CHARACTERIZATION AND FORMATION OF IODINATED DISINFECTION BYPRODUCTS FROM ORGANIC AND INORGANIC PRECURSORS

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Environmental Sciences and Engineering in the Gillings School of Global Public Health.

Chapel Hill
2020

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ABSTRACT

Kirsten Elaine Studer: Characterization and Formation of Iodinated Disinfection Byproducts from Organic and Inorganic Precursors
(Under the direction of Howard Weinberg)

Many drinking water sources are impacted by medical waste that contain iodinated contrast media (ICM) or their biodegradation products. When dissolved iodine enters a drinking water treatment plant, it can be a precursor to iodinated disinfection byproducts (I-DBPs) in the finished water, such as iodinated trihalomethanes (I-THMs) and iodoacids (IAs), that have higher relative toxicity compared to regulated DBPs. The goal of this research was to advance our understanding of I-DBP formation in drinking water and evaluate strategies to reduce consumer exposure.

The first objective was to develop a method for resolving and quantifying four IAs from nine other haloacetic acids in a drinking water matrix, since current methods lack sensitivity and quality assurance for measuring their occurrence. With a multi-step extraction process that utilizes liquid-liquid and solid-phase extractions, four IAs were reproducibly concentrated from ng/L to the µg/L levels required for detection.

The second objective was to evaluate medical waste-impacted surface waters in the US state of North Carolina that are used as downstream drinking water sources. The surface waters receiving such wastewater residues showed measurable levels of organic iodine and downstream drinking water treatment plants had I-DBPs in the finished water. Non-target chemical analysis

confirmed that ICMs and their transformation products were responsible for at least a portion of the total organic iodine load in the drinking water source.

In the third objective, organic iodine was also tracked through each unit process in the drinking water treatment plant to evaluate I-DBP formation. Utilizing adsorption isotherms, an optimized use of powdered activated carbon was proposed to meet the plant's current usage objective for taste and odor control while decreasing I-DBP formation in the finished water. When free chlorine was applied at any location in the treatment plant and total dissolved iodine was present, I-THMs formed. Once in the distribution system, I-THMs degraded in the presence of residual disinfectant leading to spatial variability in the water delivered to the consumers.

This research demonstrates that I-DBP precursors can originate from anthropogenic sources thus increasing the need for drinking water source protection together with enhanced treatment processes to ensure a high-quality drinking water for consumers.

To my father.

ACKNOWLEDGMENTS

The Weinberg laboratory has provided me with an excellent setting in which to study environmental chemistry and the public health impacts of drinking water. I am grateful for Howard Weinberg's mentorship and guidance during my time at UNC. Christian Zwiener also provided me with a wonderful opportunity to teach and learn in Germany from October 2017 to March 2018 with the Teach@Tübingen Teaching Fellows program. I would also like to acknowledge the support and guidance of Rebecca Fry, Orlando Coronell, Steven Whalen, and Aaron Salzberg for serving as additional mentors and contributing to this research. I would also like to thank Glenn Walters and D.J. Fedor for designing and building the NOMinator 3000 Reverse Osmosis System. During the months of June to August 2018, I received valuable assistance from Lauren Sprouse, a talented undergraduate researcher, who, under my mentorship, collected water and extracted samples for instrumental analysis. I am also grateful to the North Carolina Urban Water Consortium for their help in identifying and contacting drinking water utilities during this study. The NC Department of Environmental Quality Division of Water Resources was also instrumental in the collection of the reservoir samples. I would also like to thank the funding sources that have made my studies possible. The work shown in Chapters 3 and 4 was supported by the North Carolina Water Resources Research Institute [WRRI Project 16-10-W] and the National Institute of Environmental Health Sciences (NIEHS) Environmental Biostatistics Training Grant [T32ES007018]. During my graduate studies I also received partial support from the Daniel A. Okun Scholarship in Environmental Sciences and Engineering and The J. Don and Margie Smith Summer Research Fellowship.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACS	American Chemical Society
AGC	Automatic gain control
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
BCIM	Bromochloroiodomethane
BDIM	Bromodiiodomethane
BDOC	Biological dissolved organic carbon
BrAA	Bromoacetic acid
Br-HAAs	Brominated haloacetic acids
BrIAA	Bromoiodoacetic acid
Br_2AA	Dibromoacetic acid
CAS	Chemical Abstracts Service
CCL 4	Contaminant Candidate List 4
CDBM	Chlorodibromomethane
CDIM	Chlorodiiodomethane
CHO	Chinese hamster ovary
ClAA	Chloroacetic acid
ClIAA	Chloroiodoacetic acid
Cl_2AA	Dichloroacetic acid
Cl_3AA	Trichloroacetic acid
Ct	Product of the disinfectant dose (C) and the contact time (t)
CW	Clearwell

CWA	Clean Water Act
DBIM	Dibromoiodomethane
DBPs	Disinfection byproducts
DCBM	Dichlorobromomethane
DCIM	Dichloroiodomethane
DOC	Dissolved organic carbon
DN	Dissolved nitrogen
DPD	N,N-diethyl-p-phenylenediamine
DWTP	Drinking water treatment plant
ECD	Electron capture detector
EDTA	Ethylenediaminetetraacetic acid
EEM	Excitation-emission matrix
EI	Electron ionization
FW	Finished water
GAC	Granular activated carbon
GC	Gas chromatography
GC-ECD	Gas chromatography electron capture detector
GC/MS	Gas chromatography mass spectrometry
GC-NCI-MS	Gas chromatography negative chemical ionization mass spectrometry
GC-PEI-MS	Gas chromatography positive electron ionization mass spectrometry
HAAs	Haloacetic acids
HAA5	Five regulated haloacetic acids
HAA9	Nine chlorine- and bromine-containing haloacetic acids

HAA13	Thirteen chlorine-, bromine-, and iodine-containing haloacetic acid
HCB	Hexachlorobenzene
HOBr	Hypobromous acid
HOCl	Hypochlorous acid
HOI	Hypoiodous acid
HRMS	High resolution mass spectroscopy
IA	Iodoacids
IA4	Four iodoacids (IAA, ClIAA, BrIAA, and I_2AA)
IAA	Iodoacetic acid
I_2AA	Diiodoacetic acid
IC	Ion chromatography
ICM	Iodinated contrast media
ICP	Inductively coupled plasma
ICR	Information collection rule
I-DBPs	Iodinated disinfection byproducts
IDL	Instrument detection limit
I-HAAs	Iodinated haloacetic acids
IHSS	International Humic Substances Society
IO_3^-	Iodate
IS	Internal standard
I-THM	Iodinated trihalomethanes
KI	Potassium iodide
LC	Liquid chromatography

LC_{50}	Lethal concentration for 50 percent cell death
LGW	Laboratory grade water
LLE	Liquid-liquid extraction
MCL	Maximum contaminant level
MDL	Method detection limit
MFE	Molecular feature extraction
MGD	Million gallons per day
MIB	2-Methyl isoborneol
MRT	Maximum residence time
MS	Mass spectrometer
MtBE	Methyl tert-butyl ether
m/z	Mass per charge
NC DEQ	North Carolina Department of Environmental Quality
NH_2Cl	Monochloramine
NHBrCl	Bromochloramine
NOM	Natural organic matter
NPDES	National Pollutant Discharge Elimination System
NTU	Nephelometric turbidity unit
PAC	Powdered activated carbon
PQL	Practical quantification limit
PFAS	Per-/poly-fluoroalkyl substances
PTFE	Polytetrafluoroethylene
Q/TOF	Quadrupole time-of-flight

RO	Reverse osmosis
RPM	Rotation per minute
SDWA	Safe Drinking Water Act
SIM	Single ion monitoring
SPE	Solid-phase extraction
SOP	Standard operating procedures
SPI	Septum-equipped programmable injector
SWAP	Source water assessment program
TBM	Tribromomethane
TCM	Trichloromethane
TDI	Total dissolved iodine
TDII	Total dissolved inorganic iodine
TDOI	Total dissolved organic iodine
TDOX	Total dissolved organic halides
THM	Trihalomethane
THM4	Four regulated chlorine- and bromine-containing trihalomethanes
TIC	Total ion count
TIC_Tox	Theoretical calculation using concentration and toxicity for comparison
TIM	Triiodomethane
TMDL	Total maximum daily loads
TOC	Total organic carbon
TOI	Total organic iodine
TOX	Total organic halogens

TP	Transformation product
UMCR 4	Fourth Unregulated Contaminant Monitoring Rule
UNC	University of North Carolina at Chapel Hill
US EPA	United States Environmental Protection Agency
USGS	United States Geological Survey
UV	Ultra Violet
WWTP	Wastewater treatment plant

CHAPTER 1 : INTRODUCTION

1.1 Drinking Water Treatment and Public Health

Drinking water is a complex environmental matrix since it often contains natural organic matter (NOM), salts, and anthropogenic micropollutants that remain after the water has undergone treatment for human consumption. NOM is a complex mixture of humic and nonhumic substances that are naturally present in all bodies of surface water and are produced from decomposition of living matter, waste production from biological activity and runoff from soils (Frimmel and Christman, 1988). An environmental matrix can also include anions such as chloride, bromide, and iodide from natural or anthropogenic sources. Other anthropogenic contributions can include pesticides, fertilizers, medical waste, industrial waste, mining waste, and domestic sewage. A conventional drinking water treatment plant includes coagulation/flocculation, sedimentation, filtration, and disinfection (Crittenden et al., 2012) for physical removal of organic and inorganic particles and chemical inactivation of bacteria, viruses and protozoa.

The primary objective of disinfection in drinking water is to control pathogens and most commonly involve the use of a chemical oxidant such as chlorine or chloramines both of which react with NOM, salts, and some anthropogenic chemicals in water producing disinfection byproducts (DBPs) that can vary widely in type and concentration depending on source water quality and treatment conditions. The formation of DBPs from reactions with NOM has been studied for a subset of byproducts for which analytical methods have been developed.

The United States Environmental Protection Agency (US EPA) regulates the quality of surface water through the Clean Water Act passed in 1972 and drinking water through the Safe Drinking Water Act (SDWA) that was introduced in 1974. The main objective of the SDWA is to ensure safe drinking water for the public using federal drinking water regulations on pathogens, disinfectants, DBPs, inorganic and organic chemicals, and radionuclides. Due to the known health effects of DBPs and availability of analytical methods, the US EPA currently regulates four trihalomethanes (THMs, the sum concentrations of which are known as THM4) to a maximum contaminant level (MCL) for a locational running annual average not to exceed of 80 μg/L and the sum of five haloacetic acids (HAAs, the sum of which are known as HAA5) to an MCL for a locational running annual average not to exceed 60 μg/L under the Stage 2 Disinfectants and Disinfection Byproduct Rule (US EPA, 2006). The four regulated THMs shown in Figure 1-1 are chloroform, bromodichloromethane, dibromochloromethane, and bromoform. The five regulated HAAs shown in Figure 1-1 are chloroacetic acid, bromoacetic acid, dichloroacetic acid, dibromoacetic acid, and trichloroacetic acid. The US EPA also regulates bromate (MCL of 10 μg/L) and chlorite (MCL of 1.0 mg/L). The maximum residual disinfectant levels for free chlorine and chloramines are both 4.0 mg/L as Cl_2.

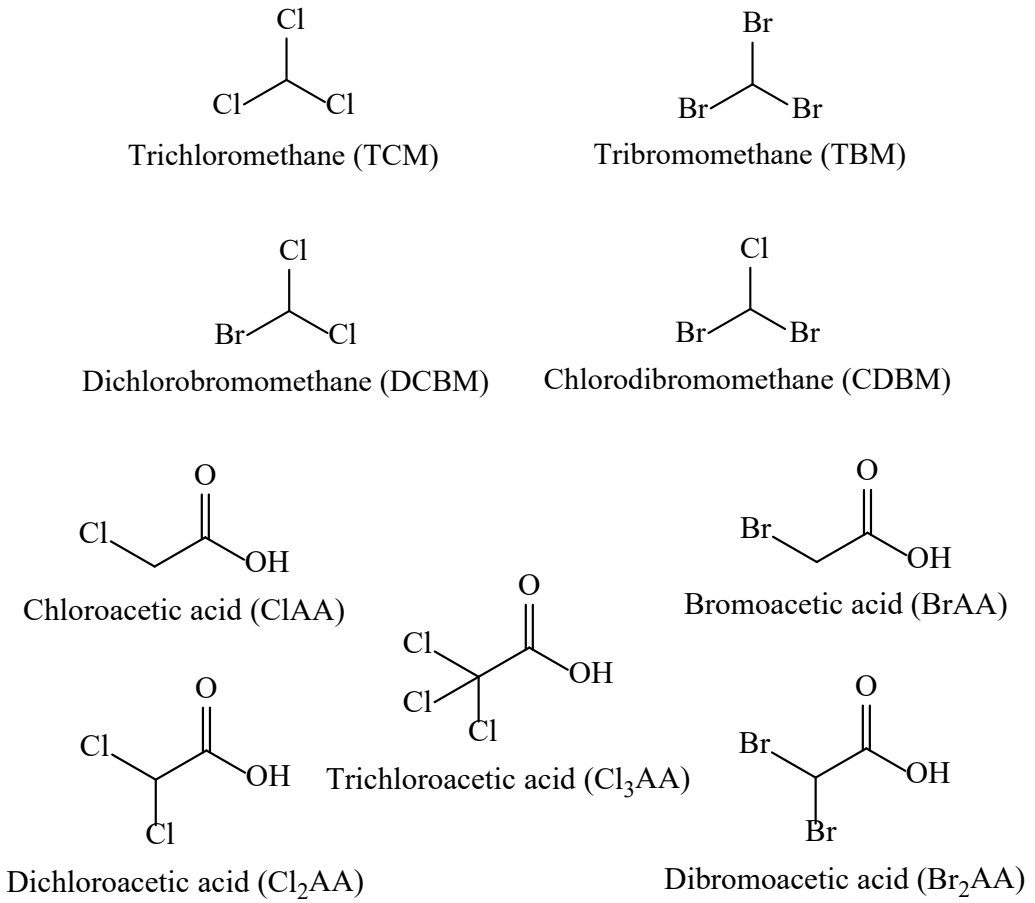

Figure 1-1: Structures for the US EPA regulated trihalomethanes and haloacetic acids.

The two most common disinfectants used for drinking water treatment are chlorine, usually in the form of hypochlorous acid (HOCl), and monochloramine (NH_2Cl). HOCl is a strong oxidizing agent and in water is most effective as a disinfectant when in the non-ionized form, while chloramine is a weaker oxidizing agent that is most often generated from the reaction of free chlorine with an ammonium salt (Benjamin, 2002). The effectiveness of disinfectants with different oxidation strengths can be compared using equivalent C.t values, the product of the disinfectant dose (C) and contact time (t) to obtain a specific pathogen kill (Crittenden et al., 2012).

According to the US EPA Surface Water Treatment Rule (US EPA, 2006b), all drinking water treatment systems must meet the C.t requirements which are calculated as log removal based on contact time and dosage of a disinfectant needed to inactivate surrogate viruses and bacteria. The minimum log removal and/or inactivation of bacteria, viruses and protozoa is achieved by filtration type, disinfectant dose, and contact time (Kawamura, 2000). Unless the turbidity of the source water is less than 1 nephelometric turbidity unit (NTU), the US EPA's Surface Water Treatment Rule specifies that treatment of surface waters for producing drinking water must include filtration. The type of filtration and any pre-treatment selected can greatly remove or at least reduce the amount of DBP precursors that were not removed by a previous process.

One form of pre-treatment before filtration is the addition of powdered activated carbon (PAC). Previous studies have utilized PAC for micropollutant removal from soils and waters, utilizing the physical removal by adsorption mechanism. Within a drinking water treatment, PAC is typically added for the removal of taste and odor compounds associated with algal blooms, which have limited health consequences but are typically public perception problems (Najm et al., 1991). PAC has also been used for targeted micropollutant removal from water such as pesticides (Kearns et al., 2014; Thompson et al., 2016), per-/poly-fluoroalkyl substances (PFAS) (Hansen et al., 2010), pharmaceuticals (Kovalova et al., 2013), and personal care products (Tursi et al., 2018), some of which are included on the Fourth Unregulated Contaminant Monitoring Rule (UMCR 4) (US EPA, 2016), or on the Contaminant Candidate List 4 (CCL 4) (US EPA, 2017). PAC can be added at multiple stages in a water treatment plant as either a dry powder or a pre-wetted slurry and can be sourced from coal, wood, coconut shells, bamboo and other carbonaceous materials that are chemically or physically activated to increase surface area for

maximum adsorption potential (Najm et al., 1991). PAC is typically characterized as carbon particles with high area to volume ratios that pass through an 80-mesh sieve or smaller (ASTM, 2019).

Since there are no US EPA drinking water regulations on taste and odor compounds, many treatment plants develop their own optimized dose procedures based on in-house jar testing for geosmin and 2-methyl isoborneol (MIB), which are responsible for earthy and musty odors, respectively. These in-house dose response curves are highly sensitive to source water quality changes such as increased NOM that can alter adsorption potential through competition and interference.

The US EPA also requires significant reductions in total organic carbon (TOC), a surrogate for NOM concentrations, as part of the drinking water treatment rules to limit pathogens and DBP formation (US EPA, 1974 and 2006). TOC has traditionally been removed through coagulation and flocculation of particles followed by clarification through sedimentation and media filtration (Crittenden et al., 2012). Enhanced coagulation, where a pre-oxidation step with ozonation is used to change the NOM properties, or chlorination (HOCl), can be used for increased TOC removal. Coagulation/flocculation works on solubility principles by changing the ionic strength of the water and decreasing the solubility of the DBP precursors. A polymer can also be added to the coagulant to aid in the removal of ions by destabilizing colloidal suspensions (Viessman and Hammer, 2005). This step is usually followed by media filtration.

Stage 1 and 2 Disinfectant and DBP Rules require the use of conventional filtration treatment to remove a specified percentage of TOC including DBP precursors. Rapid filtration processes are commonplace in US drinking water utilities and a variety of uniform-size, granular filter media has been used to improve clarity of water by removing particles. The common

naturally occurring filter media types include sand, anthracite coal, garnet, and ilmenite (Crittenden et al., 2012). Granular activated carbon (GAC) is a type of filter media used in conventional treatment plants when adsorption and filtration are combined in one-unit process. While the GAC filters require a large footprint for the filter tanks and back wash waste tanks, this is an effective and less costly option for organic matter removal compared to membrane filtration (Schideman et al., 2010). There are issues with dissolved organic carbon (DOC) breakthrough in filters that can lead to DBP formation if the filter is not maintained and back-flushed periodically (Kennedy et al., 2015). Many utilities also add chlorine prior to filtration to control biological activity on the filter.

Due to DBP regulations some water utilities are switching to chloramines, where the primary disinfection is still performed by HOCl but the addition of ammonia quenches the free chlorine and creates the secondary disinfectant monochloramine, a less reactive oxidant. Some of the DBP formation potential research with chloramines cannot be directly applied to a water treatment plant since the reactions studied under laboratory-controlled conditions are conducted mostly with preformed chloramines and so much less is known about pure chloramine DBPs (Dyksen et al., 2007; Seidel et al., 2005). Chloramination as a secondary disinfectant can create many new types of DBPs not formed by free chlorine in addition to those produced during chlorination but at decreased levels due to chloramine's lower oxidative strength.

Once the treated, so-called finished, water leaves the plant it enters the distribution system that includes pipes, pumps, and storage tanks. Corrosion control chemicals, such as orthophosphate and polyphosphates, are also added to maintain the integrity of the pipe materials and reduce leaching of lead and copper (Crittenden et al., 2012). To maintain the chemical stability of the disinfectant and reduce deposition of calcium carbonate in the distribution system,

lime or sodium hydroxide is added to the finished water to maintain an US EPA recommended pH between 6.5 and 8.5 (US EPA, 2006a). The residual disinfectant should ensure continued protection against pathogens, but microbial regrowth can occur due to stagnation and changes to water chemistry (Ji et al., 2015; Ling et al., 2018).

Stagnation can be determined within a distribution system by conducting tracer tests to establish locational water age and to determine the maximum residence time (MRT) for residual disinfectant needs. Residual disinfectant must be below the 4.0 mg/L as Cl_2 at all points in the distribution system but still present at the MRT location to control microbial regrowth. For the acceptable levels of DBPs at the consumer tap, the US EPA initially required a running annual average of THM4 and HAA5 data that was not to exceed the MCL for the Stage 1 Disinfectants and DBP Rule. To avoid possible violations, some utilities would select their sampling locations based on the desired DBP concentration so that they did not exceed the MCL. Stage 2 Disinfectants and DBP Rule now requires that a locational running annual average not exceed the MCL so that DBP concentration compliance must be met at each location. These monitoring sites were established through an initial distribution system evaluation and based on the four highest THM4 and the four highest HAA5 locations in a peak historical month (US EPA, 2006a).

To reduce the DBP formation potential in drinking water, the highest quality water source should be chosen prior to any treatment strategy. Decreasing its TOC by coagulation/flocculation/filtration or membrane technologies will reduce DBP formation potential with any subsequently used disinfectant. By removing disinfectant demand from the treated water, the dose of the disinfectant to achieve pathogen inactivation and continued residual will be lower and fewer DBPs will form.

1.2 Surface Water Quality Impacts

With many drinking water sources compromised by point and non-point anthropogenic inputs resulting from drought stress, storm-water runoff, and upstream wastewater treatment plant discharges, a wide range of chemical contaminants are now present in surface water sources. Waste streams from hospitals and medical facilities are often regulated no differently than other municipal wastewater sources even though they may contain biologically active anthropogenic chemicals that are not removed by conventional wastewater treatment plants. As the effluents from these plants often feed into downstream drinking water sources, it is imperative to determine the fate of these medical residues not only for their potential direct impact on human and ecosystem health but also because they may react with disinfectants used in water treatment to form DBPs with potentially harmful health impacts.

