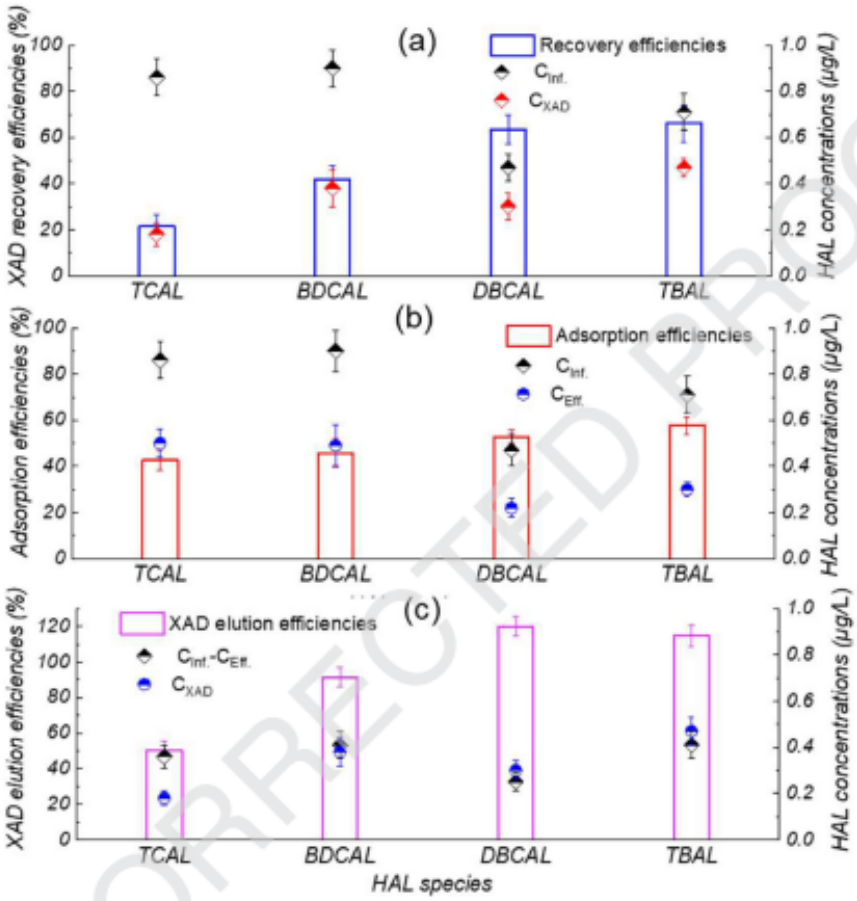

Fig. 5 – XAD recovery, adsorption, and elution efficiencies of haloacetaldehydes (HALs)

and genotoxic DBPs studied to-date, with iodoacetic acid being the most genotoxic of all DBPs studied (Richardson et al., 2004; Richardson et al., 2008; Wagner and Plewa, 2017; Jeong et al., 2016; Xia et al., 2018; Gonsioroski et al., 2020). IAA is also tumorigenic in mice (Wei et al., 2013). Thus, while iodo-HAAs are typically found at low or sub-μg/L levels (Krasner et al., 2006; Allen et al., 2022; Cuthbertson et al., 2019; Richardson et al., 2008; Krasner et al., 2006), they are quite toxicologically important. Further, although the toxicity of the bromo-chloro-HAAs is not as high as nitrogen-containing DBPs (N-DBPs), HAA concentrations are significantly higher, such that their potential health risks should not be ignored (Wagner and Plewa, 2017).