The Clean Water Act (CWA) regulates pollutant discharge into surface water through the National Pollutant Discharge Elimination System (NPDES) permit program with the primary objective to restore and maintain the water quality (US EPA, 1972). Under the SDWA, states must also perform source water assessment on regulated contaminants, which is often informed by the NPDES permits (US EPA, 1974).

Using NPDES permit information and surface water sampling data, states are required to list impaired and threatened surface waters to the US EPA every two years with identification of the pollutants associated with impairment (US EPA, 1972). The lists must be submitted to the US EPA under CWA Section 303(d) and the states must also provide proposed actions to reduce impairment by developing total maximum daily load (TMDL) allowances for each pollutant identified. Within North Carolina, the Source Water Assessment Program (SWAP) overseen by the NC Department of Environmental Quality (NC DEQ) addresses the contamination of public

water supplies by determining the TMDL allowances for water quality management (North Carolina Department of Environmental Quality, 1999).

Based on the NC SWAP reports, source waters are rated as good, threatened, or impaired for categories of aquatic life, fish consumption, recreation, and water supply. Impairment assessments include pathogens, heavy metals (mercury, lead, cadmium, arsenic), turbidity, oxygen depletion, fecal coliform, pH, polychlorinated biphenyls, dioxin, chlorophyll-A, nitrate/nitrite, and chloride (salinity). NC DEQ has mapped the impaired surface waters using the SWAP assessments for 2012 and 303(d) for 2010 seen in Figure 1-2 (North Carolina Division of Water Resources, 2017).

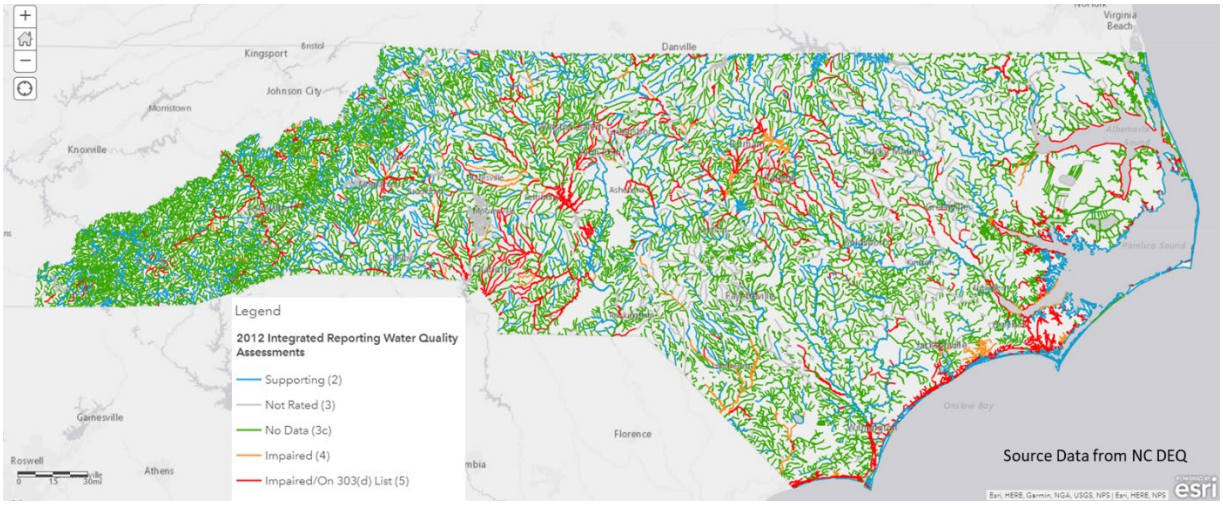

Figure 1-2: NC surface water designations in 2012 for impaired and impaired/on US EPA 303(d) list for 2010.

Within a specific watershed there can be multiple water quality designations based on the SWAP report assessment and surface water monitoring. Figure 1-3 shows the variable water quality designations for the Jordan Lake watershed from a 2012 assessment (North Carolina Division of Water Resources, 2017).

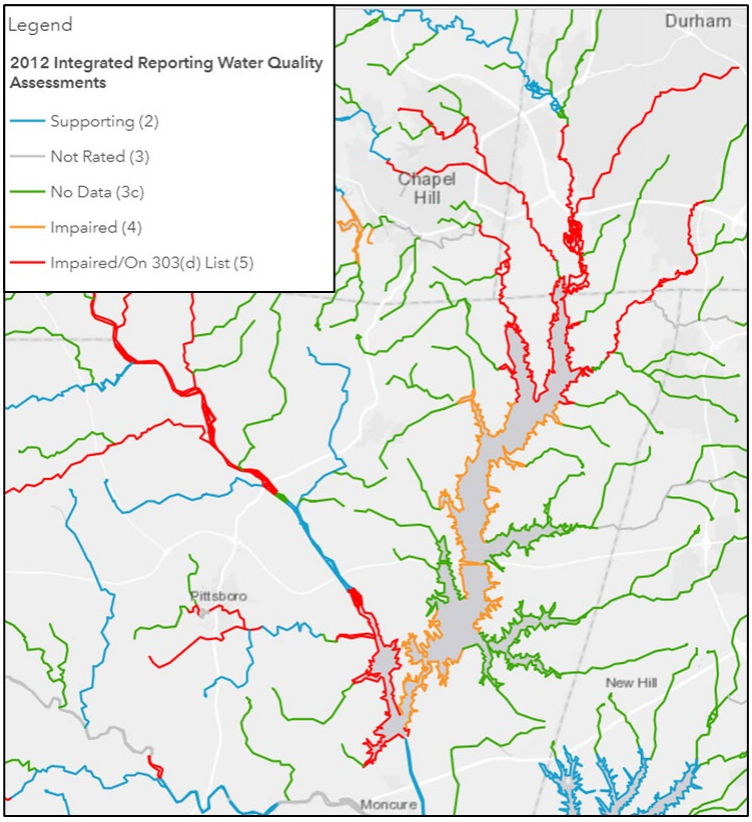

Figure 1-3: Jordan Lake source water NC SWAP report designations in 2012 for impaired and impaired/on US EPA 303(d) list for 2010.

These NC SWAP reports focus on chronic point sources of pollution with a calculated susceptibility rating determined from the contaminant and inherent vulnerability ratings that can miss more diffused runoff or smaller discharge from septic tank drain fields. The reports do not comprehensively reflect the impact on surface waters receiving treated wastewater from medical facilities since they do not account for pharmaceutical contaminants. Within NC, there are numerous wastewater treatment plants that receive waste from regional hospitals that are upstream of drinking water treatment plants. Table 1-1 shows the major drinking water sources impacted by treated medical waste from large regional hospitals in NC. This list only includes wastewater treatment plants within NC and drinking water sources within NC.

Table 1-1: NC drinking water sources impacted by upstream wastewater treatment plants (WWTPs) receiving regional hospital wastewater.

Drinking Water Source (Town)	Regional Hospital	Upstream WWTPs
B. Everett Jordan Lake (Cary/Apex and Chatham)	Duke University Hospitals University of North Carolina (UNC) Hospitals	Mason Farm WWTP South Durham WWTP Triangle WWTP
Haw River (Burlington and Pittsboro)	Moses H. Cone Memorial Hospital Alamance Regional Medical Center	TZ Osborne WWTP North Buffalo WWTP
Cape Fear River (Fayetteville and Wilmington)	WakeMed Cary Hospital Cape Fear Valley Medical Center Highsmith-Rainey Specialty Hospital	Mason Farm WWTP South Durham WWTP Triangle WWTP Pittsboro WWTP Western Wake Regional WWTP
Falls Lake/Neuse River (North Durham/Raleigh)	UNC Health Care Hillsborough Campus	North Durham WWTP Town of Hillsborough WWTP
Yadkin River (Winston Salem/Clemmons)	Wake Forest Baptist Hospital Novant Health Medical Park Hospital	Muddy Creek WWTP

Since wastewater treatment plants are not designed for enhanced removal of pharmaceuticals and personal care products, medical residues either intact or transformed can be discharged from these wastewater treatment plants into NC surface waters. Discharging treated wastewater with anthropogenic pollutants shifts the burden of treatment to downstream communities that would need to upgrade their systems to remove the contaminants if deemed a public health risk.

1.3 Disinfection Byproducts

Over 600 chlorine- and bromine-containing DBPs resulting from the reaction of chlorine or chloramine with NOM have been identified, but widespread occurrence data for most of them in drinking waters remains unknown and many others have not yet been identified. This is evidenced by the mass imbalance of total organic halogen (TOX) compared to that of the identified DBPs in the same water (Richardson and Postigo, 2011; Stevens et al., 1990).

Existing and routinely used methods for HAA and THM extraction from drinking water focus on the chlorinated and brominated species, since they are typically present at low μg/L levels in drinking water (Brophy et al., 2000; Ells et al., 2000; Magnuson and Kelty, 2000; Sarrion et al., 2000; Weinberg et al., 2002). US EPA Methods 551.1 for THM4 and 552.3 for HAA5 use a liquid-liquid extraction (LLE) from water then instrumental analysis by gas chromatography (GC) separation and an electroconductivity detector (ECD). The ECD is ideal for halogenated DBPs due to the electronegativity of halogen atoms. Since HAAs are of low volatility, a methylation step is needed with a derivatizing agent to decrease the boiling point of the analytes (Domino et al., 2003). DBP analyte concentrations are based on calibration curves prepared with standards and analytes are confirmed by retention time and matrix spikes. These GC methods, however, only account for a fraction of the DBPs that are produced, many of which are likely to be non-volatile and thus not amenable to GC, or have high polarity leaving them in water after extraction. A technique to recover more analytes in a single analysis is solid-phase extraction (SPE) followed by liquid chromatography (LC) and non-target screening with high-resolution MS. This has been used previously for environmental water analysis where complex matrices create challenges for identifying unknown species and separating the numerous

constituents (Fischer et al., 2012; Krauss et al., 2010; Moschet et al., 2013; Zedda and Zwiener, 2012).

One of the largest datasets available on DBP occurrence is from the US EPA's Information Collection Rule (ICR), which provided information for the development of the Safe Drinking Water Act (US EPA, 1994). The ICR data includes water quality, microbial and DBP occurrence making it is one of the most comprehensive and diverse databases to date due to the number of utilities that participated and the variety of locations across the US (Obolensky et al., 2007). The ICR surrogate analyte groups included TOC, uniform formation condition TOX, absorbance at UV 254 nm, and biological dissolved organic carbon (BDOC). Water quality parameters also monitored through the ICR were bacteria, protozoa, coliphage, viruses, aldehydes, bromide, chlorate, chlorite, disinfectant residual, pH, temperature, ammonia, alkalinity, and turbidity. The DBPs that were monitored included THM4, HAA5, haloacetonitriles, cyanogen chloride, chloral hydrate, and bromate.

Additional DBP occurrence data comes from the US EPA's unregulated contaminant monitoring rule (UCMR), which updates every five years and is currently on UCMR 4 and requires utilities to monitor 30 unregulated contaminants (US EPA, 2016). The DBP focus for UCMR 4 is on brominated HAAs with monitoring required for bromide and HAA9 that includes HAA5 as well as tribromoacetic acid, bromochloroacetic acid, bromodichloroacetic acid, and chlorodibromoacetic acid. Iodine-containing DBPs (I-DBPs) were not included in the ICR or the UMCRs.

In 2002, an US EPA structural-activity analysis designated iodo-trihalomethanes (I-THMs) and other DBPs not monitored in the ICR as "high priority" DBPs (Weinberg et al., 2002). In this Nationwide Occurrence Study that looked at a diverse set of source waters and

treatments, Weinberg et al. (2002) found that THM4 concentrations were not strong indicators for the formation of other halogenated DBPs, including I-THMs.

According to Chowdhury et al. (2009), active research on DBP formation has led to the development of over 118 predictive models. The majority of the models are empirically based; however, recent attempts to predict DBP formation have combined mechanistic relationships and empirical data (Brown et al., 2011; Sadiq and Rodriguez, 2004). Real limitations exist on the success of the DBP predictive models due to the availability of reliable data and the capability of external validation for the constructed relationship or correlation. Many models that have been developed for DBP formation in one source water lack the ability to generalize to other water sources. Overall, the key water quality characteristics connected to DBP formation, particularly THMs, have been TOC levels, UV-absorbance at 254nm, pH, temperature, bromide concentration, disinfectant dose, disinfectant contact time, and disinfectant residual (Obolensky and Singer, 2008; Sadiq and Rodriguez, 2004; Ye et al., 2009).

Once the finished water enters the distribution system, additional changes to DBP concentrations can occur. Due to variable flow-rates, pipe biofilms, hydraulic residence times (water age), and residual disinfectant doses, DBP concentrations can fluctuate within the system (Brown et al., 2011). With residual free chlorine, THMs and HAAs can continue to form within the distribution systems leading many drinking water treatment plants to add ammonia to the finished water to form chloramines as the secondary disinfectant with a lower THM4 and HAA5 formation potential (Baribeau et al., 2006; Seidel et al., 2005; Speitel et al., 2010; Wang et al., 2015).

DBP formation potential reactions on a laboratory scale often use a concentrated precursor matrix to ensure the DBPs formed are above the analytical method detection limits or

practical quantitation limits (PQL). One technique used for increasing the NOM precursors for such lab-scale studies uses filtration of a large volume of surface water followed by a reverse osmosis (RO) membrane a process which concentrates the DBP precursors achieving high recoveries (80–99%) of NOM while preserving the integrity of the original source water through the collection of the retentate (Kitis et al., 2001; Song et al., 2009). The advantage of using a concentrated natural water in place of a commercially available NOM standard is that the source water quality can be preserved for multiple experiments over an extended time period while being a closer representation of the complex mixtures that are typically disinfected in water treatment.

To ensure the NOM concentrate is representative of the source water and to compare to other source waters, excitation-emission matrix (EEM) fluorescence spectroscopy can be used to determine the chemical characteristics of the organic carbon and organic nitrogen present in wastewater, surface water, and drinking water. EEM fluorescence spectroscopy allows for the characterization of NOM using the ratio of emission to excitation intensity of known fluorophores at specific wavelengths for the hydrophobic acid fraction (Peak A, $\lambda ex/\lambda em \sim 260/380–420nm$), humic-like fraction (Peak C, $\lambda ex/\lambda em \sim 350/420– 480nm$), and hydrophobic base (or protein-like) fraction (Peak T, $\lambda ex/\lambda em \sim 220/303$ nm) (Mcknight et al. 2001; Stedmon et al. 2003; Murphy et al. 2008). Comparing the EEM peak intensities as a function of TOC in an experimental matrix to other source waters can provide more information on the DBP formation potential for more than one water type since more information is known for the organic carbon precursors that compose NOM (Hwang et al., 2001; Yang et al., 2008).

1.4 Iodinated Disinfection Byproducts

Both inorganic and organic iodine precursors for I-DBPs have been measured in drinking water sources that show formation of I-THMs and iodinated haloacetic acids (I-HAAs) in the finished water (Duirk et al., 2011; Richardson et al., 2008). Occurrence data on iodoacids are rare due to the absence of a validated analytical method in a drinking water matrix. Various groups of iodo-DBPs have been identified by either broad screening methods without validated quantitative results or by targeted compound analysis (Krasner et al., 2006; Liu et al., 2013; Plewa et al., 2008; Postigo et al., 2017; Richardson et al., 2008; Shi and Adams, 2009). While these methods can provide some quantitative information on iodoacids, they do not have the concentration factors and chromatographic methods needed for simultaneous extraction and quantification of HAA9 and iodoacids (IAs). The Nationwide DBP Occurrence Study included I-THMs which were highest in finished waters treated with chloramines (Weinberg et al., 2002). In a follow up to the 2002 occurrence study, samples were collected from the same plants with I-THM formation and found that I-HAA were also present in the finished water (Weinberg et al., 2011), which suggests I-THMs are good surrogates for the overall I-DBP group. However, widespread occurrence data has not been collected for I-DBPs since they are not currently regulated.

Generally, I-DBPs are formed from chloramine disinfection of source drinking water containing iodide, with a shorter contact time increasing their formation (Bichsel and von Gunten, 1999). In the initial step for I-DBP formation, hypoiodous acid (HOI) is formed by the oxidation of iodide, which is fast for both chlorine (k=4.3x10^{8} M^{-1} s^{-1}) and monochloramine (k=2.4x10^{10} x[H^{+}] M^{-2}s^{-1}) (Bichsel and von Gunten, 1999). However, in the presence of chloramine the half-life of HOI is much longer and is on the order of hours due to the slow

reaction of HOI with NH_2Cl ($k_{NH2Cl+HOI}$=2x10^{-3} M^{-1} s^{-1}) compared to hypochlorous acid (HOCl) ($k_{HOCl+HOI}$= 8.2 M^{-1} s^{-1} and $k_{HOCl+HOCl+HOI}$= 8.3x10^4 M^{-1} s^{-1}) under drinking water conditions (pH range 6 to 9) (Bichsel and von Gunten, 1999). Comparatively, the oxidation rates of bromide by chlorine to form HOBr (k=2950 M^{-1} s^{-1}) (Farkas et al., 1949) and by monochloramine to form NHBrCl ($k_{NH2Cl+Br}$= 2.8 x10^6 M^{-2} s^{-1}) (Trofe et al., 1980) are very fast. Given this relatively long half-life for HOI, there is abundant time for I-DBPs to form in the presence of chloramine especially when the reaction rate between HOI and NOM is orders of magnitude higher than the oxidation of HOI by chloramine to iodate (IO_3^-) (Bichsel and von Gunten, 2000; Duirk et al., 2011). Chlorination of iodine-precursors also forms HOI but chlorine then rapidly oxidizes HOI to iodate, which greatly reduces iodine incorporation in DBPs (Bichsel and von Gunten, 1999). Additionally, during chloramination, formation of I-DBPs is strongly dependent on the order of addition of chlorine and ammonia due to the fast oxidation of HOI to iodate by chlorine. These DBP formation potentials are determined using controlled laboratory conditions (i.e., disinfectant dose, temperature, contact time, etc.) that can provide insight to potential treatment changes based on a fully characterized water sample with natural and/or anthropogenic precursors present to determine how changing individual parameters can impact the DBPs generated.

When organic iodine compounds are oxidized by HOCl in the presence of other organic matter in high-concentration laboratory experiments, I-DBPs such as I-THMs and I-HAAs can be formed likely because of high rates for dehalogenation reactions or halogen-substitution reactions and availability of reaction rich sites on the organic matter (Duirk et al., 2011; Wendel et al., 2014). Based on the presented HOCl+HOI reaction rates, the presence of excess HOCl would be expected to rapidly oxidize the HOI to iodate (IO_3^-) but the presence of organic matter provides an opportunity for iodide incorporation and the formation of organic I-DBPs. Iodine

precursors can also be compared through incorporation rates from a subset of DBPs using the methods used by others for measuring bromine incorporation (Cowman and Singer, 1996; Francis et al., 2010; Hua and Reckhow, 2012).

Many lab-based assessments on I-DBP formation from chlorine and chloramine have been conducted due to the detection of I-DBPs in finished water where iodide was not detected (Richardson et al., 2008). One type of organic iodine compound used in I-DBP formation potential reactions is iodinated contrast media (ICM) from medical waste in wastewater that were observed in source water for a drinking water treatment plant with I-DBPs (Duirk et al., 2011). Duirk et al. (2011) also looked at I-DBP formation potentials in laboratory grade water (LGW) at one contact time (72 hrs) for chlorination and chloramination of iopamidol, a common iodinated contrast media (ICM) but did not include NOM characterization that could provide insight to specific I-DBP formations. Allard et al. (2016) looked at I-DBP formation from ICMs with UV+HOCl and NH_2Cl in the presence of an International Humic Substances Society (IHSS) standard NOM, but the water prepared from those derived standards is not actually used for drinking and they are not comparable across other sources due to the lack of NOM characterization before and after disinfection. Wendel et al. (2014) identified some DBPs of ICMs in purified water; however, in the presence of other DBP precursors, such as NOM, formation pathways may be altered. More information is needed to determine what conditions are favorable for I-DBP formation from an organic iodine source such as ICMs.

1.5 Sources of Iodine

Inorganic iodine in surface waters can originate from geologic formations (Fuge and Johnson, 1986), hydraulic fracturing (Harkness et al., 2015), seawater intrusion (Oh et al., 2010),

atmospheric depositions (Fuge and Johnson, 1986), and manufacturing waste (Nguyen et al., 2012; Pal and Nayak, 2016). Levels in U.S. drinking water sources have been measured as high as 230 µg/L with a median of 10.3 µg/L (Fuge and Johnson, 1986; Weinberg et al., 2011).

Although many studies view the incorporation of iodine from an inorganic species as the major pathway for generation of I-DBPs, researchers have also described the importance of organic sources of iodine in this same regard (Bichsel and von Gunten, 2000; Duirk et al., 2011; Peng et al., 2016). Organic iodine sources from medical facility waste (Kormos et al., 2011; Richardson et al., 2008; Steger-Hartmann et al., 1999), enzymatic halogenation (Blasiak and Drennan, 2009), or livestock operations (Hladik et al., 2016) also add to the total iodine load in surface waters. One specific occurrence study of 23 U.S. cities observed significant I-DBPs in drinking waters whose sources contained low or non-detectable iodide concentrations (Richardson et al., 2008). A follow-up study implicated ICMs from medical waste as reacting with the drinking water disinfectant chloramine to form I-DBPs (Duirk et al., 2011).

Seitz et al. (2006) looked at full-scale unit processes for the removal of ICMs within the drinking water treatment system using coagulation/flocculation, ozonation, filtration, and activated carbon adsorption, but did not determine the fate of the iodine or the formation of I-DBPs in the finished water. Four ICMs, iomeprol, iopromide, iohexol, and iopamidol, were identified in the source water ranging in concentration from 80 to 399 ng/L and a 70% decrease was observed over all treatment units.

Commonly used ICMs that share the triiodinated benzene ring shown in Figure 1-4, are applied at high doses (60 to 120 g as I per dose) during diagnostic medical procedures and are eliminated, without metabolization of the parent compound, through urine and feces over 24 hrs after administration (Pérez et al., 2006). These organic iodine compounds are widely and

globally used (75 million patient applications/year) and are very persistent through conventional wastewater treatment plants since they do not have adequate mechanisms for complete ICM removal (Hollender et al., 2009; Ternes and Hirsch, 2000).

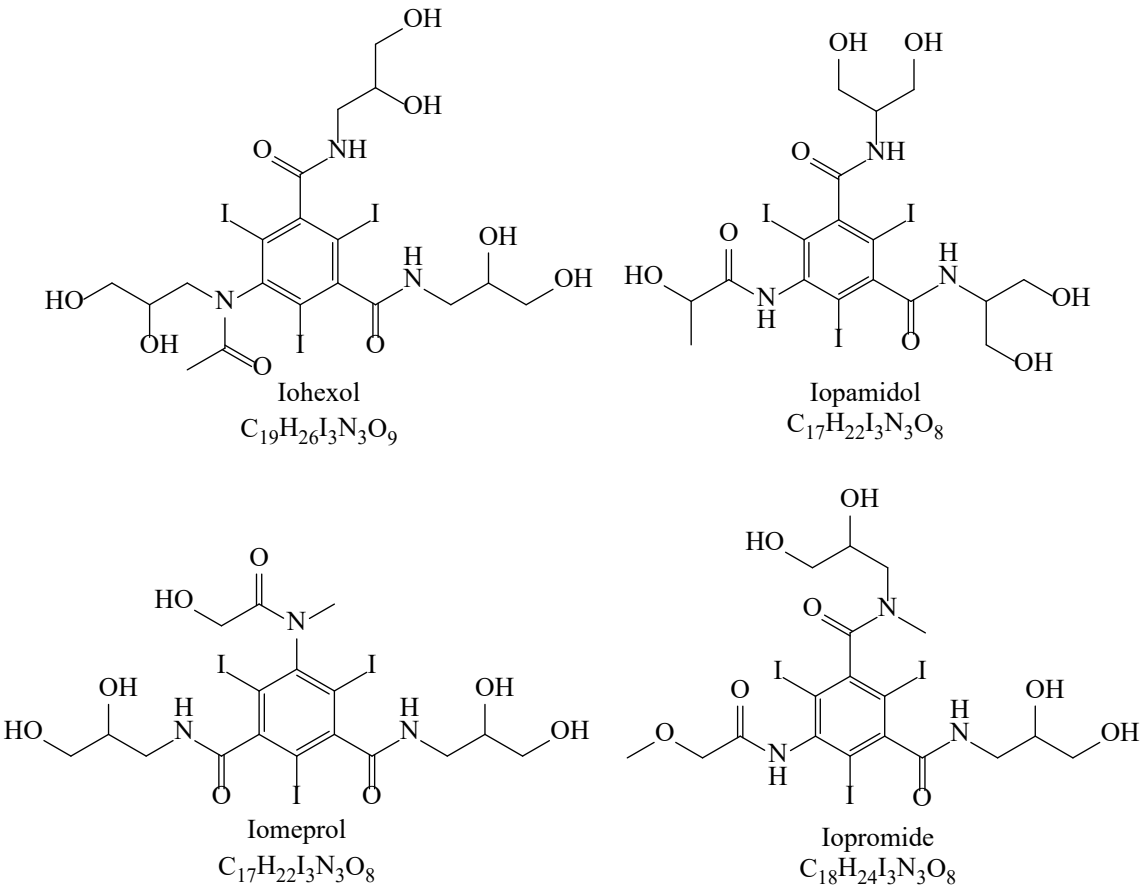

Figure 1-4: Examples of iodinated contrast agents used in medical imaging and found in US surface waters.

Currently, the waste streams from most hospitals and other medical facilities in the U.S. are not monitored or regulated even though contrast media are very persistent and conventional wastewater treatment plants simply do not have adequate mechanisms for complete iodine removal (Hollender et al., 2009; Ternes and Hirsch, 2000). Additionally, the parent ICM

compounds can be altered during the wastewater treatment as well as by solar photolysis before they reach the downstream drinking water plant intake.

Previous research groups have investigated the discharge rates of iodinated contrast agents to the environment and determined that they include multiple biotransformation products in wastewater effluents; however, the high-molecular weight, triiodinated structure is conserved with only the amide moieties altered (Drewes et al., 2001; Kormos et al., 2011; Schulz et al., 2008; Ternes and Hirsch, 2000). Once the biotransformed contrast agents are discharged to a surface water, further transformation can occur due to UV photolysis (Fabbri et al., 2016). While Duirk et al. (2011) showed that ICMs present in a drinking water source could lead to I-DBP formation in the finished water, they did not look for transformed ICMs or link them back to specific point sources. With the multiple transformation products possible, using a targeted analysis would provide an incomplete picture of the organic iodine being discharged or circulating within a surface water. Due to the numerous possible transformations, a targeted screening for ICMs may miss intact tri-iodinated compounds due to the mass alterations even though they may still be an organic iodine precursor for I-DBPs.

If the ICM or their biotransformed products reach the drinking water treatment plant (DWTP), further transformation can occur in the presence of a strong oxidant such as is used in algal bloom treatment or disinfection. Previous experiments have suggested a dehalogenation reaction or a halogen-substitution reaction where an iodine on the ICM ring structure is substituted with a chlorine allowing the iodine to form the iodinating agent HOI (see Figure 1-5) (Wendel et al., 2016). Based on the Section 1.4 reaction rates, the presence of excess HOCl should quickly oxidize the HOI to iodate but the presence of organic matter could also provide an opportunity for iodide incorporation to produce I-DBPs.

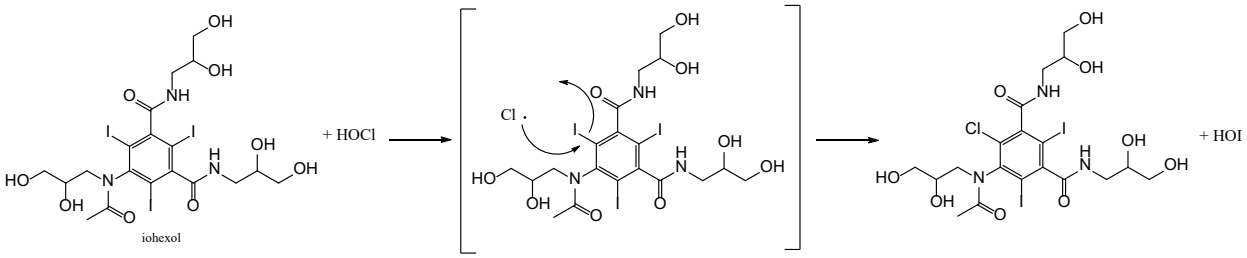

Figure 1-5: Proposed halogen-substitution reaction of iohexol with HOCl for HOI formation (based on Wendel et al. 2016).

Conventional drinking water treatment does not have a removal mechanism that specifically targets inorganic or organic iodine. In order to determine if an existing treatment option can be optimized for iodine removal, the changes in their concentration and species needs to be determined for each unit process within a treatment plant.

1.6 Toxicological Studies

Inorganic and organic precursors have been shown to change the types of DBPs formed and consequently impact the overall toxicity of the DBPs formed (Richardson, 2008; Hua, 2008). After the first DBPs were identified in treated drinking water (Rook, 1974; Symons et al., 1975), investigations began on determining their health effects (Keysser, 1976). Exposure to DBPs occurs at low concentrations for extended periods of time, with chemicals present in mixtures that have multiple exposure routes (Villanueva et al., 2015). Most epidemiological studies correlating DBP exposure to a cancer endpoint only focus on the exposure to the regulated THMs and HAAs, even though many more DBPs are present in the complex mixture (Arbuckle et al., 2002; Cantor et al., 1998; Hildesheim et al., 1998; Hoffman et al., 2008; MacLehose et al., 2008; Morris et al., 1992; Wang et al., 2007). The exposure routes to DBPs include ingestion of disinfected drinking water, dermal absorption and inhalation from bathing and swimming.

Epidemiological studies have indicated a weak correlation between chlorinated DBPs and various types of human cancer, with bladder, colon, and rectal cancers being the primary disease endpoints (Cantor et al., 1998; Hildesheim et al., 1998; Villanueva et al., 2007). I-DBPs have not been included in epidemiological studies due to the lack of occurrence data.

According to Plewa et al. (2004), I-DBPs are over 250 times more cytotoxic than the regulated chloroacetic acid and so the US EPA has identified these compounds as DBPs of emerging toxicological interest (Richardson, 2003). One cytotoxicity assay that has been extensively used in assessing individual DBPs is the Chinese Hamster Ovary (CHO) cell assay (Plewa et al., 2012; Yang et al., 2014). Even though the CHO assay uses reproductive cells that are not the target organ for carcinogenesis and do not undergo metabolism while *in vitro* (Plewa and Wagner, 2009), there is valuable information that can be gained from using the mammalian cells for cyto- and genotoxicity tests. In a comparison of LC_{50} from the CHO assay and a human colon cell assay, DeAngelo and others showed significant correlation using the Pearson coefficient between single chemical haloacetimides, haloacetonitriles, halonitromethanes, and haloacetic acids tested in the two separate assays (DeAngelo, 2013; DeAngelo et al., 2007; Lyon et al., 2014). The individual LC_{50} data from the CHO assay have also been used to identify the forcing agents that may be driving the toxicity of the DBP mixtures, based on their concentrations and assuming additive properties (Krasner et al., 2016; Zeng et al., 2016).

Based on the relative cytotoxicity observed in the CHO assay, I-DBPs are significantly more toxic than brominated DBPs and both iodinated and/or brominated DBPs are significantly more cytotoxic than chlorinated DBPs (Wagner and Plewa, 2017). For example, unregulated dichloroiodomethane (DCIM) is almost 4 times more cytotoxic than regulated TCM based on the comparison of the LC_{50} established by the CHO assay.

The cytotoxicity data from the CHO assay can also be used to compare the theoretical toxicity of a mixture using single chemical LC_{50} values and their respective molar concentrations, assuming additive properties. Similar to the National Research Council's risk assessment of potable water reuse (National Research Council, 2012), the sum of the products of each individual DBP total ion count (TIC) for an concentration and the individual LC_{50} value for toxicity (TIC-Tox) technique is a powerful theoretical tool for predicting how the toxicity of mixtures will change based on the individual species present (Plewa et al., 2017; Wendel et al., 2014; Yang et al., 2014). More research is needed to focus on the formation and occurrence of I-DBPs, since there is compelling evidence that there may be elevated biological effects from human exposure to them even though there is very limited information available on their occurrence or formation potentials in drinking water treatment plants.

1.7 Research Gaps

Due to the human and environmental importance of I-DBPs and their formation during disinfection, the origin of iodine in source waters should be examined so that approaches for limiting the impact on drinking water quality can be controlled. The current gaps in I-DBP research include 1) the need for sample preparation and instrumental methods for extracting and detecting iodoacids at their level of occurrence with other haloacetic acids at high concentrations; 2) the lack of source tracking information on organic iodine in the environment and its impact on drinking water quality; and 3) the need to identify and optimize drinking water treatments for I-DBP precursor removal.

As for the iodine precursors for I-DBP formation, a direct link between ICMs, their biotransformed derivatives and I-DBP formation has not yet been established in a drinking water

treatment plant downstream of the wastewater discharge. By only looking at parent ICMs, there may be potential I-DBP precursors that are not accounted by measuring only the parent ICM but an aggregate concentration of total dissolved organic iodine (TDOI) may provide more information for predicting I-DBP formation potential in finished drinking water.

The fate of organic iodine once it enters a drinking water treatment plant is not clearly understood except for the increased potential for I-DBP formation in the finished water. In order to determine which treatment option to optimize for total dissolved iodine (TDI) removal, the changes in concentration and species needs to be determined for each unit process within a treatment plant. Additional information on TDI changes will help optimize treatment for increased removal before it can transform into cytotoxic I-DBPs and decrease exposure for increased public health protection.

Addressing these knowledge gaps in I-DBP research can move the field of drinking water quality and public health forward through method development, source identification, and removal optimization.

1.8 Research Hypotheses

The central hypothesis of this research is that organic iodine from medical waste can be precursors for I-DBPs in downstream drinking water treatment plants. To test this central hypothesis, the following specific hypotheses were pursued:

1. A robust and reproducible analytical method for the quantification of iodoacids from drinking water can be developed using a multi-step extraction and analysis by gas chromatography mass spectrometry (GC/MS).

2. Surface waters receiving treated hospital waste effluent can show elevated levels of iodine and drinking water treatment plants using these impacted surface waters may generate I-DBPs.

3. An existing unit process within the drinking water treatment plant can be optimized for iodine precursor removal to limit iodine incorporation into DBPs and reduce the theoretical relative cytotoxicity in finished water.

Figure 1-6 shows the relationships and step-wise investigation between the central hypothesis and the three individual hypotheses.

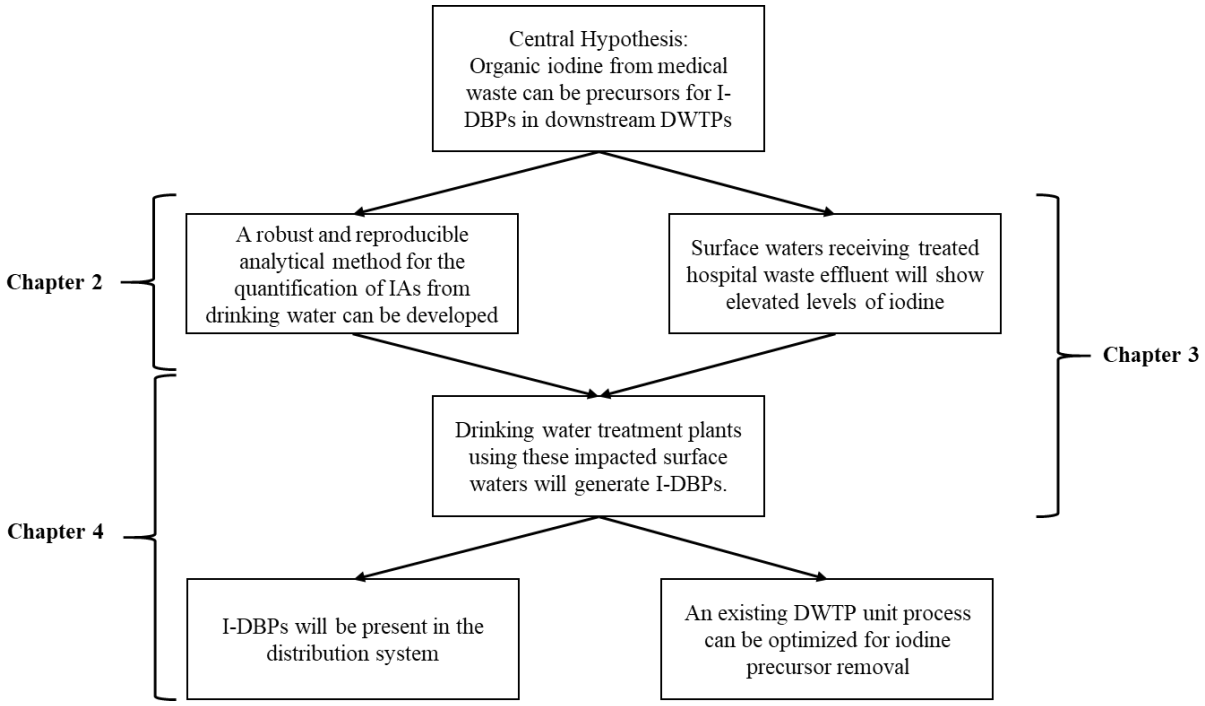

Figure 1-6: Hypothesis structure for the investigation of organic iodine from medical waste as precursors for I-DBPs in downstream drinking water treatment plants.

1.9 Research Objectives

The overall goal of this research is to advance our understanding of iodinated disinfection byproduct formation in drinking water in order to limit public exposure. In support of this overall research goal, the following specific objectives were pursued:

Objective 1: Development of a sensitive and quality assured method for the extraction of iodoacids from drinking water matrices for quantification;

Objective 2: Determination of whether the quality of surface water receiving wastewater effluent is affected by hospital waste, which may contribute iodine to drinking water sources which may ultimately form iodinated DBPs after disinfection;

Objective 3: Identification and optimization of the treatment strategy for TDI removal with powdered activated carbon addition at the drinking water plant and evaluation of the changes to TDI speciation throughout the drinking water treatment plant.

1.10 Dissertation Organization

This dissertation is comprised of five chapters, three of which are written as standalone papers for publication. Chapters 2, 3, and 4 represent original unpublished research.

Objective 1 is addressed in Chapter 2 and is being prepared for publication under the title "Sensitive Determination of Iodoacids from Drinking Water Using Gas Chromatography-Mass Spectrometry," with co-author Howard S. Weinberg.

Objective 2 is presented in Chapter 3 and a publication is currently being prepared with the title "Iodine source tracking in surface waters receiving wastewater effluent: Implications for iodinated disinfection byproducts in downstream drinking water production" with coauthors Christian Zwiener and Howard S. Weinberg.

Objective 3 is addressed in Chapter 4 and a publication is currently being prepared with the tentative title of "Tracking the transformation of total dissolved iodine within a drinking water treatment plant: Can PAC be used to limit I-THM formation?" with coauthors Christian Zwiener and Howard S. Weinberg.

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CHAPTER 2 : SENSITIVE DETERMINATION OF IODOACIDS FROM DRINKING WATER USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

2.1 Introduction

When using chemical oxidants for drinking water disinfection, they react with natural organic matter (NOM), anthropogenic pollutants, and inorganic contaminants in the water to form disinfection byproducts (DBPs). In the presence of bromide and/or iodide, the speciation among DBPs can change depending on the disinfectant. For example, chloramine can react with iodide to form iodine-containing DBPs (Bichsel and von Gunten, 1999). Inorganic iodine in U.S. surface waters are reported in the 0.01 to 73.3 μg/L range originating from geologic formations (Fuge and Johnson, 1986; Weinberg et al., 2011), hydraulic fracturing (Harkness et al., 2015), seawater intrusion (Oh et al., 2010), atmospheric depositions (Fuge and Johnson, 1986), and manufacturing waste (Nguyen et al., 2012; Pal and Nayak, 2016). Organic iodine sources from medical facility waste (Kormos et al., 2011; Richardson et al., 2008; Steger-Hartmann et al., 1999), enzymatic halogenation (Blasiak and Drennan, 2009), and livestock operations (Hladik et al., 2016) also add to the total iodine load in surface waters and there is evidence that they provide iodine as a DBP precursor (Duirk et al., 2011).

Iodinated disinfection byproducts (I-DBPs) are associated with chloramine disinfection of source drinking water containing iodide and NOM, with a shorter contact time increasing the formation (Bichsel and von Gunten, 2000, 1999). The path of formation for I-DBPs includes the oxidation of iodide to form hypoiodous acid (HOI), which can either be further oxidized to iodate (IO_3) or react with NOM to form I-DBPs (Bichsel and von Gunten, 1999). The

incorporation of iodide into DBPs has not been as widely studied as bromide but could provide insight into the formation of I-DBPs based on the source water iodide concentrations (Obolensky and Singer, 2005). One specific occurrence study of 23 U.S. cities measured I-DBPs, including iodoacids, in disinfected drinking waters despite low or non-detectable iodide concentrations in the source water and a follow-up study implicated medical imaging compounds reacting with the drinking water disinfectant chloramine to form I-DBPs (Duirk et al., 2011; Richardson et al., 2008).

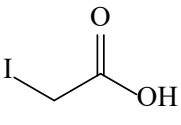
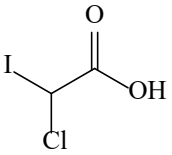
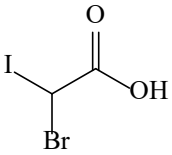
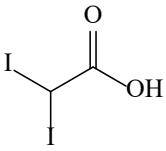
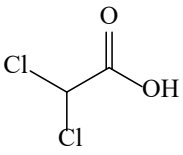
Iodoacids (IAs) are one group of I-DBPs and a subset of all haloacetic acids (HAA), nine of which (HAA9) contain either chlorine, bromine, or both halogens and only five of which (HAA5) (including chloroacetic acid) are regulated in drinking water in the US (US EPA, 2006). According to Plewa et al. (2004), I-DBPs are over 250 times more cytotoxic than the regulated chloroacetic acid (Plewa et al., 2004) and so the U.S. Environmental Protection Agency (US EPA) has identified these compounds as DBPs of emerging toxicological interest (Richardson, 2003).

Existing and routinely used methods for HAA extraction from drinking water focus on the chlorinated and brominated species (HAA9: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, bromoacetic acid, dibromoacetic acid, tribromoacetic acid, bromochloroacetic acid, bromodichloroacetic acid, and chlorodibromoacetic acid), since they are typically present at low μg/L levels in drinking water (Ells et al., 2000; Magnuson and Kelty, 2000; Sarrión et al., 2000). US EPA Method 552.2 quantifies the regulated HAA5 using a 5-fold concentration factor by liquid-liquid extraction (LLE) and analysis of the extract by gas chromatography with electron capture detection (GC-ECD) (Domino et al., 2003). Since the levels of iodide in natural waters are typically lower than bromide (Jones et al., 2012; Watson et

al., 2012), iodoacids formed from the reaction of chloramine with iodine need a much greater concentration factor for environmentally relevant concentrations to be detected.