As shown in Fig 3, XAD resins were not as effective for extracting HAAs from water as compared to THMs, with recoveries of most HAAs 20-50% and recoveries of most THMs 50-4-110%, except TCM 6.4%). This is similar to recoveries by liquid-liquid extraction reported by our group (17-45%) (Cuthbertson et al., 2020). Although HAAs are significantly less volatile than THMs, their recoveries by XAD resins were much lower. Therefore, it is the polarity and not the volatility that determined their adsorption onto the resins. As shown in Fig S1 and Table S8, HAA recoveries were strongly and very highly significantly correlated with log K_{ow} ($r = 0.85$, $P < 0.001$) and were highly significantly correlated with MW ($r = 0.67$, $P < 0.01$) and pK_a ($r = -0.63$, $P < 0.05$). Many HAAs have pK_as <2 (Table S2), such that a significant portion will be in a protonated state at the low pH used for extraction. The ionic forms are much more water soluble and should not adsorb well onto XAD resins like the neutral forms. Further, the relationship between recovery and log K_{ow} is consistent with previous research indicating that recovery mainly depends on their hydrophobicity, with more hydrophobic compounds (THMs) better retained on DAX-8 resins than more hydrophilic compounds (HAAs) (Le Roux et al., 2017). The micro-reticular XAD resins specifically adsorb relatively non-polar organic compounds, with negatively charged compounds, like HAAs, adsorbed at low efficiencies (Kool et al., 1981; Ringhand et al.,1987). Lau et al. (2021) also reported low recoveries of these HAAs (< 6%) for XAD resin extracts taken to dryness.

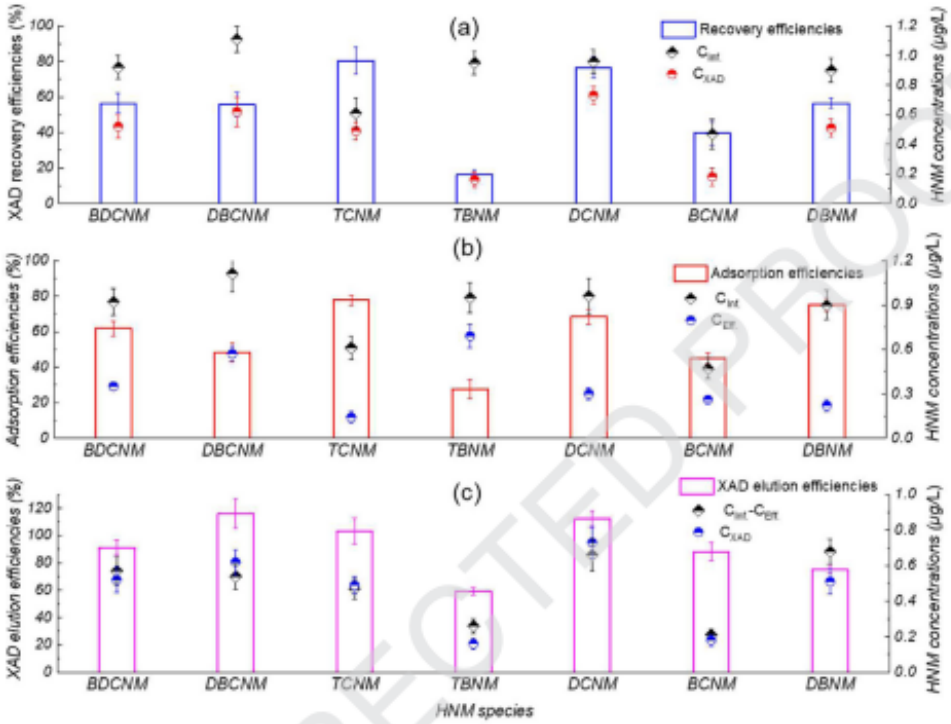

Fig. 6 – XAD recovery, adsorption, and elution efficiencies of halonitromethanes (HNMs)

2.3. *Recoveries of 9 haloketones*

Haloketones are frequently reported in chlorinated water (Krasner et al., 2006; Allen et al., 2022), but can undergo base-catalyzed hydrolysis (Kali et al., 2021). No cytotoxicity or genotoxicity studies have been conducted yet in mammalian cells for this class, so their toxicological importance relative to other DBPs is not currently known.