Although the iodoacids can be detected on a GC-ECD, a concentrated extract of chloraminated drinking water will contain a mixture of IAs and HAA9, the latter at orders of magnitude higher concentration making resolution of IAs from them highly challenging. The physiochemical similarities among haloacetic acids and the iodoacids sub-set preclude an extraction method that could isolate this specific subgroup. Table 2-1 compares the physiochemical properties of four iodoacids (IA4) with dichloroacetic acid, one commonly occurring HAA, to demonstrate this. For example, all are highly water-soluble and will be present in their ionic form at the pH of disinfected water (pH of 6 to 8). Based on the differences in boiling points of the methyl ester derivatized acids (Table 2-1); however, there is the ability to chromatograph the analytes despite their other physiochemical similarities.

Table 2-1: Physiochemical properties of 5 HAAs relevant in method development for analysis of iodoacids in drinking waters.

	Iodoacetic Acid (IAA)	Chloroiodoacetic Acid (ClIAA)	Bromoiodoacetic Acid (BrIAA)	Diiodoacetic Acid (I_2AA)	Dichloroacetic Acid (Cl_2AA)
CAS Number	64-69-7	53715-09-6	71815-43-5	598-89-0	79-43-6
Structure					
Molecular Formula	$C_2H_3IO_2$	$C_2H_2ClIO_2$	$C_2H_2BrIO_2$	$C_2H_2I_2O_2$	$C_2H_3Cl_2O_2$
Molecular Weight (g/mol)	185.95	220.39	264.84	311.85	128.94
Boiling Point (°C)*	262.1±23.0	267.4±25.0	272.1±25.0	320.1±32.0	201.1
Methyl Ester Boiling Point (°C)**	163.7	195.5	217.3	244.3	140.9
Calculated pK_a*	3.18±0.10	1.47±0.10	1.67±0.10	2.24±0.41	1.37±0.10
Log K_{OW}**	0.85	1.03	1.12	1.53	0.52
Water Solubility (mg/L)	$2.43x10^4$	$1.13x10^4$	$5.41x10^3$	$1.29x10^3$	$2.74x10^5$
Dipole Moment (D)***	1.91	1.87	1.81	1.86	1.99

*Scifinder, (Scifinder Scholar, 2017); **Calculated by EPI Suite (US EPA, 2017); ***Calculated by Chem3D (Cambridge Software, PerkinElmer, Cambridge, MA, USA)

Occurrence data on iodoacids are rare due to the absence of a validated analytical method in a drinking water matrix. Various groups of I-DBPs have been identified by either broad screening methods without validated quantitative results or by targeted compound analysis (Krasner et al., 2006; Liu et al., 2013; Plewa et al., 2008; Postigo et al., 2017; Richardson et al., 2008; Shi and Adams, 2009). While these methods can provide some quantitative information on

iodoacids, they do not have high enough concentration factors or appropriate chromatographic methods needed for simultaneous extraction and quantification of HAA9 and IA4 in a single sample. Positive confirmation of the presence of iodine in the chromatographic peaks during quantification is also necessary to ensure correct DBP identification during method validation. Moreover, the EPA and other methods developed for HAA5 and HAA9 (Domino et al., 2003; Richardson et al., 2008) do not account for the unstable nature of iodoacids throughout collection, preservation, and extraction and are, therefore not suitable for IA analysis.

This chapter presents a fully quality assured method that successfully extracts both IAs and HAA9 from a model and drinking water at orders of magnitude difference between their concentrations and successfully chromatographs 13 species. Four iodoacids, namely iodoacetic (IAA), chloroiodoacetic (ClIAA), bromoiodoacetic (BrIAA), and diiodoacetic acids (I_2AA) were selected for this method development based on their occurrence levels observed in a limited U.S. survey (Weinberg et al., 2002).

2.2 Experimental Section

2.2.1 Materials

Laboratory grade water (LGW) was prepared in-house from a Dracor system (Durham, NC, USA), which pre-filters inlet 7 MΩ-cm deionized water to 1 μm, quenches residual disinfectants, reduces total organic carbon to less than 0.2 mg as C/L with activated carbon, and removes ions to 18 MΩ-cm with mixed bed ion-exchange resins. Sampling and extraction glassware were soaked for 8 hrs in a 5% nitric acid solution, triple-rinsed with LGW, and baked in an 180ºC oven until dry. All chemicals and materials used for the analysis of iodoacids in drinking water are listed with their sources and purity in Table 2-2.

Table 2-2: Materials for the analysis of iodoacids.

Chemical/Material	Grade/Purity/Concentration	Supplier
2,3-dibromopropionic acid (internal standard)	Supelco, 1000 µg/mL in MtBE	Sigma-Aldrich (St. Louis, MO, USA)
2-bromobutyric acid (acid surrogate)	Supelco, 1000 µg/mL in MtBE	Sigma-Aldrich (St. Louis, MO, USA)
Ammonium hydroxide (NH_4OH)	28-30%, certified ACS	Fisher Scientific (Pittsburgh, PA, USA)
Ammonium sulfate ($(NH_4)_2SO_4$)	99.5%	Mallinckrodt (Staines-upon-Thames, UK)
Ascorbic acid ($C_6H_8O_6$)	SigmaUltra, ≥99%	Sigma-Aldrich (St. Louis, MO, USA)
Bromoiodoacetic acid (BrIAA)	85-90%	CanSyn (Toronto, Ontario, Canada)
Chloroiodoacetic acid (ClIAA)	90-95%	CanSyn (Toronto, Ontario, Canada)
Carbitol (Di(ethylene glycol) ethyl ether)	≥99%	Sigma-Aldrich (St. Louis, MO, USA)
Diazald (N-Methyl-N-(p-tolylsulfonyl)nitrosamide)	99%	Sigma-Aldrich (St. Louis, MO, USA)
Diiodoacetic acid (I_2AA)	>95%	CanSyn (Toronto, Ontario, Canada)
EPA 552.2 Acids Calibration Mix	Supelco, 200-2000 µg/mL in MtBE	Sigma-Aldrich (St. Louis, MO, USA)
Chloroacetic acid (ClAA)	614.0 µg/mL	
Dichloroacetic acid (Cl_2AA)	596.6 µg/mL	
Trichloroacetic acid (Cl_3AA)	199.3 µg/mL	
Bromoacetic acid (BrAA)	407.9 µg/mL	
Dibromoracetic acid (Br_2AA)	200.6 µg/mL	
Tribromoacetic acid (Br_3AA)	1962 µg/mL	
Bromochloroacetic acid (BrClAA)	400.0 µg/mL	
Bromodichloroacetic acid ($BrCl_2AA$)	375.3 µg/mL	
Chlorodibromoacetic acid ($ClBr_2AA$)	1009 µg/mL	

Table 2-2 continued		
Filters for real water samples	0.45 µm nylon membrane	Fisher Scientific (Pittsburgh, PA, USA)
Iodoacetic acid (IAA)	Puriss p.a. ≥99.5%	Sigma-Aldrich (St. Louis, MO, USA)
Magnesium sulfate ($MgSO_4$)	Anhydrous, powder, ≥98%	Fisher Scientific (Pittsburgh, PA, USA)
Methanol	Chromasolv®, 99.9%	Sigma-Aldrich (St. Louis, MO, USA)
Methyl tert-butyl ether (MtBE)	Chromasolv®, 99.9%	Sigma-Aldrich (St. Louis, MO, USA)
Nitric acid (HNO_3)	Certified ACS plus, 70%	Fisher Scientific (Pittsburgh, PA, USA)
Potassium hydroxide (KOH)	45% w/w	Fisher Scientific (Pittsburgh, PA, USA)
Potassium iodide (KI)	99.0%, certified ACS	Sigma-Aldrich (St. Louis, MO, USA)
Potassium bromide (KBr)	99%, certified ACS	Sigma-Aldrich (St. Louis, MO, USA)
Silicic acid (H_4O_4Si)	Purum p.a ≥99%	Sigma-Aldrich (St. Louis, MO, USA)
Sodium hydroxide (NaOH)	Pellets, certified ACS, ≥97%	Fisher Scientific (Pittsburgh, PA, USA)
Sodium hypochlorite (NaOCl)	5.65-6%	Fisher Scientific (Pittsburgh, PA, USA)
Sodium sulfate (Na_2SO_4)	Anhydrous, granular, 99.3%	Fisher Scientific (Pittsburgh, PA, USA)
Sodium sulfite (Na_2SO_3)	99%, certified ACS	Fisher Scientific (Pittsburgh, PA, USA)
Sulfuric acid (H_2SO_4)	Certified ACS plus, 95-98%	Fisher Scientific (Pittsburgh, PA, USA)
Strata Florisil (FL-PR) cartridges	500 mg / 3 mL	Phenomenex (Torrance, CA, USA)

2.2.2 Model Waters for Stability Tests

The model water was prepared from a diluted NOM concentrate (112 mg/L as C) produced by reverse osmosis (RO) of a surface water with mid-range dissolved organic carbon (DOC) of 7.3 mg/L as C.

A RO system (schematic shown in Figure 2-1 and operating procedures in Appendix C) comprised a series of filters, a cation-exchange cartridge (CG8-H Cation Exchange Resin,

ResinTech, West Berlin, NJ, USA), two pump and pipe systems for raw and filtered water, respectively, and the RO membrane. It was operated by first collecting raw water (University Lake, Chapel Hill, NC, USA) in the reservoir using a hose connected to a tap and pipe that directly connects to the raw water source. Using pump 1, the raw water was then filtered through filters 1, 2 and 3 (10, 5 and 1 µm, respectively, Filtration Solutions, Lake Wylie, SC, USA) before passing through a hydrogen-saturated cation exchanger. The water was then filtered again (filter 4, 0.45 µm) and collected in the second reservoir. Using pump 2, the filtrate was pumped through a spiral-wound polyamide thin-film composite RO membrane (FILMTEC^{TM} XLE-4021 Membrane, DOW Chemical Company, Midland, MI, USA). While the retentate was recycled back into the second reservoir, the RO filtrate was returned to the source water.

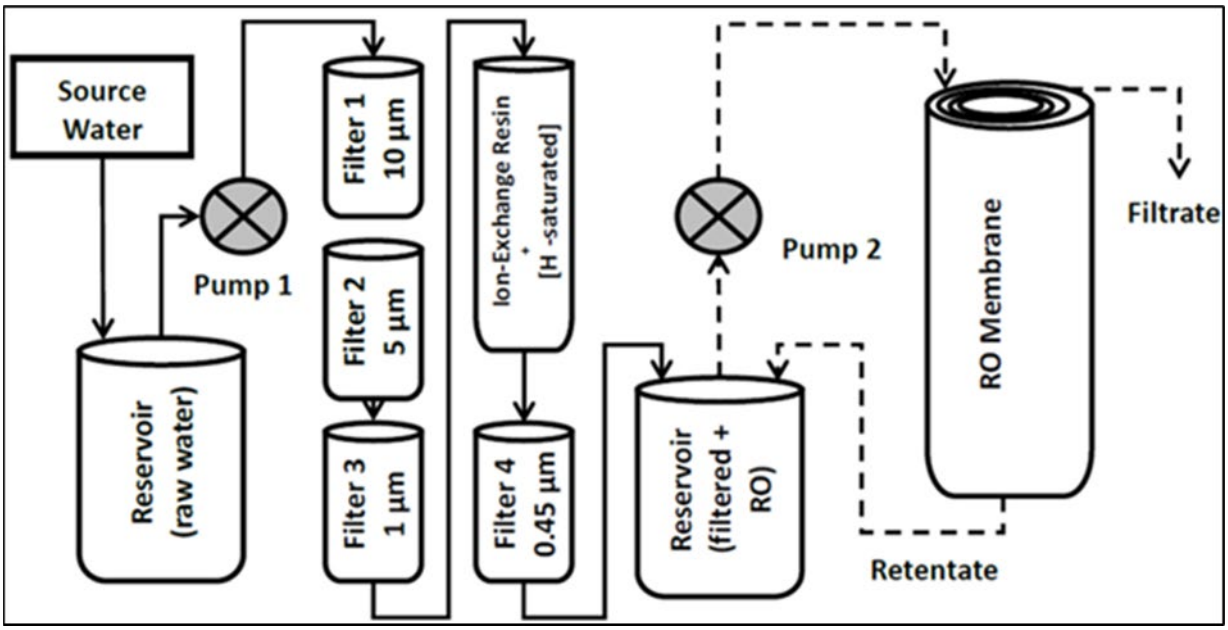


Figure 2-1: Schematic of the reverse osmosis system.

For the IA4 stability tests, the model water was diluted to 5 mg/L DOC as C and spiked with the inorganic anions iodide (200 µg/L) and bromide (1000 µg/L). The effects due to the

presence of quenched chloramines at 4 mg/L as Cl_2 were also determined by adding the stochiometric amount of quenching agent ascorbic acid (9.9 mg). Additional discussion of ascorbic acid as a quenching agent is presented in the Results and Discussion Section 2.2.7. Monochloramine was produced by adding ammonium sulfate to free chlorine in a Cl_2/N weight ratio of 3/1. The target analyte concentrations used for the stability tests were 20 ng/L IAA, 14 ng/L each of ClIAA and BrIAA, 8 ng/L I_2AA and between 10 to 50 µg/L HAA9 (based on the concentration range in the Supelco EPA 552.2 calibration mix).

2.2.3 Method Detection Limit

To determine the method detection limit (MDL) and the practical quantification limit (PQL) for each of the IA4 species in the presence of HAA9, eight-replicate real-water matrixes (DOC of 5 mg/L as C) from the NOM concentrate and spiked at the same IA4 and HAA9 levels as the stability tests described in Section 2.2.2. were extracted and analyzed by GC-ECD and gas chromatography negative chemical ionization mass spectrometry (GC-NCI-MS) verified the peak retention times and the presence of iodine (described in Section 2.3.2). The same acid surrogate (2-bromobutyric acid) concentration (100 ng/L) was added to all samples prior to extraction to monitor the extraction process and <30% variation in peak-area was targeted for all analyzed samples to meet the quality control criteria. The internal standard (pre-derivatized 2,3-dibromopropionic acid, methyl ester) was added to the final extract to account for any variations during the sample injection. Triplicate standrad calibration curves for the MDL analysis using 0, 20, and 50 ng/L for each IA4. The MDL for each iodoacid species was calculated by multiplying the standard deviation of the mean relative area (analyte absolute area/internal standard absolute area) for the analyzed sample set with the Student t-value apropriate for a 99% confidence level

according to the US EPA method (US EPA, 1986). The PQL was calculated from the analyte mean relative area with a signal to noise (S/N) of 10 to prevent random error.

The instrument detection limit (IDL) for gas chromatography positive electron ionization mass spectrometry (GC-PEI-MS) (discussed in Section 2.3.1) was determined using 8 replicte injections of an IA4 mixture in MtBE with each analyte at 46.6 μg/L (based on the extraction concentration factor of 35 ng/L) and the internal standard at 100 μg/L. This concentration was selected based on S/N ratio of >10. The IDL for each iodoacid species on the GC-PEI-MS was calculated using the same method as previously decribed for the MDL and outlined by Parra and Taylor (2014).

2.2.4 Drinking Water Treatment Plants Sampling

Finished drinking water samples were collected from seven drinking water treatment plants. Plants 1 to 6 were selected based on the National Occurrence Study, which found I-DBP formation in the finished waters (Weinberg et al., 2002). Plant 7 was selected because of its highly-impaired source water due to three upstream wastewater treatment plants and whose source water contained organic iodine (approximately 10 μg/L as I). Samples were collected in 1 L bottles containing ascorbic acid as the quenching agent, then kept at 4°C until extraction within 48 hrs. Duplicate samples were extracted with IA4 standard addition levels at 20 to 100 ng/L.

2.2.5 Source Water Quality Assessment of Drinking Water Treatment Plants

The source water from the seven drinking water treatment plants outlined in Section 2.2.4 (Plants 1-7) were sampled for bromide, iodine, and TOC. Total dissolved iodine was determined from a 0.45 μm-filtered (Fisher Scientific, Pittsburgh, PA, USA) aqueous sample by targeting the

127 mass per charge (m/z) ion on the ICP-MS in no-gas mode with a 100 µg/L tellurium (125 m/z) internal standard (Takaku et al., 1995). Samples were made basic with 5% ammonium hydroxide (Fisher Scientific, Pittsburgh, PA, USA) to increase iodine signal stability. Bromide and total organic carbon (TOC) were determined using Standard Method 4110 and 5310, respectively (APHA, 1999).

2.2.6 Drinking Water Formation Potential

24 L of settled water (pH 7.5 and DOC 1.1 mg/L as C) from a conventional drinking water treatment plant using the same source water as outlined in Section 2.2.2 were collected before the filtration and disinfection processes. Bench-scale DBP formation potential reactions using real waters and a controlled disinfectant dose simulate the conditions for iodoacid formation and can, therefore, be used to demonstrate the applicability of this analytical method in the practice of drinking water treatment. The collected water was filtered in 1 L batches through 0.45 µm nylon filters using a Buchner filtration system and the filtrates combined for a single homogenous sample. Six 4-L acid-washed amber bottles with Teflon-lined septa and caps were used as reaction vessels for chloramination with potassium iodide spikes at 15, 50, and 100 µg/L as I^-; with one 4-L bottle each for 15 and 50 µg/L as I^- spiked samples and four 4-L bottles for the standard addition using the 100 µg/L as I^- spiked sample for quantitation. These levels of iodide were selected based on iodide occurrence data in surface water used as a drinking water source (Richardson et al., 2008) and its PQL for this new method. The monochloramine dose was the same for each reaction vessel at 3.0 mg/L as Cl_2 with an $N:Cl_2$ weight ratio of 1:3. At the end of the 24 hr contact time, each reaction vessel was quenched of any remaining disinfectant using 9.9 mg of ascorbic acid. Duplicate samples from each bottle were extracted with standard

addition duplicates taken from the four bottles spiked with 100 µg/L as I^-, with the IA4 levels at 20, 35, 50, and 100 ng/L. Prior to extraction, 1-L amber sample bottles were filled with quenched water, then chilled for at least 4 hr at 4˚C (see Figure 2-2 for Experimental Design Schematic). Total organic iodine (TOI) was measured in the spiked, chlorinated samples by fractionation of total organic halogens into TOI using the method of adsorption, pyrolysis, and collection (Hua and Reckhow, 2006) followed by quantitation with the ICP-MS.

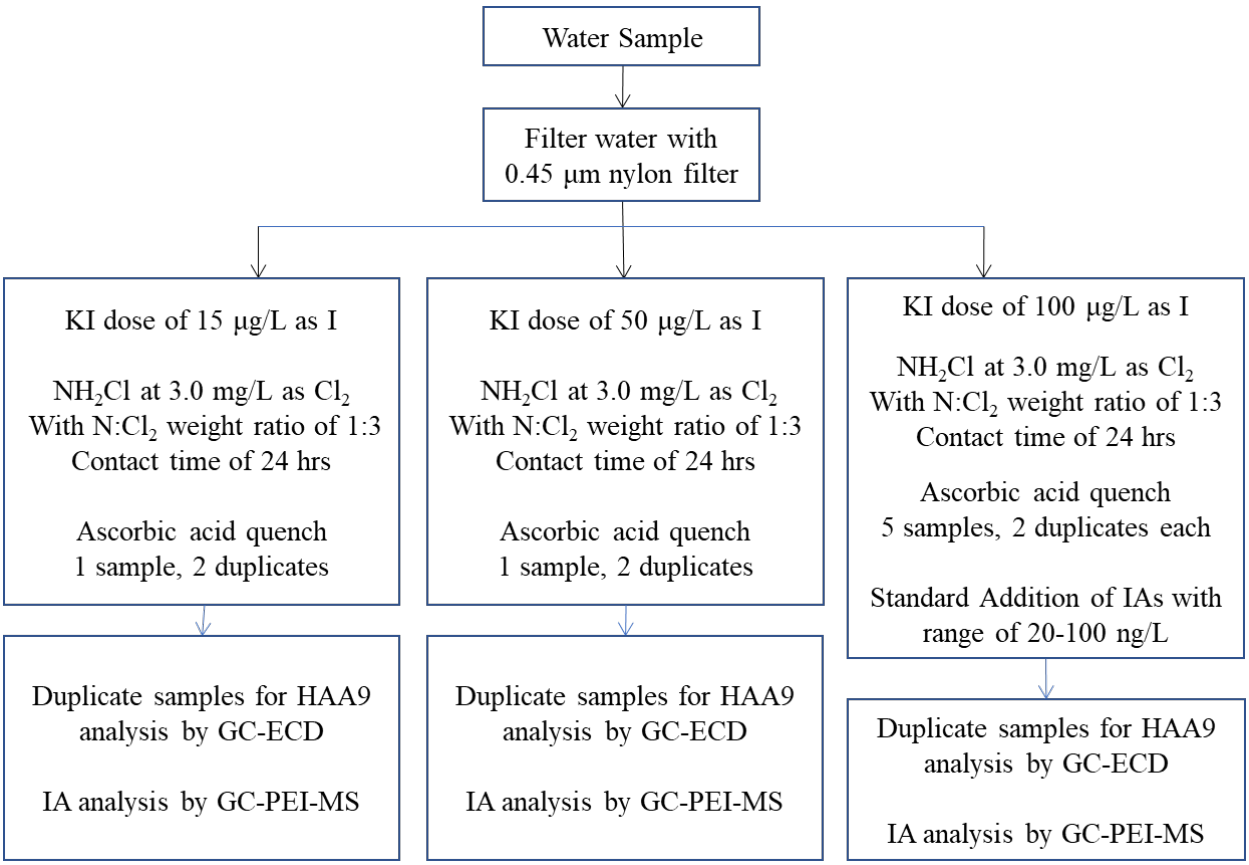


Figure 2-2: Experimental schematic for iodoacid (IA) formation potential in drinking water where KI is potassium iodide, NH_2Cl is monochloramine, HAA9 refers to chlorinated- and brominated-haloacetic acids, GC-ECD is gas chromatography electron capture detector, and GC-PEI-MS is gas chromatography positive electron ionization mass spectrometry.

2.2.7 Dechlorinating Agent

US EPA Method 552.3 calls for ammonium chloride (NH_4Cl) as the dechlorinating or quenching agent of disinfected drinking water for HAA9 analysis. However, NH_4Cl does not remove chloramines and will even form chloramines if used to remove residual HOCl (Domino et al., 2003). Ascorbic acid will form ammonium chloride and dehydroascorbic acid when monochloramine is quenched as shown in Figure 2-3 (Urbansky et al., 2000). To ensure any residual disinfectant is reduced and DBP formation stopped, a stochiometric amount of ascorbic acid was added based on the residual oxidant level. This approach was confirmed by selecting several drinking water samples and adding an equivalent ratio of quenching agent to an extra aliquot of that sample, which resulted in no measurable residual chlorine or chloramine. Stability tests for IA4 were performed over a 7-day period in a drinking water matrix with chloramine quenched by ascorbic acid.

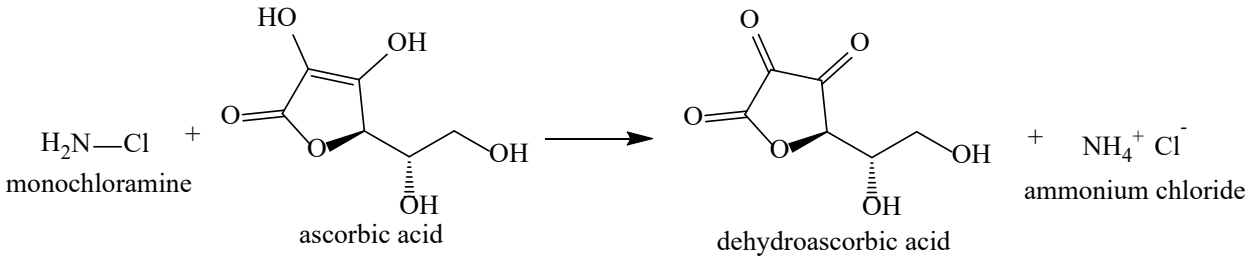


Figure 2-3: Quenching of monochloramine with ascorbic acid.

2.2.8 Drinking Water Treatment Plant Survey

To further validate the IA4 method, seven drinking water treatment plants were surveyed for iodine in their source water and iodoacids in the finished water. Total iodine and total bromide for the source waters were determined by targeting ions at m/z 80 and 127, respectively on an ICP-MS in no-gas mode with a 100 µg/L tellurium (125 m/z) internal standard (Takaku et

al., 1995). The TOC of the finished water from each plant was measured following Standard Method 5310 after quenching with sodium sulfite (APHA, 1999).

2.2.9 Extraction Materials

After many iterations and evaluations of methodological approaches, a multistep approach was developed that allowed for simultaneous increase in concentration factor without increasing detector signal interference or baseline noise. The extraction for iodoacids required a 1L aqueous sample and adapted a combination of general approaches for the analysis of polar analytes of low molecular weight (Weinberg et al., 2011). In preparation for extraction, the 1L sample is spiked with 100 ng/L 2-bromobutyric acid (acid surrogate) so that the entire analytical method can be monitored for each individual sample. The sample is then split into two 500 mL aliquots placed in 1 L amber bottles with Teflon-lined septa to which approximately 30 mL of concentrated sulfuric acid (18.4 M H_2SO_4) is added to obtain a pH below 0.5 to ensure that the target analytes are in their protonated form. 100 g anhydrous sodium sulfate is then added to improve transfer of the HAAs to 100 mL of the methyl tert-butyl ether (MtBE) extraction solvent and an immediate two-minute liquid-liquid extraction (LLE) is undertaken by shaking the bottles contents. The sample was then transferred to a 1 L separatory funnel and the layers were allowed to separate for 5 min before the aqueous phase was drained to waste. The use of the separatory flask allowed for a clear visual of the layers and provided more control for separating the aqueous phase compared to the amber bottle. The MtBE from each of the two extractions were then combined in a 250 mL amber bottle with a Teflon-lined septum and dried using anhydrous magnesium sulfate.

The combined MtBE extract was then loaded onto a preconditioned 500 mg Strata Florisil solid phase cartridge at 1.5 mL/min using a manually operated 24 port Visiprep^{TM} solid phase extraction (SPE) manifold (Sigma-Aldrich, St. Louis, MO, USA) with appropriate connectors and Teflon transfer lines. This was to retain and concentrate the target analytes on the SPE cartridge and allow the impurities from the first LLE step that were not absorbable to the solid phase to drain to waste. Throughout the loading, the sample bottles and SPE cartridges were shielded from the light to protect the iodine-containing compounds from photolyzing (Gottardi, 2001). After washing each cartridge with 6 mL of MtBE containing 10% methanol and drying the cartridges under vacuum for 10 min, 3 mL of acidic methanol (90 % methanol : 10 % concentrated sulfuric acid) was used to elute the retained analytes under vacuum to maximize solvent recovery. Previous attempts to methylate the iodoacids using acidic methanol did not produce evidence of methyl esters by GC-ECD or GC-PEI-MS and the loss of analytes is most likely due to decomposition from prolonged exposure to elevated temperature and light. Elution with acidic methanol improved the recovery of all four IAs when compared to methanol. The eluent was back-extracted into 1 mL MtBE containing 100 µg/L of 2,3-dibromopropionic acid, methyl ester as internal standard (IS) in the presence of 7 mL of a super saturated sodium sulfate solution. The mixture was then vortexed in a 15 mL graduated centrifuge tube for two minutes. The top 4 mL of solvent was removed then anhydrous magnesium sulfate was added to remove water and sodium bicarbonate was added to neutralize the solution. The solution was mixed and allowed to settle before transferring 500 µL of the MtBE to a new vial for subsequent derivatization of the recovered halo-acids. Since methanol is completely miscible in water and MtBE is only sparingly soluble, there will be minimal methanol in the top layer of MtBE after separation has occurred.

2.2.10 Derivatization

(Safety Considerations: Diazomethane is a strong respiratory irritant and potential explosive hazard. Derivatization work should be conducted in a laboratory hood at all times.) Conversion of the extracted acids into their methyl esters prior to GC analysis was required to increase their volatility and lower their polarity. Although HAA9 can reach close to 100% methylation when extracted from water and derivatized with diazomethane after 15 min (Brophy et al., 2000), a reaction time of 1 hour was used in this new method to account for the potential hindering effected of the larger iodine atom (1.33 Å diameter) compared to both chlorine (0.99 Å) and bromine (1.14 Å). 250 µL freshly prepared yellow diazomethane (see Appendix D for generation details) was added to each 500 µL sample extract in a clear 4 mL glass vial and the reaction allowed to proceed for 1 hr at 4ºC in the dark. Then, after equilibrating to room temperature and ensuring the presence of a yellow color to indicate that sufficient derivatizing agent had been added, excess diazomethane was quenched using 10-20 mg of silicic acid which was indicated by the final colorless extract. The extracts were then transferred to 2 mL amber autosampler vials with 250 µL inserts and stored at -15 ºC until analysis. The sample preparation and extraction methods are summarized in Figure 2-4 and shows a concentration factor of 1333. Since the four IAs are present in drinking water at concentrations orders of magnitude lower than HAA9, the large concentration factor was needed to ensure detection.

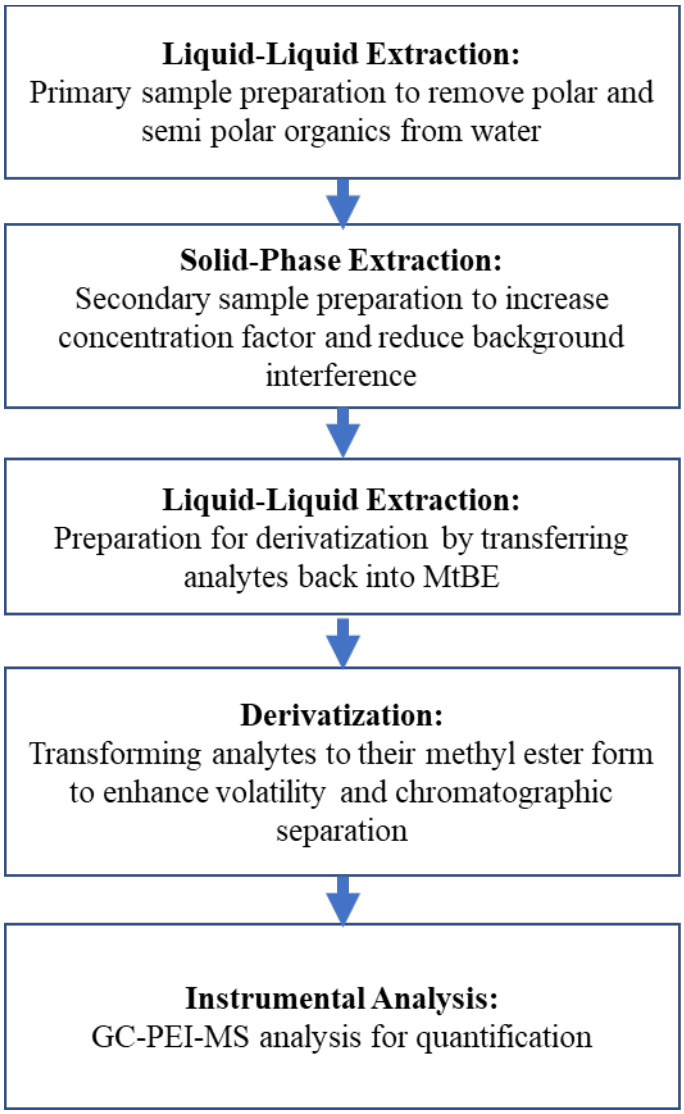


Figure 2-4: Simplified flowchart for iodoacids extraction and concentration from water.

2.2.11 Standard Addition

Standard addition of the four iodoacids to aqueous samples accounted for variable extraction efficiency across different matrices. Calibration curves were constructed for the 4 IAs by spiking each analyte into the quenched drinking water matrix at the same concentrations ranging from 20 to 100 ng/L and extracting along with the unspiked sample. For quantification of iodoacids in the sample, the mean relative area was obtained from the absolute area of each

analyte divided by the absolute area of the internal standard (2,3-dibromoproprionic acid, methyl ester) from the same chromatogram. The challenge with this approach when analyzing samples from different water types is to know with some degree of certainty that some waters will have similar matrix effects and allow for the calibration in one type of sample to be used for determination of analyte concentration in another sample. The addition of the surrogate acid standard (2,3-dibromopropionic acid) and an examination of its analytical response across various water samples can be used to confirm the IA4 extraction recovery between different matrixes. If the acid surrogate recovery changes (>30%) between sample types, then standard addition curves are needed for each matrix.

2.3 Instrumentation

2.3.1 GC-Positive Electron Ionization-MS

A Varian 3800 GC connected to a Saturn 2000 ion trap MS (Varian, Inc., Walnut Creek, CA, USA) with an internal ionization source was used for routine analysis of the extracts. An extract volume of 1 μL was injected through a Varian 1079 injection port fitted with a deactivated glass septum-equipped programmable injector (SPI) liner (Restek, Bellefonte, PA, USA) and held at 90˚C for 0.1 min then ramped to 180˚C at 100˚C·min^{-1}. Changes in injection port temperature were achieved by the SPI with carbon dioxide (CO_2) cooling. Helium was used as the carrier gas set at a constant flow rate of 1.0 mL·min^{-1} on a Rxi®-5Sil MS column with 30 m length ×0.25 mm I.D., 0.25 μm film thickness (Restek, Bellefonte, PA, USA). The column temperature program began at 37˚C and was held for 21 min, ramped to 136˚C at a rate of 5˚C·min^{-1}, held for 3 min, then ramped to 250˚C at 20˚C·min^{-1} and held for 2.5 min for a total run time of 52 min. The transfer line temperature was set at 240˚C, the trap manifold at 80˚C, and the

ion trap at 180˚C. After a 12 min solvent delay, the ion trap was operated using single ion monitoring (SIM) scans in electron impact ionization (EI) mode with axial modulation voltage set at 4.0, emission current at 10 µA, a target ion count (TIC) of 65000, with automatic gain control (AGC) turned on. Fragmentation patterns and M^+ fragments for IAs were identified using a full scan MS method (m/z from 110 to 340D) at high concentrations (10 mg/L) to select the appropriate SIM ions for targeting in routine analysis (Figure 2-5). The SIM scan segments were determined from M^+ fragments with a range of ±2 m/z for each analyte listed on Table 2-3, which also includes the acid surrogate and internal standard at M^+ of 153 and 167 m/z, respectively. Detailed explanations of the fragmentation pattern for each iodoacid using GC-PEI-MS are shown in Table 2-4. Data were analyzed using Varian MS Workstation software v. 6.6.

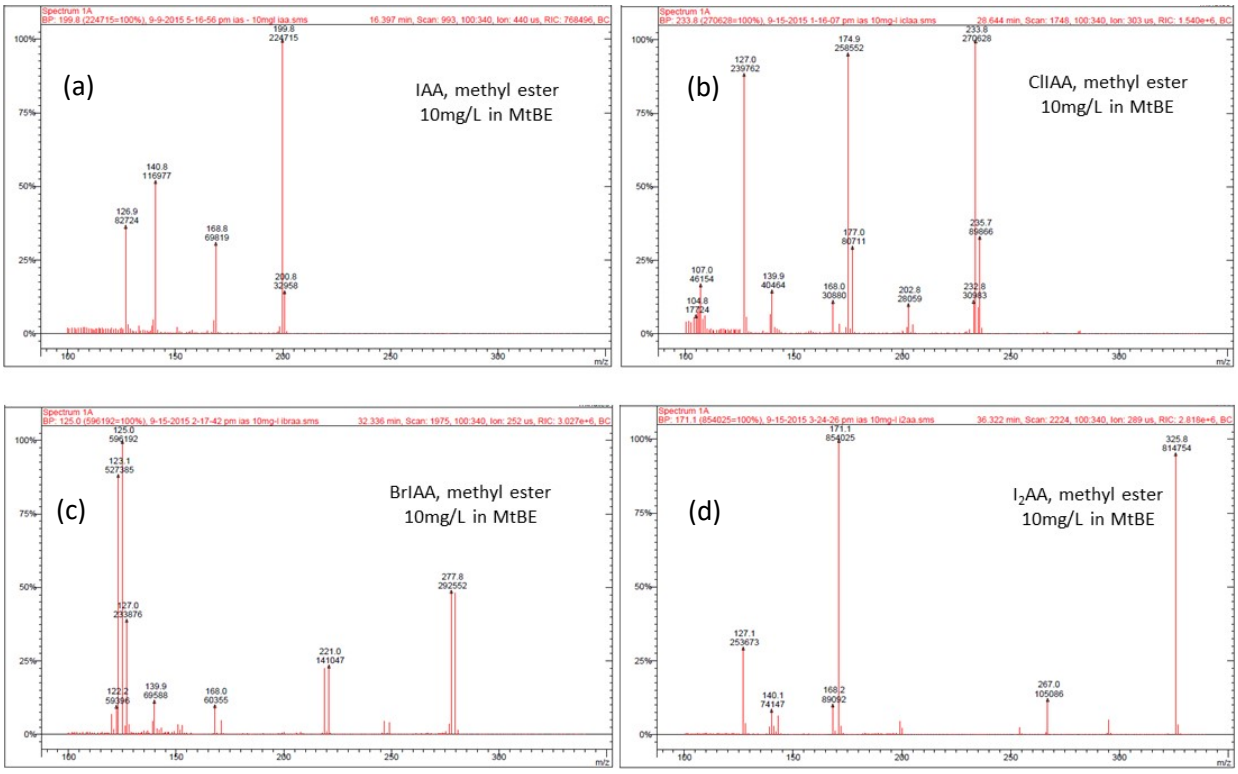


Figure 2-5: Positive electron ionization (EI+) fragmentation patterns for methyl esters of (a) iodoacetic acid; (b) chloroiodoacetic acid; (c) bromoiodoacetic acid; and (d) diiodoacetic acid prepared from standards at 10 mg/L.

Table 2-3: Single ion monitoring (SIM) scan segments and ranges for GC-PEI-MS.

Scan Segment	Start (min)	End (min)	Low Mass (m/z)	High Mass (m/z)
Filament Off	0	12.50	-	-
IAA	12.50	19.50	198	202
Acid Surrogate	19.50	23.00	151	155
ClIAA	23.00	30.00	232	236
Internal Standard	30.00	31.75	165	169
BrIAA	31.75	33.00	276	280
I_2AA	33.00	36.50	324	328
Filament Off	36.50	52.00	-	-

Table 2-4: Explanation of fragmentation patterns for iodoacids using GC-PEI-MS.

	IAA, methyl ester	**ClIAA, methyl ester**	**BrIAA, methyl ester**	**I_2AA, methyl ester**
	$C_2H_2IO_2$-CH_3	C_2HIClO_2-CH_3	C_2HIBrO_2-CH_3	$C_2HI_2O_2$-CH_3
RT (min)	16.4	28.6	32.3	36.3
M+ (m/z)	200	234/236	278/280	326
methoxy group (m/z) loss of -OCH_3	169	203/205	247/249	295
ester group (m/z) loss of -$C(O)OCH_3$	141	175/177	219/221	267
methoxy group and I (m/z) loss of -OCH_3 and –I	N/A^	N/A	N/A	168
ester group and I (m/z) loss of -$C(O)OCH_3$ and -I	N/A	N/A	N/A	140
methoxy group and Cl (m/z) loss of -OCH_3 and -Cl	N/A	168	N/A	N/A
ester group and Cl (m/z) loss of -$C(O)OCH_3$ and -Cl	N/A	140	N/A	N/A
methoxy group and Br (m/z) loss of -OCH_3 and -Br	N/A	N/A	168	N/A
ester group and Br (m/z) loss of -$C(O)OCH_3$ and -Br	N/A	N/A	140	N/A
(m/z) Iodine	127	127	127	127
(m/z) loss of -I	< mass range (73)	107/109	151/153	200

^N/A=not applicable; *<mass range=lower than the scan m/z range collected

2.3.2 GC-Negative Chemical Ionization-MS

A ThermoQuest TRACE GC 2000 Version 1.5.6 and Finnigan PolarisQ Version 1.4.1 ion trap MS with PAL Autosampler and XCalibur^{TM} 1.4 SR1 software was used for confirming the identity of the iodoacids through negative chemical ionization (NCI). The same column type and manufacturer as described for EI was used on this separate instrument ensuring analyte identification with similar relative retention times observed during PEI. The oven temperature program for the GC-NCI-MS instrument was the same as outlined above for the GC-PEI-MS. The injection port was held at 180˚C with a constant flow of helium at 1.0 mL·min^{-1}. The transfer line temperature was set at 260˚C and the ion trap at 150˚C. After a 5 min solvent delay, the mass spectrometer was operated in full scan NCI mode using methane as reagent gas at 1.8 mL·min^{-1} and with the m/z scan range from 100 to 340 Dalton.

2.3.3 GC-ECD

HAA9 are routinely extracted and analyzed at quantitation levels around 1 µg/L, using EPA Method 552.3 (Domino et al., 2003) or earlier versions. To evaluate the impact of this new method on HAA9 analysis, the extracts were also analyzed using a Hewlett Packard 6890 series GC equipped with an HP 7683 series injector and G2397A µ-ECD detector (Agilent Technologies, Santa Clara, CA, USA). The same temperature method was used as specified for the GC-NCI-MS, with a 30m Zebron ZB-1 column of 0.25 mm I.D. and 1.0 µm film thickness (Phenomenex, Torrance, CA, USA). The ECD temperature was maintained at 300°C. With the concentration factor needed for the iodoacids, the PQLs for the HAA9 were lowered to 0.01 µg/L.

2.3.4 Instrumental Analysis

With the increased concentration factor compared to EPA Method 552.1, the iodoacid methyl esters can be overwhelmed by signal response for the other HAAs if an ECD is used. With a negative chemical ionization mass-spectrometer, a single ion (SIM) mode for iodine at 127 mass/charge ratio can be used to resolve iodinated-HAA methyl esters from the TIC chromatogram. The GC-NCI-MS method was, therefore, developed to provide confirmation for IAs and complete resolution of the 13 analytes, which included the four iodoacids that were orders of magnitude lower than the nine other haloacetic acids.

2.4 Results and Discussion

Two separate instruments were used in the development of this method to confirm then quantify the 4 iodoacid species: GC-PEI-MS and GC-NCI-MS. Confirmation of iodinated analyte chromatographic resolution from HAA9 methyl ester peaks in the absence of an isotopic pattern for iodine and iodine-containing fragments, required NCI due to its increased sensitivity for small polar molecules and the strong electronegativity of iodine. Quantitation of 4 iodoacids for routine analysis was performed with GC-PEI-MS using individual SIM segments. To determine the HAA9 concentrations, the same extracts were analyzed with GC-ECD.