Results presented in Fig 4 demonstrate that most haloketones can bond to the XAD resins effectively under acidic conditions (pH 1). The recoveries of 1,1-dibromopropanone (11DBP), 1-bromo-1,1-dichloropropanone (1B11DCP), and 1,1,3,3-tetrabromopropanone (1133TeBP) were up to 66%, 84%, and 65%, respectively; these DBPs have a relatively high adsorption owing to their high MW and pK_a, while 1,1-dichloropropanone (11DCP), chloropropanone (CP), 1,3-dichloropropanone (13DCP), and 1,1,3-trichloropropanone (113TCP) recoveries were relatively low (17-37%). In addition, the elution of most haloketones from XAD resins was 80-100%, except 1,3-dichloropropanone, which had a low recovery (20%). A low recovery for 1,1-dichloropropanone was reported by Lau et al. (2021), even though its log K_{ow} value is >0 (Table S3). Thus, this illustrates that the octanol water partition coefficient is not the only factor that determines recovery of DBPs by XAD resins. Haloketones will not dissociate in acidic conditions, thus, factors affecting their XAD recoveries are not related to pK_a, but rather to log K_{ow} and MW, which show significant correlations between recovery and log K_{ow} ($r = 0.72$, $P < 0.05$) and MW ($r = 0.71$, $P < 0.05$) (Fig S2 and Table S8).

In addition, it should be noted that the recovery of haloketones by XAD resins was generally lower than by liquid-liquid extraction reported by our group (Cuthbertson et al., 2020), which was 51-128%, with the exception of chloropropanone (32%).

2.4. *Recoveries of 4 haloacetaldehydes*

Like haloketones, haloacetaldehydes are also easily hydrolyzed, and their cytotoxicities and genotoxicities are 100-1000 times that of THMs and HAAs (Jeong et al., 2014; Wagner and Plewa, 2017). Trichloroacetaldehyde is one of the most commonly occurring species (Krasner et al., 2006; Allen et al., 2022), is listed as possible human carcinogen by the U.S. EPA, and has a WHO guideline limit of 10 μg/L (U.S. EPA, 2006; U.S. EPA, 2006; Jeong et al., 2014; Kali et al., 2021). Mean adsorption and recovery for haloacetaldehydes is shown in Fig 5.

XAD resin recovery for trichloroacetaldehyde (TCAL) was only 22%, but was somewhat higher (56-58%) for bromodichloroacetaldehyde (BDCAL), dibromochloroacetaldehyde (DBCAL), and tribromoacetaldehyde (TBAL). A previous paper showed much higher recoveries by liquid-liquid extraction in ultrapure and tap waters (>160%) (Cuthbertson et al., 2020), which were proposed to be due to the transformation of other DBPs to form these haloacetaldehydes or by reactions occurring with precursors. The recoveries of

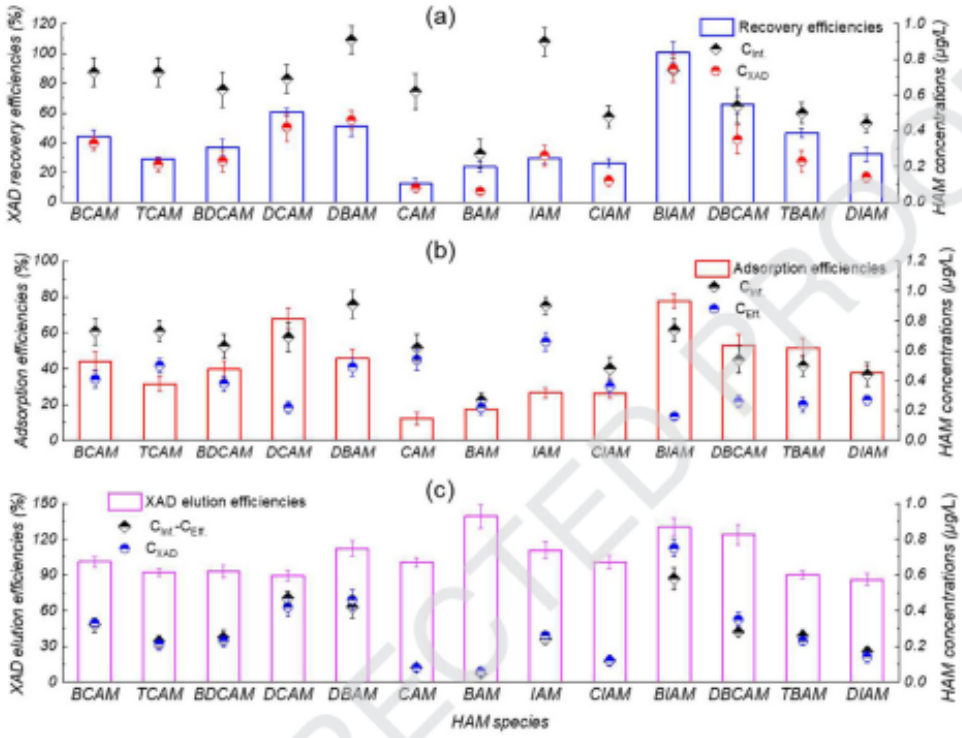

Fig. 7 – XAD recovery, adsorption, and elution efficiencies of haloacetamides (HAMs)

BDCAL and DBCAL by XAD resins that we measured (46% and 53%, respectively) were similar to liquid-liquid extraction recoveries (55-56%) reported by Lau et al. (2021), but were much higher than XAD resin recoveries reported by these authors after extracts were evaporated to dryness (13%).

Like haloketones, haloacetaldehydes are in a neutral form in acidic solution, such that pK_a should not impact their recovery by XAD resins. On the other hand, log K_{ow} and MW were important factors. The recoveries for HALs showed strong and significant correlations with log K_{ow} ($r = 0.97$, $P < 0.05$) and MW ($r = 0.96$, $P < 0.05$) (Fig S3; Table S8).

2.5. *Recoveries of 7 halonitromethanes*

Halonitromethanes can be formed by chlorine or chloramine disinfection and are enhanced when pre-ozonation is used before chlorination or chloramination (Allen et al., 2022; Ioannou et al., 2016; Richardson et al., 2008; WHO, 2011). While trichloronitromethane (also known as chloropicrin) has been commonly measured in many studies (due to the ease of incorporating it with other DBPs in EPA Method 551), the 7 halonitromethanes shown in Fig 6 have also been measured in drinking water (Weinberg et al., 2002; Krasner et al., 2006; Cuthbertson et al., 2019; Cuthbertson et al., 2020; Allen et al., 2022). They are potent mammalian cell cytotoxins and genotoxins (Plewa et al., 2004; Wagner and Plewa, 2017) and are mutagenic in Salmonella bacterial cells (Kundu et al., 2004). XAD resin recoveries for halonitromethanes are shown in Fig 6.

As can be seen from Fig 6, the adsorption of most halonitromethanes (56-80%) were also relatively high, except tribromonitromethane (TBNM) (28%), and they were better recovered compared to liquid-liquid extraction (38-52%) (Cuthbertson et al., 2020). Moreover, the XAD elution of most halonitromethanes was >80%, except for dibromonitromethane (DBNM) and TBNM. Moreover, TBNM could not be eluted efficiently, which reduced its recovery further to <20%. A relatively low recovery (26%) of TBNM was also found with liquid-liquid extraction (Cuthbertson et al., 2020). These results indicate that the toxicity of some halonitromethanes might be underestimated using XAD resin extraction, which is important because DBNM and TBNM are among the most toxic of the halonitromethane class (Plewa et al., 2004a). MW was an important factor for the recovery of HNMs as seen by the statistically significant correlation ($r = -0.77$, $P < 0.05$; Fig S4 and Table S8) between MW and recovery. Log K_{ow} did not show a significant correlation (Table S8).