2.4.1 Stability

The linear dynamic concentration range measured by relative area for each of the iodoacids was obtained up to 1 µg/L in the model water (N=3), with the mean and standard deviation shown in Table 2-5 for iodoacetic acid. The four iodoacids (spiked at 10 to 40 µg/L to allow for direct LLE followed by derivatization) were evaluated for pre-extraction stability in

both quenched and unquenched chloraminated (residual of 3.2 mg/L as Cl_2 with a pH of 6.6) matrices. Stoichiometric amounts of ascorbic acid were used as the quenching agent. No significant differences in IA concentrations were observed over seven days when iodoacids were spiked into LGW without pH adjustment as quantified by GC-ECD. When spiked into an unquenched chloraminated model water, there was an initial period of decreasing iodoacid concentration followed by formation over the seven-day period (Figure 2-6). Over 7 days, IAA decreased by 5%, ClIAA increased by 12%, BrIAA increased by 6%, and I_2AA increased by 19% but these changes will be impacted when additional inorganic precursors are present in an unquenched matrix. When iodoacids were spiked into unquenched model water containing iodide (100 μg/L) and bromide (500 μg/L), there was a preliminary decrease in concentration followed by an increase in concentration for all four iodoacids (Figure 2-7). IAA increased by 11%, ClIAA increased by 31%, BrIAA increased by 4%, and I_2AA increased by 66% over the 7-day period. This may be due to an initial decomposition of the IAs from the residual chloramines followed by the formation of IAs from the DOC, iodide, bromide, and residual disinfectant and may also point to a difference in decomposition rates compared to formation rates of IAs. The final stability test involved a quenched model water matrix where IAA decreased 25%, ClIAA increased 4%, BrIAA decreased 6% and I_2AA decreased 22% over seven days (Figure 2-8). Based on these changes, I_2AA appears to decompose to form IAA with the loss of an iodine which may be due to auto-decomposition from charge instability of two large electronegative atoms rather than specific matrix effects. Ascorbic acid as the quenching agent was a critical component of a quality-controlled method as it prevented continued formation of iodoacids in the presence of iodide and bromide.

Table 2-5: Linearity of the iodoacetic acid methyl ester from a standard addition curve extracted from LGW using the GC-ECD method (N=3).

IAA Spike Concentration (ng/L)	Relative Area*				
	Mean	Upper Value	Lower Value	Standard Deviation	Relative Standard Deviation (%)
0	0.99	1.14	0.84	0.15	15.1
20	2.15	2.30	1.99	0.16	7.43
50	3.45	3.66	3.23	0.22	6.28
250	12.3	12.5	12.1	0.21	1.71
1000	46.2	46.9	45.5	0.69	1.49

*Relative Area = Absolute Area of Analyte Divided by the Absolute Area of the Internal Standard in Each Sample

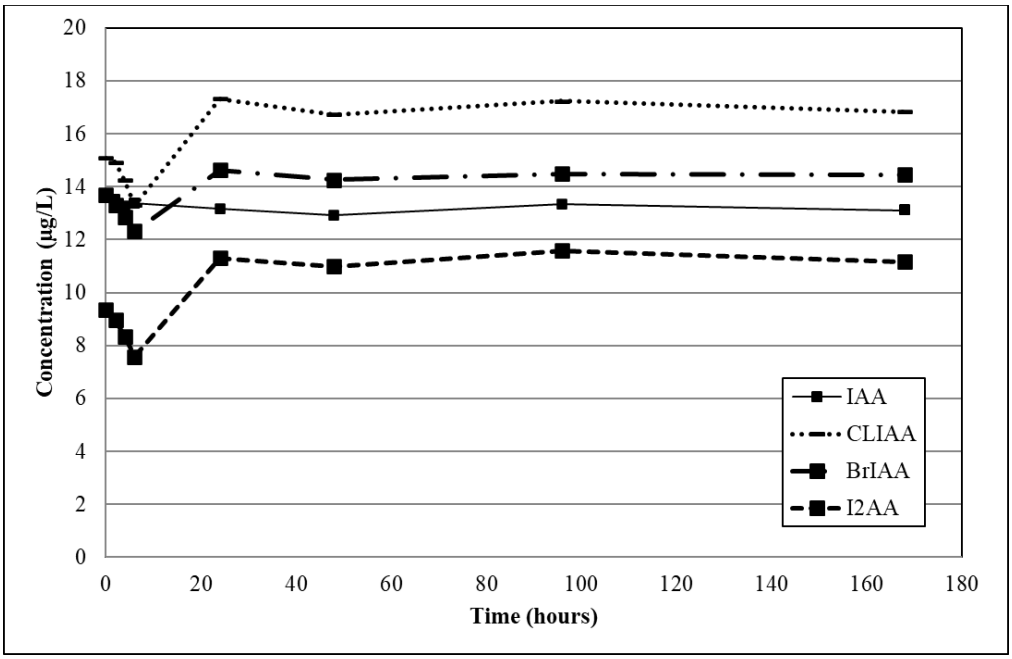


Figure 2-6: Iodoacid stability test in unquenched, chloraminated model-water (n=3 for each time point).

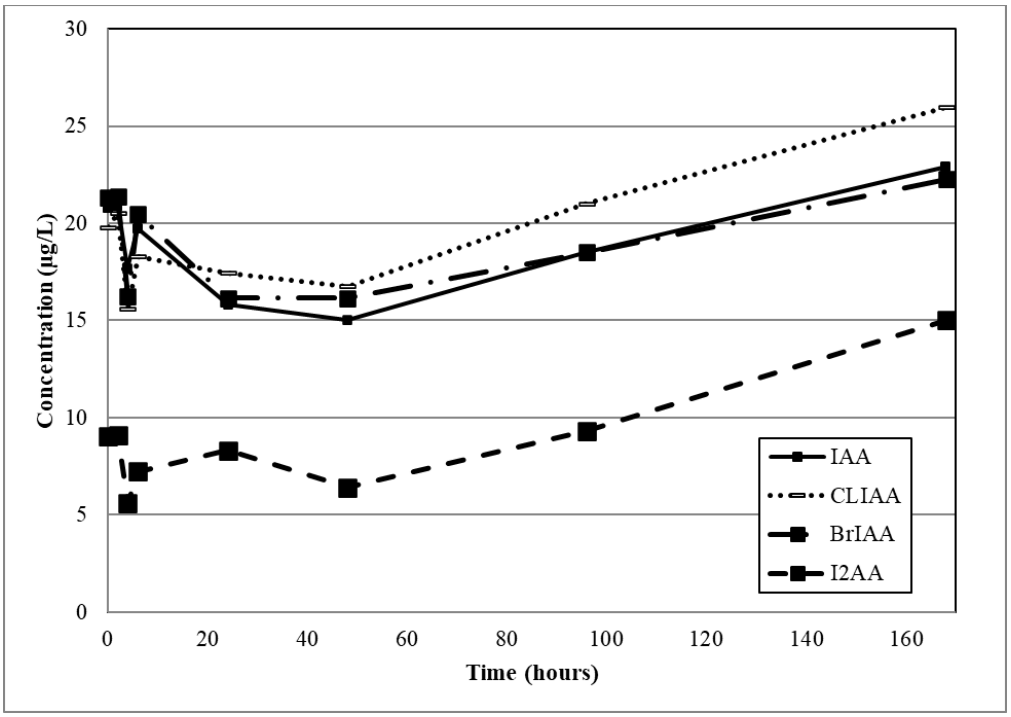


Figure 2-7: Iodoacid stability test in unquenched, chloraminated model-water with bromide and iodide (n=3 for each time point).

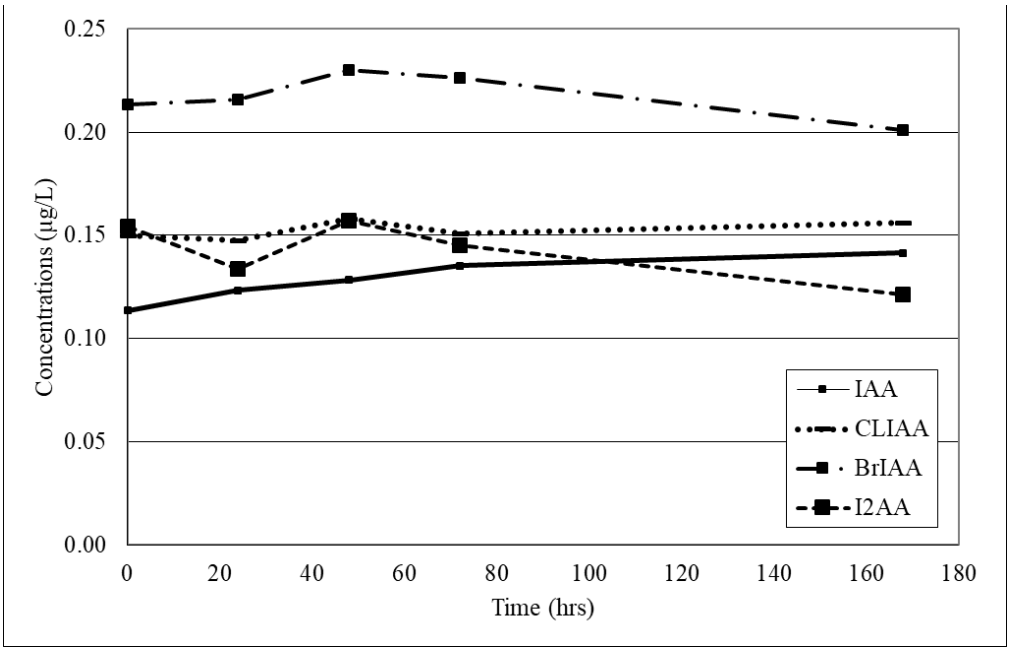


Figure 2-8: Iodoacid stability test in quenched, chloraminated model-water (n=3 for each time point).

2.4.2 Analysis of IA4 Standards

With the extraction, concentration, and derivatization processes optimized, the IAs were analyzed for routine analysis on a GC-PEI-MS ion trap in SIM mode targeting the M^+ ions identified for each analyte (as described in Table 2-3). The same extracts were also analyzed by GC-ECD to quantify the HAA9. An example TIC chromatogram and individual SIM segments using PEI-MS with derivatized IA4 standards are shown in Figure 2-9 and the chromatogram for Plant 7 finished water extract is shown in Figure 2-10. With the coextracted HAA9 at µg/L concentrations and elevated matrix interferences due to the large concentration factor, the SIM mode was needed to target the M^+ ions at the lower IA4 concentrations. Figure 2-10 shows the overall ion intensity for the SIM mode compared to the full scan mode has decreased by a factor of 100 and the noise surrounding the IAA peak at 16.4 min and the I_2AA peak at 36.3 min is significantly reduced. The TIC and m/z 127 fragments associated with chromatographic peaks from NCI-MS are shown in Figure 2-11, where the four IA analytes were added before extraction at 100 ng/L and resolved from the drinking water matrix with HAA9 present at µg/L. Since the negative chemical ionization easily dissociates iodine ions from small molecular weight organics, the m/z 127 fragment can be used to resolve the iodoacids from the co-extracted HAA9.

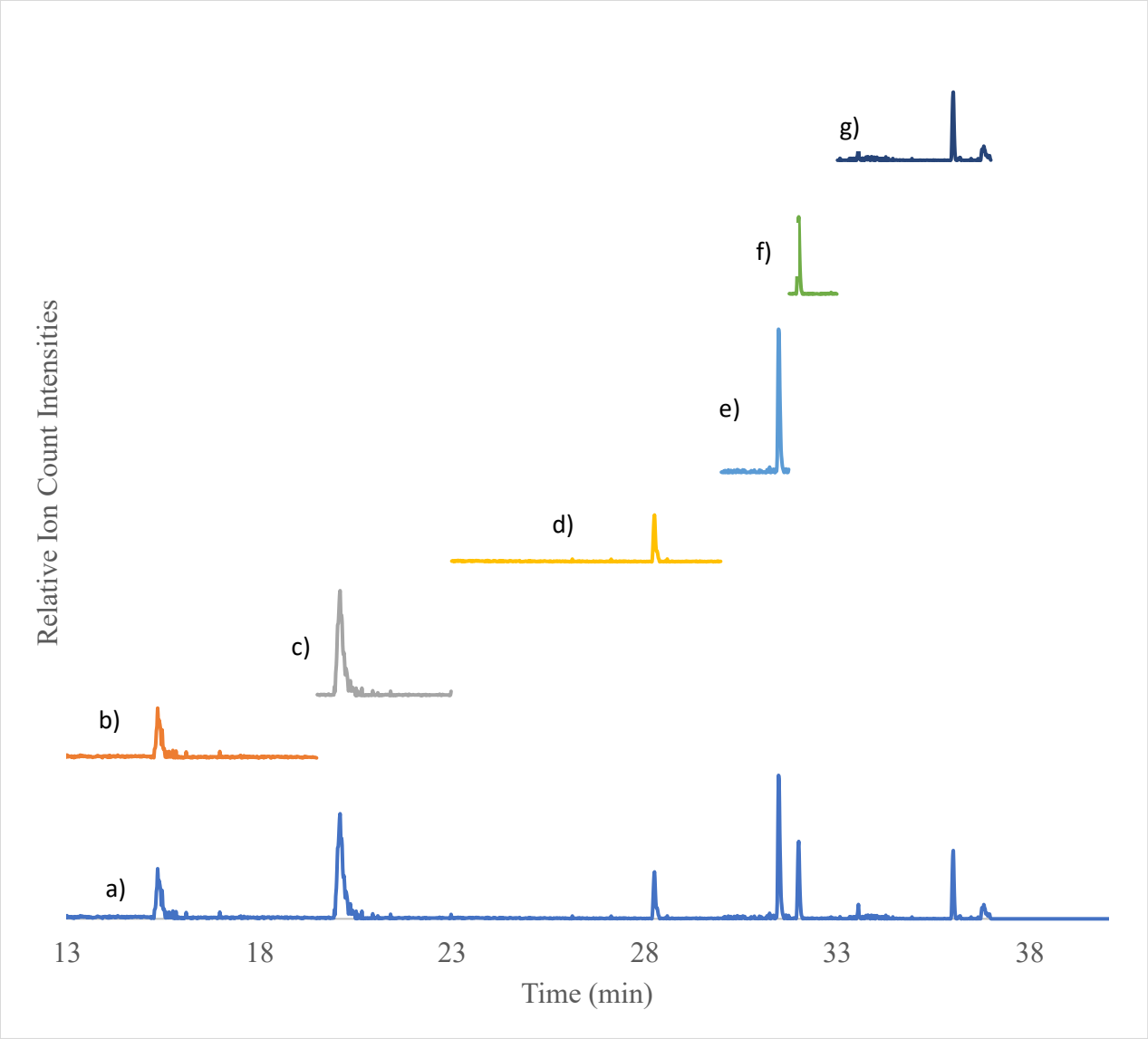
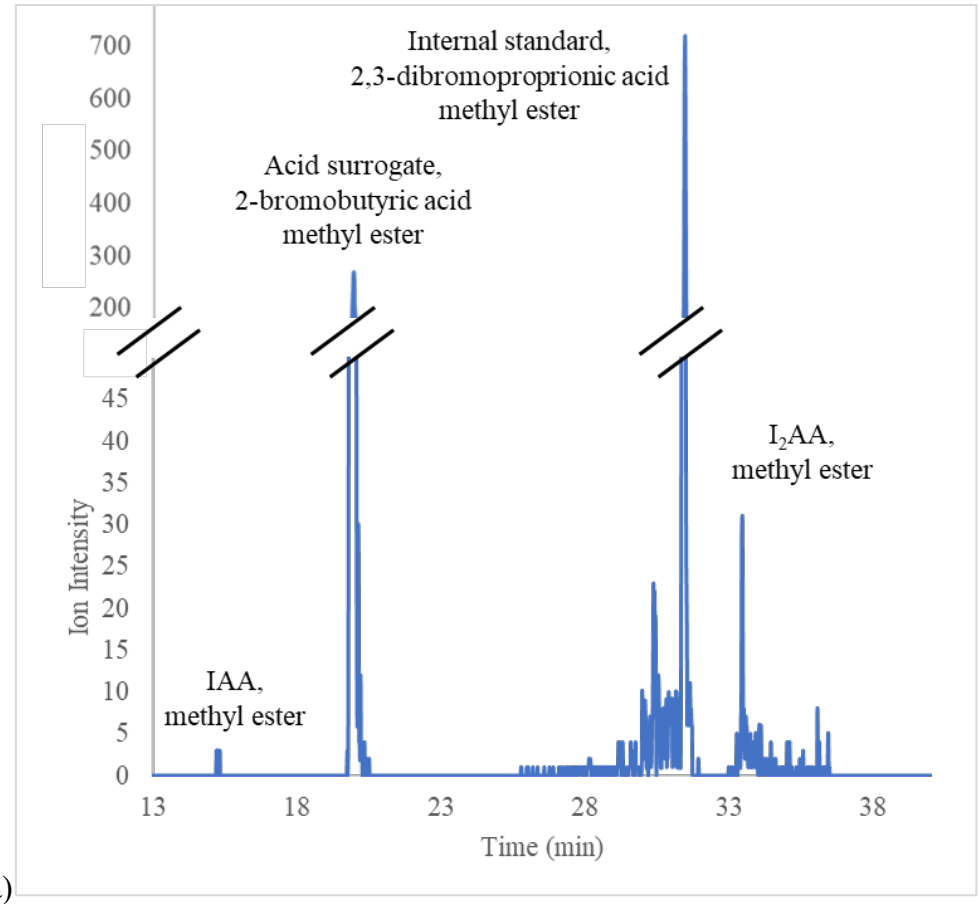
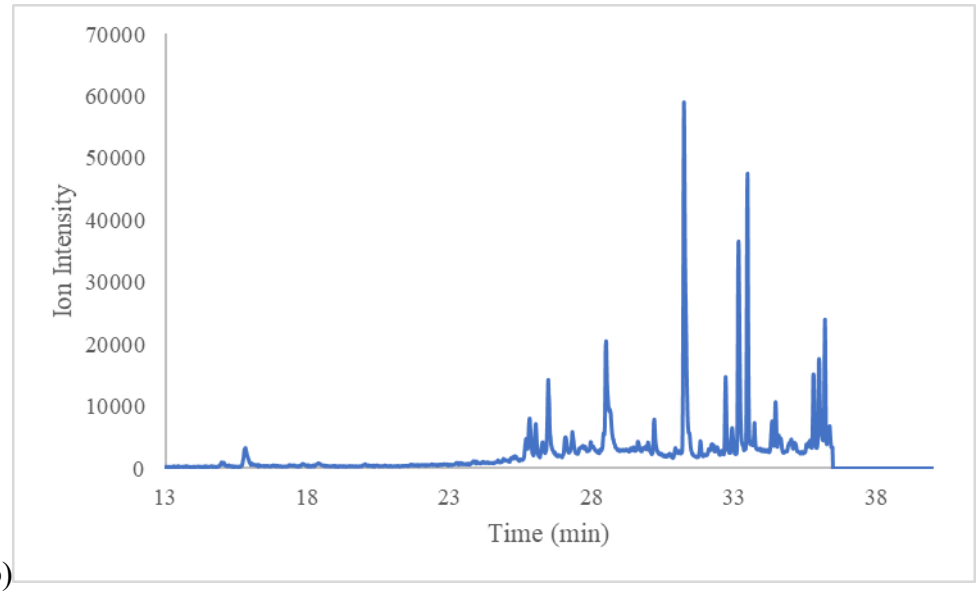


Figure 2-9. Single ion monitoring segments for iodoacids detection by GC-PEI-MS. a) Total ion count (TIC) chromatogram of derivatized iodoacid standards each at 133.3 µg/L in MtBE with internal standard + acid surrogate; b) iodoacetic acid, methyl ester (m/z = 198-202 m/z); c) acid surrogate, 2-bromobutyric acid, methyl ester (m/z = 151-155); d) chloroiodoacetic acid (m/z = 232-236); e) internal standard, 2,3-dibromoproprionic acid, methyl ester (m/z = 165-169); f) bromoiodoacetic acid, methyl ester (m/z = 276-280); and g) diiodoacetic acid, methyl ester (m/z = 324-328).



a)



b)

Figure 2-10: GC-PEI-MS chromatogram for Plant 7 finished water: a) Full scan mode (m/z from 100 to 340) b) Single ion monitoring mode (Table 2-3 shows each SIM segment).

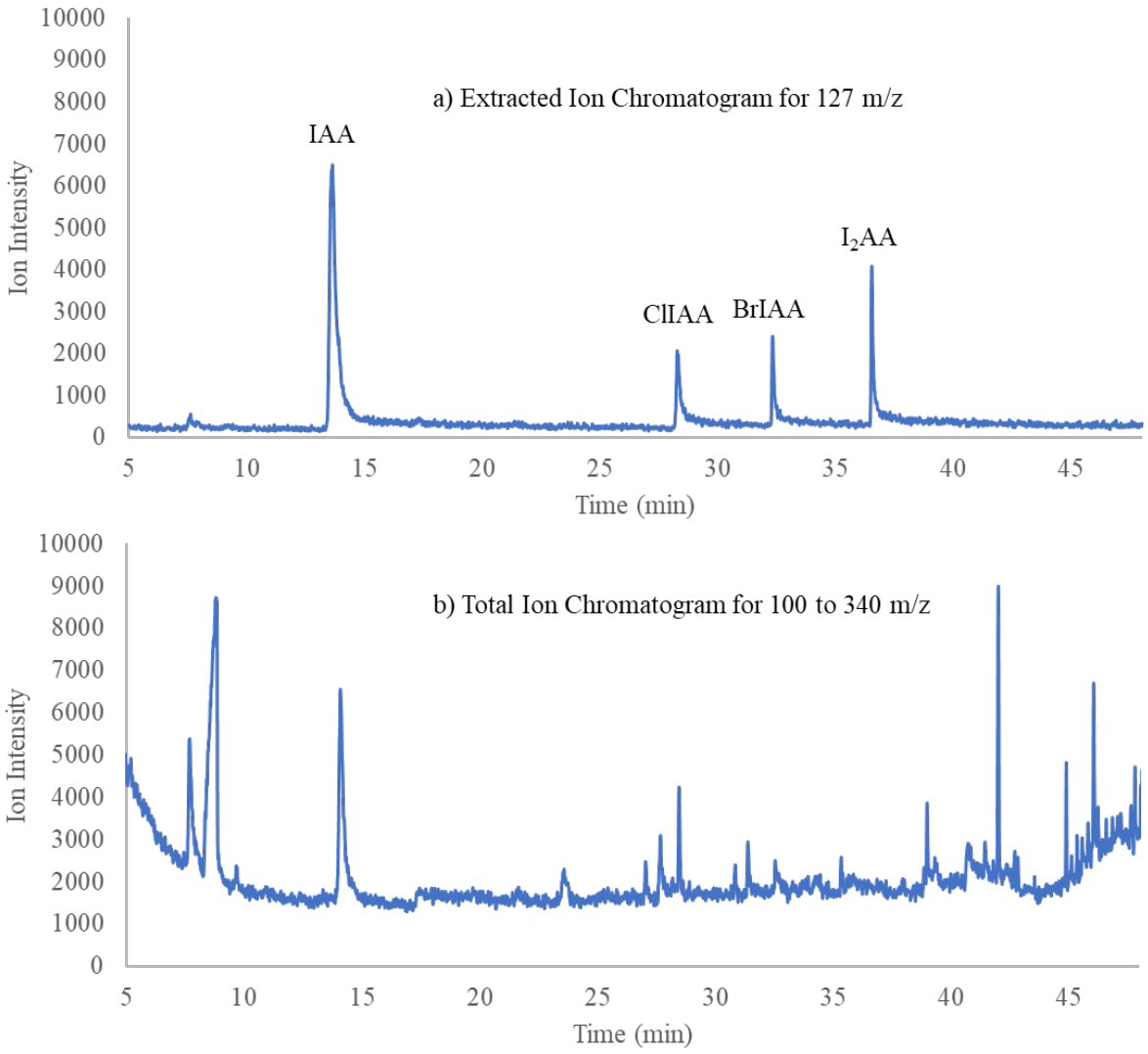


Figure 2-11: Confirmation of the presence of IA4 in the derivatized from an extract of a chloraminated drinking water that had been spiked with 100 ng/L of each species: a) An extracted ion chromatogram at m/z 127 showing four iodoacids extracted from a drinking water matrix that were spiked individually at 100 ng/L and; b) a total ion count chromatogram of the same sample using a full scan (m/z from 100 to 340) by GC-NCI-MS analysis.