2.6. *Recoveries of 13 haloacetamides*

Haloacetamides are typically found in chlorinated and chloraminated drinking water (Krasner et al., 2006; Allen et al., 2022), and they also can be formed directly from chlorami-

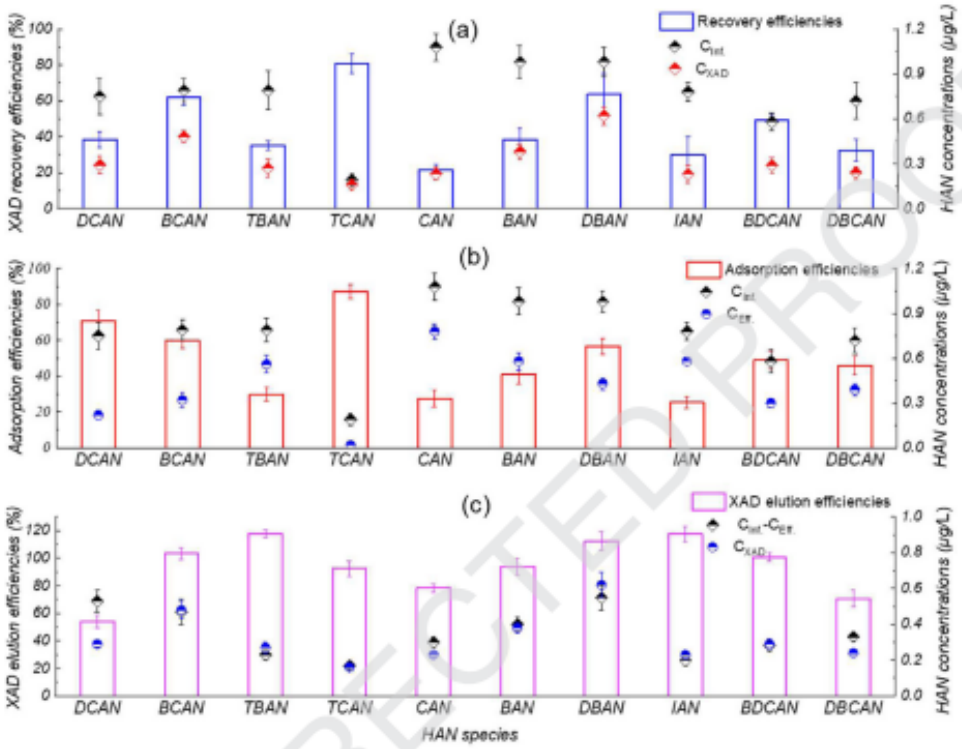

Fig. 8 – XAD recovery, adsorption, and elution efficiencies of haloacetonitriles (HANs)

nation reactions with NOM (Huang et al., 2012) and also by hydrolysis of haloacetonitriles (Reckhow et al., 2001; Farré et al., 2012; WHO, 2011). As many plants in the U.S. and in other countries have switched from chlorine to chloramine to lower regulated DBPs, the increased formation of haloacetamides is a concern. The commonly detected species of haloacetamides are dibromoacetamide (DBAM), dichloroacetamide (DCAM), and trichloroacetamide (TCAM) (Krasner et al., 2006; Farré et al., 2012; Allen et al., 2022; Cuthbertson et al., 2019; Yang et al., 2014). Although haloacetamides have not yet been regulated in any country, they are much more cytotoxic and genotoxic than the regulated THMs and HAAs and are among the most cytotoxic DBPs studied to-date (Wagner and Plewa, 2017).

The XAD recovery of most haloacetamides was 40-60% (Fig 7), and was especially low for chloroacetamide (CAM), bromoacetamide (BAM), and iodoacetamide (IAM) (<40%). This result was similar to a previous study, which reported lower recoveries for CAM and BAM compared to other haloacetamides by liquid-liquid extraction (Kosaka et al., 2016). The recoveries of CAM, BAM, and IAM were also very low by liquid-liquid extraction (1-3%), with other haloacetamides having somewhat higher recoveries (22-66%) (Cuthbertson et al., 2020).

Fig 8

As weak alkaline substances, most haloacetamides will have a significant proportion in a positively-charged ionic state at pH 1 (Cuthbertson et al., 2022), making them relatively difficult to adsorb onto XAD resins. For example, CAM and DCAM have a reported pK_a of -0.26 (Wada and Takenaka, 1971). No significant correlations were observed for the recovery of haloacetamides and log K_{ow} or MW (Table S8).