2.4.3 Detection Limits

The MDL, as defined by the EPA to show 99% confidence in a measured concentration (US EPA, 1986), were determined for the 4 iodoacids in a spiked water matrix (Section 2.2.3) using a GC-ECD taken through the method described in Section 2.2.3 and shown in Table 2-6.

The MDL values for the iodoacids on a GC-ECD were found to be 9, 8, 11, and 6 ng/L for IAA, ClIAA, BrIAA, and I_2AA, respectively.

Table 2-6: Method detection limit (MDL) data for four iodoacids using GC-ECD analysis.

Sample	IS	IAA	ClIAA	BrIAA	I_2AA
MDL 1					
Time (min)	20.4	31.3	40.4	43.3	48.0
Area	57.6	49.8	27.6	25.4	15.4
S/N (Area)	11.5	10.0	5.5	5.1	3.1
Relative Area (Area/IS Area)	1.0	0.9	0.5	0.4	0.3
Concentration (ng/L)	N/A*	17.8	17.9	20.5	12.0
MDL 2					
Area	89.5	88.3	31.9	44.3	27.8
S/N (Area)	11.5	10.0	5.5	5.1	3.1
Relative Area (Area/IS Area)	1.0	1.0	0.4	0.5	0.3
Concentration (ng/L)	N/A	20.3	11.5	11.0	6.1
MDL 3					
Area	57.1	59.2	23.2	27.8	21.0
S/N (Area)	11.5	10.0	5.5	5.1	3.1
Relative Area (Area/IS Area)	1.0	1.0	0.4	0.5	0.4
Concentration (ng/L)	N/A	21.3	13.1	10.9	7.3
MDL 4					
Area	57.6	69.9	33.0	43.1	22.4
S/N (Area)	11.5	10.0	5.5	5.1	3.1
Relative Area (Area/IS Area)	1.0	1.2	0.6	0.7	0.4
Concentration (ng/L)	N/A	25.0	18.5	16.7	7.7
MDL 5					
Area	46.1	42.0	20.5	27.1	17.7
S/N (Area)	11.5	10.0	5.5	5.1	3.1
Relative Area (Area/IS Area)	1.0	0.9	0.4	0.6	0.4
Concentration (ng/L)	N/A	18.7	14.4	13.1	7.6
MDL 6					
Area	90.2	67.6	29.7	35.6	24.3
S/N (Area)	11.5	10.0	5.5	5.1	3.1
Relative Area (Area/IS Area)	1.0	0.7	0.3	0.4	0.3
Concentration (ng/L)	N/A	15.4	10.7	8.8	5.3

Table 2-6 continued					
Sample	**IS**	**IAA**	**ClIAA**	**BrIAA**	**I_2AA**
MDL 7					
Area	52.1	55.2	22.2	30.4	19.0
S/N (Area)	11.5	10.0	5.5	5.1	3.1
Relative Area (Area/IS Area)	1.0	1.1	0.4	0.6	0.4
Concentration (ng/L)	N/A	21.8	13.8	13.0	7.2
MDL 8					
Area	76.3	86.5	28.3	42.2	28.0
S/N (Area)	11.5	10.0	5.5	5.1	3.1
Relative Area (Area/IS Area)	1.0	1.1	0.4	0.6	0.4
Concentration (ng/L)	N/A	23.3	12.0	12.3	7.2
Peak Area					
Mean Relative Area	N/A	1.0	0.4	0.5	0.3
Standard Deviation	N/A	0.1	0.1	0.1	0.05
Relative Standard Deviation (%)	N/A	15.1	18.4	20.4	14.7
Concentrations (ng/L)					
Mean Concentration (ng/L)	N/A	20.5	14.0	13.3	7.6
Standard Deviation	N/A	2.9	2.7	3.5	1.8
Relative Standard Deviation (%)	N/A	15.1	20.5	27.8	26.2
PQL (ng/L)	N/A	29	27	35	18
MDL (ng/L)	N/A	8.7	8.0	10.4	5.5

*N/A = not applicable

Using the MDL concentration range for the GC-ECD as a guide, a IA4 standard calibration curve for the GC-PEI-MS was built with a concentration range of 26.7 to 133.3 µg/L (or 20 to 100 ng/L before extraction). To calculate the IDL for the GC-PEI-MS, the 46.6 µg/L calibration concentration (or 35 ng/L before extraction) was selected for 8 replicate injections based on S/N >10 for each analyte. Detailed IDL peak area data using GC-PEI-MS are shown in Table 2-7. The IDLs for the GC-PEI-MS were 5.0, 3.6, 2.3, and 4.6 µg/L for iodoacetic acid, chloroiodoacetic acid, bromoiodoacetic acid, and diiodoacetic acid, respectively. Assuming a 90% analyte recovery through the 1333 concentration factor extraction, the IDL concentrations

for the pre-extracted samples would be 3.4, 2.4, 1.5, and 3.1 ng/L for iodoacetic acid, chloroiodoacetic acid, bromoiodoacetic acid, and diiodoacetic acid, respectively.

Table 2-7: Instrument detection limit (IDL) data for iodoacids from single ion monitoring segments of the methyl esters using GC-PEI-MS analysis.

Sample	IS	IAA	ClIAA	BrIAA	I_2AA
IDL 1					
Time (min)	31.4	15.3	28.2	31.9	36.0
M+ (m/z)	167	200	234	278	326
Area	3445	602	506	762	1091
S/N (Area)	923	113	136	225	373
Relative Area (Area/IS Area)	1.0	0.17	0.15	0.22	0.32
Concentration (μg/L) in extract	N/A	46.6	46.6	46.6	46.6
IDL 2					
Area	3292	570	601	697	1205
S/N (Area)	303	61	169	197	376
Relative Area (Area/IS Area)	1.0	0.17	0.18	0.21	0.37
Concentration (ng/L) in extract	N/A	46.6	46.6	46.6	46.6
IDL 3					
Area	3217	526	618	644	1100
S/N (Area)	867	122	166	204	360
Relative Area (Area/IS Area)	1.0	0.16	0.19	0.20	0.34
Concentration (ng/L) in extract	N/A	46.6	46.6	46.6	46.6
IDL 4					
Area	3525	464	770	812	1088
S/N (Area)	332	90	198	247	167
Relative Area (Area/IS Area)	1.0	0.13	0.22	0.23	0.31
Concentration (ng/L) in extract	N/A	46.6	46.6	46.6	46.6
IDL 5					
Area	2694	324	507	678	1113
S/N (Area)	781	110	144	204	399
Relative Area (Area/IS Area)	1.0	0.12	0.19	0.25	0.41
Concentration (ng/L) in extract	N/A	46.6	46.6	46.6	46.6
IDL 6					
Area	2677	257	550	626	876
S/N (Area)	386	54	134	183	294
Relative Area (Area/IS Area)	1.0	0.10	0.21	0.23	0.33
Concentration (ng/L) in extract	N/A	46.6	46.6	46.6	46.6

Table 2-7 continued					
Sample	**IS**	**IAA**	**ClIAA**	**BrIAA**	**I_2AA**
IDL 7					
Area	3451	286	755	849	1125
S/N (Area)	475	59	227	260	393
Relative Area (Area/IS Area)	1.0	0.08	0.22	0.25	0.33
Concentration (ng/L) in extract	N/A	46.6	46.6	46.6	46.6
IDL 8					
Area	3426	301	806	742	1082
S/N (Area)	477	75	186	112	358
Relative Area (Area/IS Area)	1.0	0.09	0.24	0.22	0.32
Concentration (ng/L) in extract	N/A	46.6	46.6	46.6	46.6
Peak Area					
Mean Relative Area	N/A	0.1	0.2	0.2	0.3
Standard Deviation	N/A	0.04	0.03	0.02	0.03
Relative Standard Deviation (%)	N/A	30	14	8	10
Concentrations					
IDL (μg/L) extracted	N/A	5.0	3.6	2.3	4.6

2.4.4 Formation Potential Tests

Using the standard addition calibration curve, formation potentials of the four iodoacids were quantified using the GC-PEI-MS approach. The HAA9 species were quantified on the GC-ECD using the same extracted samples. The three different levels of iodide spiked samples prior to chloramination did result in IA formation, with IAA, ClIAA, I_2AA, and TOI increasing with increasing iodide spikes. Since the drinking water matrix used in the IA formation potential experiments did not contain bromide, there were no Br-HAAs formed. Table 2-8 shows the formation of each haloacetic acid species according to the level of iodide spiked prior to chloramination and the organic iodine concentration for each and the iodine incorporation can be determined for each iodide spike level according to equation 1 (Obolensky and Singer, 2005).

$$Iodine\ Incorporation\ Factor = \frac{\sum molar\ conc. \times \#iodide}{\sum(molar\ conc.)\times(\#halogens)} \times 100 \qquad \text{Equation 1}$$

where the molar concentration of the iodinated species and the total number of halogenated species are used to determine the iodine-incorporation factors for precursor comparisons. An example calculation for iodide incorporation percentage in HAAs using data from Table 2-8 is now given:

$$Haloacetic\ Acids\ Iodine\ Incorporation\ for\ the\ 15\,{}^{\mu g}/_{L}\ spike\ as\ I\ sample$$

$$= \left(\frac{(0.213\,nM) + (0.0933\,nM) + (0.0544\,nM \times 2)}{(0.213\,nM) + (0.0933\,nM) + (0.0544\,nM \times 2) + (27.6\,nM \times 2) + (24.9\,nM \times 3)}\right) \times 100$$

$$= 0.32\ \%$$

The incorporation factors correlated with the iodide spikes with factors of 0.46 % and 0.43 % for spikes of 50 and 100 µg/L as I, respectively.

Table 2-8: Formation potential of four iodoacids and two HAAs in a drinking water matrix with three spiked concentrations of iodide prior to chloramination (N=2).

Iodide Spike (µg/L as I)	TOI (µg/L as I)	IAA (ng/L)	ClIAA (ng/L)	BrIAA (ng/L)	I_2AA (ng/L)	Cl_2AA (µg/L)	Cl_3AA (µg/L)
15	12	39.7	20.4	<2.3	16.8	3.6	4.1
50	21	71.5	22.0	<2.3	28.1	3.4	5.0
100	31	94.9	81.4	<2.3	78.9	2.8	N/A*

*N/A due to problem with chromatography

The iodide incorporation percentages were expected to remain the same and the IA concentrations were expected to increase with increasing iodide spikes. Comparing the mass fraction of iodine in the IAs to the spiked iodide levels, only 0.3% of the 15 µg/L iodine spike and 0.2% of the 50 and 100 µg/L iodine spikes were incorporated into the haloacetic acids for IA formation but could have formed other I-DBPs. The TOI confirms that the inorganic iodide was

incorporated into I-DBPs at greater levels than observed in the IA4 formation, where the 15 μg/L iodine spike had 80% incorporation, the 50 μg/L iodine spike had 42% incorporation, and the 100 μg/L iodine spike had 31% incorporation. This suggests the formation of other I-DBPs (such as iodinated trihalomethanes) as a result of the chloramination reaction and a potential limit to IA4 and TOI formation within this formation potential matrix as the TOC concentration for each spike level was 1.1 mg/L as C. Based on these incorporation rates, the concentration of iodide in the source water does not linearly correlate to treated water IA4 concentration but more information is needed on IA4 and source water iodine levels to model the relationship.

2.4.5 Drinking Water Treatment Plant Survey

The water quality parameters for each of the 7 plants on the day of sampling are shown in Table 2-9, with total iodine concentrations ranging from 9 to 230 μg/L as I representing different impacts on the source water. For example, Plants 3, 4, and 5 are impacted by saltwater intrusion that introduces mainly inorganic iodide and bromide, but the source water for Plant 7 is impacted by upstream wastewater that receives medical waste that introduces organic iodine. The TDI concentration in addition to the source and speciation of the iodine will impact the iodine incorporation percentage observed in the finished water IAs. Table 2-10 shows the HAA9 and IA4 results in the finished water of each plant. Iodoacid species (ng/L) were quantifiable at two to three orders of magnitude lower than the HAA9 (μg/L) in each of the seven plants sampled, showing the ability to extract IAs from quenched drinking water matrices. The formation of iodoacids in four of the plants followed iodine incorporation patterns similar to those found in the bench scale experiments with iodine spikes, namely that IAA > ClIAA and BrIAA (when bromide was present) > I_2AA, with the exception of Plants 2, 6, and 7. The iodine incorporation

percentages for HAAs measured at the plants ranged from a low of 0.6% at Plant 4 and 1.8% at Plant 1. Plants 2 and 6 showed the highest levels of total iodine (53 and 230 ng/L) and bromide (0.40 and 0.68 μg/L) in the influent waters. The higher levels of influent bromide changed the relative speciation of the iodoacids due to the faster reaction rates of HOBr and HOI compared to HOCl. (Bichsel and von Gunten, 1999; Deborde and von Gunten, 2008; Trogolo and Arey, 2017) Plant 7 did not have measurable amounts of chlorine- or bromine-containing iodoacids, even though bromine-containing HAAs were present in the finished water. The main species observed in Plant 7 were IAA and I_2AA which may be due to the wastewater origin of the total iodine in the influent water. The release of iodine from an organic compound has a different HOI formation pathway compared to inorganic iodide, which may impact the incorporation mechanisms. Additionally, Plant 7 used a pre-ozonation process that forms bromate from bromide but is not at a sufficient dose to transform all of the organic iodine to iodate. Compared to Plant 4 with whose source of inorganic iodide was from saltwater intrusion and all 4 IAs were detected in the finished water, this may show the importance of the iodine source in addition to the TOC concentration and the oxidant doses on IA speciation and concentration. This survey of drinking water treatment plants shows that 4 iodoacids can be detected in finished drinking water in the presence of other HAAs by using this robust extraction and analytical method.

Table 2-9: Water quality parameters for surveyed drinking water treatment plants.

Plant Number	Impacts to Source Water	Disinfectants/ Oxidants	Influent Total Iodine (µg/L as I)	Influent Bromide (mg/L as Br)	Finished Water Total Organic Carbon (mg/L as C)
1	Connate water	Chlorine, chloramines	30	0.2	2.6
2	Connate water	Chlorine dioxide, chlorine, chloramines	53	0.4	3.7
3	Saltwater intrusion	Chlorine, chloramines	15	0.36	1.4
4	Saltwater intrusion	Ozone, chlorine, chloramines	15	0.36	1.2
5	Saltwater intrusion	Chlorine, chloramines, chlorine dioxide	19	0.13	5.8
6	Salt deposits	Chlorine, chloramines	230	0.68	Not Available
7	Wastewater discharge	Ozone, chlorine, chloramines	9*	0.35*	1.5*

*average value reported over 4 months

Table 2-10: Finished water concentrations of HAA9 and IA4 from the surveyed plants (N=2).

	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6	Plant 7
Iodoacids Concentrations (ng/L)							
IAA	291 ± 91	34 ± 0.4	114 ± 21	29 ± 4.4	92 ± 5.7	24 ± 1.1	27 ± 1.2
ClIAA	254 ± 16	31 ± 2.4	65 ± 1.5	3.5 ± 0.7	33 ± 18	38 ± 7.2	<4.0
BrIAA	104 ± 8.0	66 ± 2.0	54 ± 1.4	11 ± 1.5	58 ± 26	69 ± 11	<2.3
I_2AA	45 ± 6.5	12 ± 10	12 ± 5.0	12 ± 4.5	11 ± 12	5.7 ± 2.7	8.7 ± 0.6
HAA9 Concentrations (µg/L)							
ClAA	3.0 ± 0.47	1.9 ± 0.48	1.8 ± 0.88	2.1 ± 1.8	1.8 ± 0.57	2.8 ± 0.50	<0.01
BrAA	0.56 ± 0.22	1.8 ± 0.47	1.1 ± 0.30	0.44 ± 0.06	0.22 ± 0.03	0.51 ± 0.25	0.10 ± 0.04
Cl_2AA	7.7 ± 0.76	1.6 ± 0.04	3.8 ± 0.83	0.40 ± 0.04	6.8 ± 2.9	0.71 ± 0.07	0.32 ± 0.11
BrClAA	4.6 ± 0.57	3.1 ± 0.38	5.2 ± 0.29	0.76 ± 0.07	1.3 ± 0.56	1.2 ± 0.10	0.44 ± 0.13
Br_2AA	2.6 ± 0.44	2.7 ± 0.32	3.6 ± 0.12	1.8 ± 0.18	0.11 ± 0.05	1.4 ± 0.04	0.40 ± 0.12
Cl_3AA	1.0 ± 0.04	0.49 ± 0.04	1.3 ± 0.17	0.13 ± 0.01	1.0 ± 0.48	0.14 ± 0.02	<0.01
$BrCl_2AA$	0.70 ± 0.19	0.66 ± 0.73	1.2 ± 0.05	0.01 ± 0.05	0.04 ± 0.02	0.06 ± 0.01	0.19 ± 0.06
Br_2ClAA	0.41 ± 0.18	1.2 ± 1.3	0.74 ± 0.02	<0.01	0.04 ± 0.01	0.02 ± 0.00	0.04 ± 0.02
Br_3AA	0.02 ± 0.03	0.31 ± 0.68	0.02 ± 0.00	<0.01	0.02 ± 0.01	0.01 ± 0.00	<0.01

* ± 95% confidence interval

2.5 Conclusions

A new method for the extraction, detection, and quantification of 4 iodoacids in drinking water with the presence of other HAAs was developed using standard addition calibration curves, LLE, SPE, and GC-PEI-MS. The four iodoacids were successfully resolved from co-extractants that are typically seen at orders of magnitude higher concentration. Comparison of the acid surrogate response between samples determines when multiple standard addition curves

are needed to account for sample matrix differences. Since the detection limit for the iodoacids using this method is in the ng/L range, it can be directly used for monitoring levels of these DBPs in drinking water, which will allow for more occurrence data to be collected and subsequent evaluation of the health implications associated with chloramination of water containing iodine.

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CHAPTER 3 : IODINE SOURCE TRACKING IN SURFACE WATERS RECEIVING WASTEWATER EFFLUENT: IMPLICATIONS FOR IODINATED DISINFECTION BYPRODUCTS IN DOWNSTREAM DRINKING WATER PRODUCTION

3.1. Introduction

With many drinking water sources compromised by point and non-point anthropogenic inputs resulting from drought stress, storm-water runoff, and upstream wastewater treatment plant discharges, a wide range of chemical contaminants are now present in water sources. Waste streams from hospitals and medical facilities are often regulated no differently than other municipal wastewater sources even though they may contain biologically active anthropogenic chemicals that are not removed by conventional wastewater treatment plants. As the effluents from these plants often feed into downstream drinking water sources, it is imperative to determine the fate of these medical residues not only for their potential direct impact on human and ecosystem health but also because they may react with disinfectants used in water treatment to form disinfection byproducts (DBPs). Of particular concern are those containing iodine. According to Richardson et al. (2008), iodine-containing DBPs (I-DBPs) have enhanced cytotoxicity and genotoxicity when compared to DBPs containing bromine and chlorine in the same sub-group. For example, unregulated dichloroiodomethane (DCIM) is almost 4 times more cytotoxic than regulated trichloromethane (TCM) based on the comparison of the 50% lethal concentration (LC_{50}) established by the Chinese Hamster Ovary (CHO) cell bioassay. Current regulations and recommendations from the U.S. Environmental Protection Agency (USEPA) do not include limits on or monitoring of I-DBPs under the Stage 2 DBP rule (USEPA, 2006), the

Fourth Unregulated Contaminant Monitoring Rule (UCMR 4) (USEPA, 2016), or the Contaminant Candidate List 4 (CCL 4) (USEPA, 2017) which are the current regulatory monitoring processes in the U.S.