2.7. *Recoveries of 10 haloacetonitriles*

Haloacetonitriles are commonly reported in chlorinated and chloraminated drinking water (Allen et al., 2022; Cuthbertson et al., 2019; Muellner et al., 2007; Krasner et al., 2006; Quintiliani et al., 2018). Haloacetonitriles generally form rapidly and hydrolyze slowly (Reckhow et al., 2001; Huang et al., 2012), and exhibit orders of magnitude higher levels of cytotoxicity and genotoxicity than THMs and HAAs (Plewa et al., 2008; Wagner and Plewa, 2017; WHO, 2011).

The adsorption of haloacetonitriles was not as high as THMs, as the former are polar substances, while the latter are more non-polar. For chloroacetonitrile (CAN), bromoacetonitrile (BAN), iodoacetonitrile (IAN), and tribromoacetonitrile (TBAN), their adsorption was 25-41%, which are slightly lower than with liquid-liquid extraction (36-47%) (Cuthbertson et al., 2020). The recovery of other haloacetonitriles (except trichloroacetonitrile (TCAN)) by XAD resins was comparable to liquid-liquid extraction; while TCAN had higher recovery by XAD resins (81%) compared to liquid-liquid extraction (20%) (Cuthbertson et al., 2020).

While haloacetonitriles exhibited a poorer adsorption on resins than THMs, they were much better than HAAs, which is in good agreement with the hydrophobic character order of DBP classes: HAAs < HANs < THMs (Le Roux et al., 2017). As the genotoxicity and cytotoxicity of haloacetonitriles is much higher than THMs and HAAs (Muellner et al., 2007; Wagner and Plewa, 2017), and DCAN, BCAN, and DBAN were reported as toxicity drivers in U.S. drinking water (Allen et al., 2022), the toxicity of haloacetonitriles might be underestimated due to their relatively low XAD recoveries. No significant correlations were observed for the recovery of haloacetonitriles and log K_{ow} or MW (Table S8).

In addition, it should be noted that the elution recoveries for some haloacetonitriles (e.g., BCAN, TBAN, DBAN, IAN) were >100%, possibly a result of side-reactions among DBPs taking place, which should be investigated in future research. An isolated study of each DBP class, rather than a combination of them all, may help control this phenomenon. However, it should be noted that this may also be noticed during the enrichment step of mixed DBPs in actual water matrices.

3. Conclusions

XAD resins are a universal sorbent that can adsorb all 66 halogenated DBPs across 7 different chemical classes, but to a different degree, due to their varied chemical properties. In general, XAD are better adsorbents for THMs, HKs, and HALs, but weaker adsorbents for HAAs, HANs, and HAMs. The recoveries of TCM, IAA, CAN, IAN, and CAM by XAD resins was extremely low; thus, these resins are not ideal for their extraction and may lead to an underestimation of toxicity. The recovery efficiencies of most DBPs were comparable with their adsorption, as most are eluted effectively from XAD resins by ethyl acetate. DBP adsorption by XAD resins is a complex process. Removal (elution) efficiencies not only depend on the chemical characteristics of DBPs themselves (polarity, MW, pK_a, volatility, halogen atom species, etc.), but also depend on the chemical functional group on the surface of the XAD resins. Therein, polarity and MW are important factors that determine the recovery of halogenated DBPs by XAD resins. Future studies should investigate potential effects of the presence of NOM or suspended solids on the recovery of DBPs by XAD resins. Finally, future studies should investigate improved extraction methods, including the use of specialized SPE cartridges, which were recently shown to have better recoveries compared to XAD resins (Lau et al., 2021).

Uncited references

Mao et al., 2016, Mazhar et al., 2020, Nieuwenhuijsen et al., 1904, Plewa and Wagner, 2015, Richardson et al., 2008, Stalter et al., 2020, Summerhayes et al., 2021, Villanueva et al., 2006

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.05.001.

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