I-DBP formation starts with hypoiodous acid (HOI) formation from the oxidation of iodide, which is fast for both chlorine (k=4.3x10^{8} M^{-1} s^{-1}) and monochloramine (k=2.4x10^{10} x[H^{+}] M^{-2}s^{-1}) (Bichsel and von Gunten, 1999). However, in the presence of monochloramine (NH_2Cl) the half-life of HOI is much longer and is on the order of hours due to the slow reaction of HOI with NH_2Cl ($k_{NH2Cl+HOI}$=2x10^{-3} M^{-1} s^{-1}) compared to hypochlorous acid (HOCl) ($k_{HOCl+HOI}$= 8.2 M^{-1} s^{-1} and $k_{HOCl+HOCl+HOI}$= 8.3x10^{4} M^{-1} s^{-1}) under drinking water conditions (pH range 6 to 9) (Bichsel and von Gunten, 1999). I-DBPs are, therefore, more likely to form in the presence of monochloramine especially when the reaction rate between HOI and natural organic matter (NOM) is orders of magnitude higher than the oxidation of HOI by monochloramine to iodate (IO_3^-) (Bichsel and von Gunten, 2000; Duirk et al., 2011). Chlorination of iodine-containing precursors also forms HOI but chlorine then rapidly oxidizes HOI to iodate, which greatly reduces iodine incorporation in DBPs (Bichsel and von Gunten, 1999).

In practice, most drinking water treatment plants (DWTPs) use chlorine to achieve pathogen log removal credits within the clearwell and add ammonia to form chloramines prior to the finished water distribution. If iodide was present in the clearwell, then iodate would form from the reaction with chlorine and I-DBPs would be less likely to form. One specific occurrence study of 23 U.S. cities observed I-DBPs in drinking waters whose sources contained low or non-detectable iodide concentrations (Richardson et al., 2008). In a follow-up study, Duirk, et al. (2011) found levels up to 2.7 µg/L of four iodinated contrast media (ICMs), iomeprol, iopromide, iohexol, and iopamidol, in 10 U.S. drinking water sources whose chloraminated

drinking water had measurable I-THMs and I-HAAs at the μg/L level. Commonly used ICMs that share the triiodinated benzene ring shown in Figure 3-1, are applied at high doses (60 to 120 g as I per dose) during diagnostic medical procedures and are eliminated, without metabolization of the parent compound, through urine and feces after 24 hrs (Pérez et al., 2006).

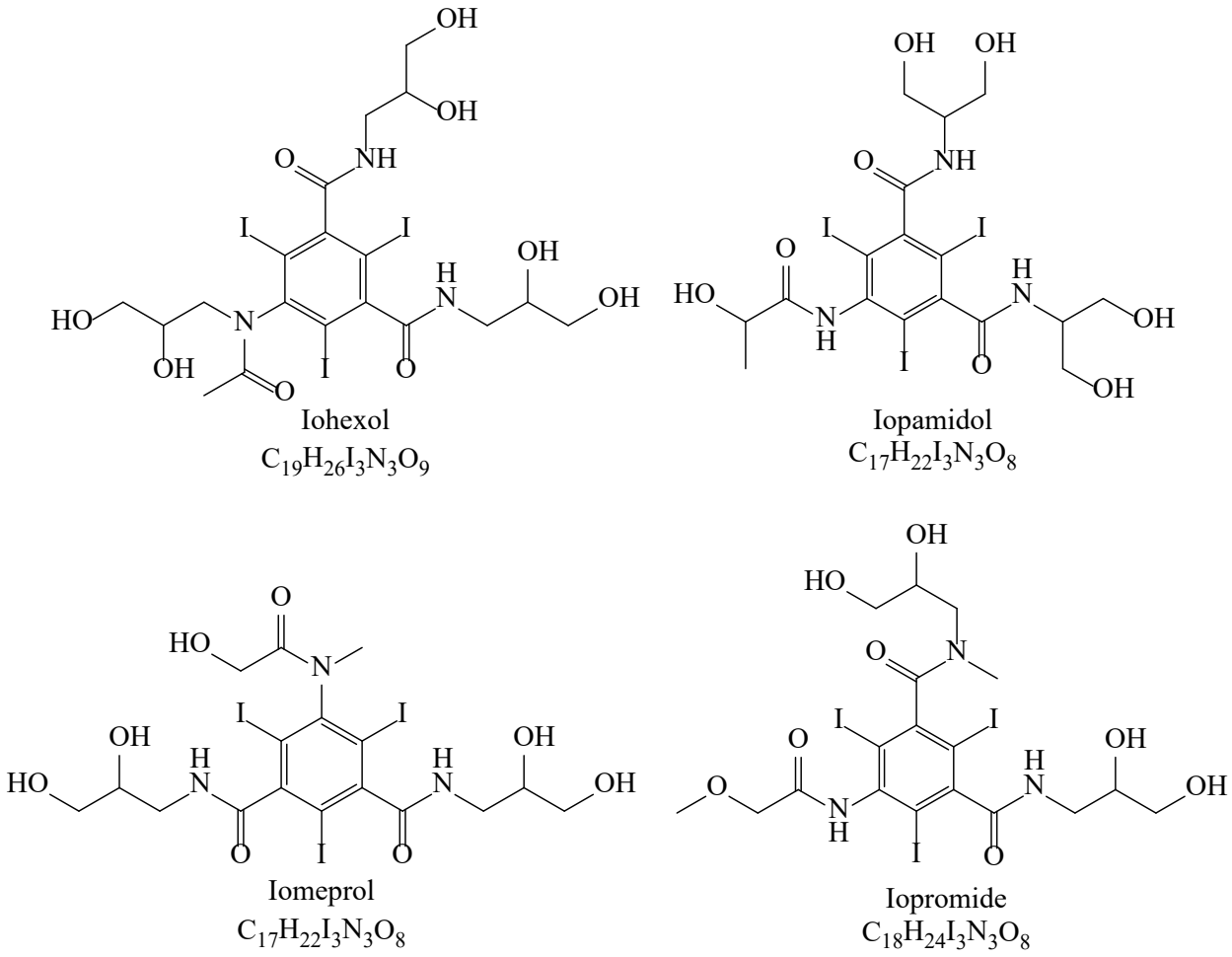


Figure 3-1: Examples of iodinated contrast agents used in medical imaging and found in US surface waters.

These organic iodine compounds are widely used globally (75 million patient applications/year) and are very persistent through conventional wastewater treatment plants since

they do not have adequate mechanisms for complete iodine removal (Hollender et al., 2009; Ternes and Hirsch, 2000). Previous research groups have investigated the discharge rates of ICMs to the environment and determined that they include multiple biotransformation products in wastewater effluents; however, the high-molecular weight, triiodinated structure is conserved with only the amide moieties altered (Drewes et al., 2001; Kormos et al., 2011; Schulz et al., 2008; Ternes and Hirsch, 2000). Once the biotransformed contrast agents are discharged to a surface water, further transformation can occur due to UV photolysis (Fabbri et al., 2016).

While Duirk et al. (2011) showed that ICMs present in a drinking water source could lead to I-DBP formation in the finished water, they did not look for transformed ICMs or link them back to specific point sources. With multiple transformation products possible, using a targeted analysis would provide an incomplete picture of the organic iodine being discharged or circulating within a surface water. If only the parent ICMs are targeted for analysis in environmental matrices, they would likely underestimate the concentration of total organic iodine (TOI) precursors that might lead to I-DBP formation in disinfected drinking water (Duirk et al., 2011). A direct link has not yet been established between these biotransformed contrast agents and the formation of I-DBPs in a drinking water treatment plant downstream of the wastewater discharge. By only looking at parent ICMs, there may be potential I-DBP precursors that are not accounted for and an aggregate concentration of organic iodine may thus provide more information for predicting I-DBP formation potential in finished drinking water.

With some of the ICM biotransformation products already identified and an unknown amount of TOI remaining, a qualitative approach is needed to determine the origin of the organic iodine. Non-target analysis using high resolution mass spectroscopy (HRMS) with a focus on iodine is the best approach to identifying the anthropogenic source(s) and has been previously

applied to identify organic iodine in seaweed (Yang et al., 2016) and lake sediments (Peng et al., 2016). Using liquid chromatography (LC) coupled with an electrospray ionization quadrupole time-of-flight (Q/TOF) MS, a systematic screening approach can determine the exact mass of the parent compound from the mass accuracy associated with the TOF and the unique isotope pattern associated with each unique analyte (Schymanski et al., 2015).

If the ICM or its biotransformed products reach the DWTP, further transformation can occur in the presence of a strong oxidant such as chlorine. When organic iodine compounds are oxidized by HOCl in the presence of other organic matter in high-concentration laboratory experiments, I-DBPs such as iodinated trihalomethanes (I-THMs) and iodinated haloacetic acids (I-HAAs) can be formed likely because of fast reaction rates for dehalogenation reactions or halogen-substitution reactions and availability of reaction rich sites on the organic matter (Duirk et al., 2011; Wendel et al., 2014). Previous experiments have suggested a dehalogenation reaction or a halogen-substitution reaction where an iodine atom on the ICM ring structure is substituted with a chlorine atom allowing the released iodine to form the iodinating agent, HOI (see Figure 3-2) (Wendel et al., 2016). Based on the HOCl+HOI and HOCl+HOCl+HOI reaction rates given earlier, the presence of excess HOCl would be expected to rapidly oxidize the HOI to iodate (IO_3^-) but the presence of organic matter could also provide an opportunity for iodide incorporation and the formation of organic I-DBPs.

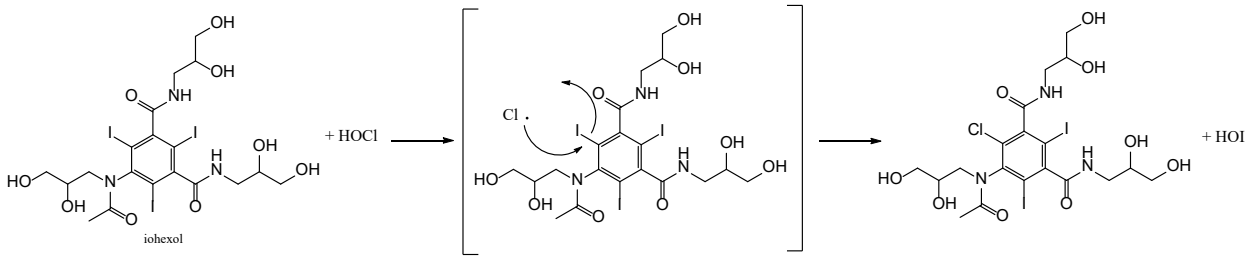


Figure 3-2: Proposed halogen-substitution reaction of iohexol and HOCl for HOI formation (intrepretation of Wendel et al., 2016).

Water quality parameter correlation analysis can also be used to confirm whether organic iodine is originating from the WWTPs by comparing concentration relationships to previously determined wastewater surrogates, such as dissolved nitrogen (DN) and conductivity. Excitation-emission matrix (EEM) fluorescence spectroscopy also allows for the characterization of organic carbon using the ratio of emission to excitation intensity of known fluorophores at specific wavelengths for the hydrophobic acid fraction (Peak A, λex/λem∼260/380–420nm), humic-like fraction (Peak C, λex/λem∼350/420– 480nm), and hydrophobic base (or protein-like) fraction (Peak T, λex/λem∼220/303 nm). Peak T can also indicate the presence of biologically derived or protein-like organic matter often seen in wastewater discharge (Gabor et al., 2014; Hudson et al., 2007; Ma, 2001).

For Chapter 3, the critical research question for the impact of ICMs and their derivatives in wastewater discharge is whether or not these chemicals will enter a downstream drinking treatment plant and remain somewhat intact through all treatment steps to become I-DBP precursors when a disinfectant is applied. The main objective was to track and link the organic iodine from wastewater discharge to the drinking water intact and finished water I-DBPs. For the study presented here, it was hypothesized that surface waters receiving treated hospital waste

effluent showed elevated levels of iodine and that drinking water plants using these impacted surface waters could generate I-DBPs.

3.2. Methods

3.2.1. Materials

All chemicals and materials are listed in Chapter 2 Table 2-2.

3.2.2. Identification of High Priority Sampling Areas

For this study, a man-made reservoir in a North Carolina watershed was selected that included three wastewater treatment plants, two of which received waste from large area hospitals that use iodinated contrast media for medical imaging procedures. Approximately 4 miles upstream of the reservoir, each of the surface waters receiving the treated wastewater discharge drained into the reservoir that served as a source of drinking water for downstream communities. According to the NC Surface Water Assessment Program Report (NC Division of Water Resources, 2017), the reservoir is highly impacted by upstream point and non-point sources and serves as the primary drinking water source for a population of approximately 160,000.

3.2.3. Sampling Strategy

Sampling sites for this case study included discharge from three wastewater treatment plants (WWTP1, WWTP2, and WWTP3) each using a four-stage Bardenpho Process treatment for nutrient removal (Crittenden et al., 2012) with sand filtration and Ultra Violet (UV) disinfection, pre- and post-WWTP discharge points into a receiving stream prior to reaching the downstream reservoir, and 9 drinking water reservoir monitoring stations designated by the NC

Department of Water Resources (Figure 3-3). Additional samples were collected within the DWTP that uses the reservoir as its source water. Reservoir characteristics are listed in the Appendix A Table A-1.

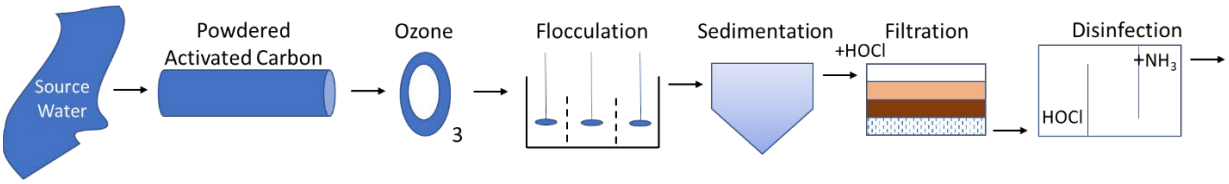


Figure 3-3 Drinking water treatment plant schematic.

Since the reservoir has a dam-controlled outflow, a river joining the reservoir after the drinking water intake was also sampled to determine if the river contributions impacted the water quality observed at the DWTP intake (Figure 3-4). The river sampling location was approximately 50 miles downstream of a wastewater treatment discharge point (WWTP 4) that has known hospital facilities in their waste collection areas. Discharge samples were not collected for WWTP 4.

Sampling locations for each of the upstream wastewater treatment plant impacting the reservoir were determined based on monitoring locations selected by the three individual wastewater utilities, with sampling points along small waterways prior to discharge and post discharge of the treated effluent (Table 3-1). Four sampling events (February, March, June, and July) occurred in 2017. Sampling of the receiving streams was conducted on the same day as the wastewater collection and all grab samples were collected at each location within the watershed, including the DWTP, within a 24 hr sampling window to ensure that weather events did not impact the analysis.

Table 3-1: Characteristics of the wastewater treatment plant (WWTP) point-sources.

Location	Average Flow (MGD)*	Impact Source
WWTP 1	5.0	None Known
WWTP 2	9.3	Regional Hospital (957-bed facility) known to use iohexol
WWTP 3	6.0	Regional Hospital (803-bed facility) known to use iohexol
WWTP 4 (upstream of River sampling location)	31	Wastewater discharge from Regional Hospital known to use iohexol

*Average flow as reported by each WWTP; MGD = million gallons per day

Since the parent ICMs can transform in the wastewater treatment plant and the aquatic environment (Kormos et al., 2011), the total dissolved iodine (TDI), total dissolved inorganic iodide (TDII), and total dissolved organic iodine (TDOI) were used to track the fate of the iodine. DWTP intake and finished water were also assessed for the presence of organic iodine and specifically I-THMs, as a surrogate for I-DBPs, to determine the impact of the ICM waste on drinking water quality following disinfection. The treatment steps used in the DWTP are shown in Figure 3-3. The NC Department of Environmental Quality, Division of Water Resources collected samples at Secchi depth from 9 monitoring stations on the reservoir labeled as A to I on Figure 3-4 (ESRI Online, Redlands, California, US) during each sampling event. The average Secchi depth for the source water samples was 0.7 m (range 0.4 to 1.8 m) for all locations and all four sampling events.

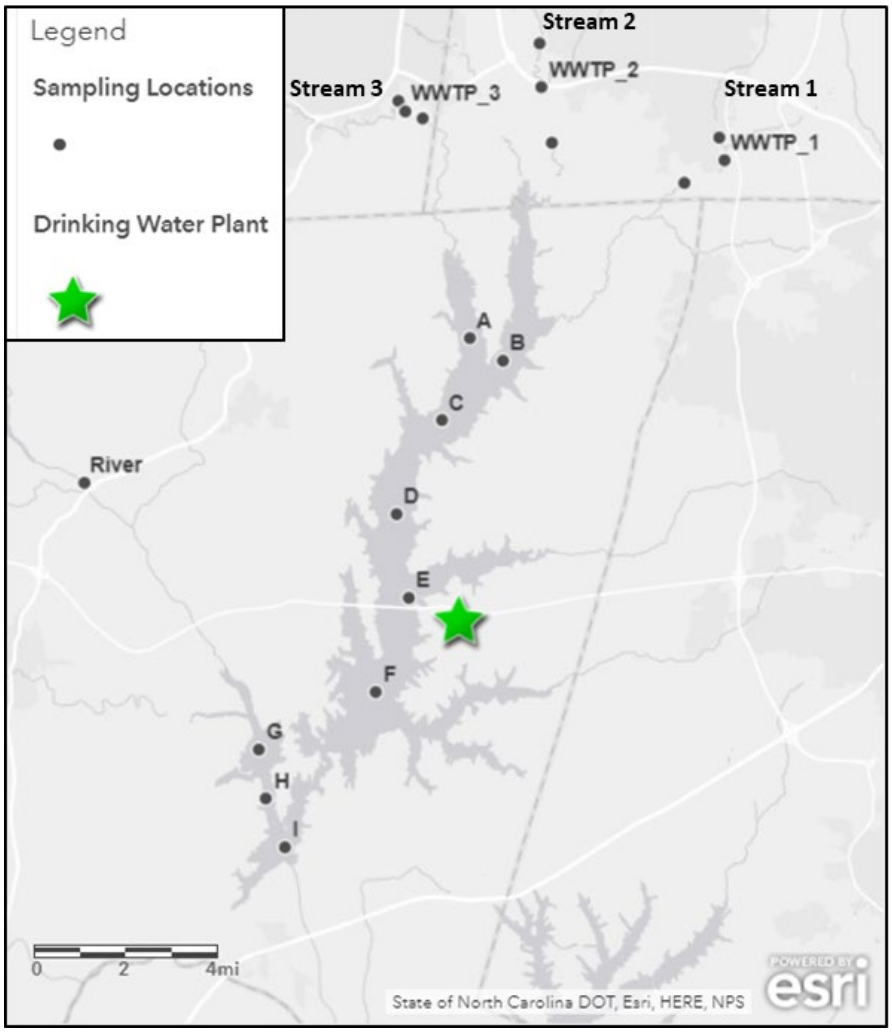


Figure 3-4: Sampling locations across the reservoir supplying the drinking water source (WWTP 4 is located 50 miles upstream of the River sampling location and not pictured on this map).

Based on U.S. Geographical Survey (USGS) stream gauges and average discharge rates for each WWTP, the percentage of wastewater by flow can be calculated (Table 3-2). With changes in precipitation events which translated to flow fluctuations prior to the four sampling events, the percentage of wastewater entering the reservoir from the three surface water streams ranged from 10 to 45% in Stream 1, 2 to 44% in Stream 2, and 8 to 40% in Stream 3.

Precipitation changes, whether drought or flood conditions, will have a significant impact on percentage of treated wastewater being introduced into the drinking water source reservoir.

Table 3-2: USGS surface-water statistics for sample locations.

Location	Flow (MGD)*	% Wastewater by Flow
Receiving Stream 1 for WWTP 1	8.4 (February) 9.0 (March) 43.3 (June) 6.2 (July)	5.0/(8.4+5.0)*100 = 37% 36% 10% 45%
Receiving Stream 2 for WWTP 2	82.7 (February) 22.0 (March) 453 (June) 11.6 (July)	9.3/(82.7+9.3)*100 = 10% 30% 2% 44%
Receiving Stream 3 for WWTP 3	12.9 (February) 15.5 (March) 96.3 (June) 35.6 (July)	6.0/(12.9+6.0)*100 = 32% 28% 5.8% 14%
River joining reservoir after DWTP intake	271 (February) 237 (March) 7,950 (June) 274 (July)	31.0/(271+31.0)*100 = 10% 12% <1% 10%
^Outlet of Reservoir	403 (February) 672 (March) 239 (June) 271 (July)	(5.0+9.3+6.0+31.0)/(403+5.0+9.3+6.0+31.0)*100 = 11.3% 7.1% 17.7% 15.9%

*MGD = million gallons per day; ^North Carolina Division of Water Resources, 2017

3.2.4. Analytical Methods

All surface water samples were collected in 500 mL high density polypropylene containers and kept on ice in a cooler until delivered to the laboratory where they were then stored in the dark at 4^{o}C for up to 14 days before processing. The treated drinking water plant samples were collected headspace-free in acid-washed, glass bottles prepared with appropriate disinfectant quenching agents for each analysis and I-THM samples were processed within 48

hrs. All samples were filtered through 0.45 µm nylon filters (Fisher Scientific, Pittsburgh, PA, USA) prior to analysis, except those intended for I-THMs, so that only dissolved levels of iodine were compared and quantified. Table 3-3 outlines the water quality parameters monitored, sample volume needed for analysis, and quenching agent used for drinking water samples.

Table 3-3: Analytical procedures used for sample analysis.

Parameter	Instrument	Sample Volume	Quenching Agent (Drinking Water Samples)
Total Dissolved Iodine (TDI)	Agilent 7500cx Inductively Coupled Plasma – Mass Spectrometer (ICP-MS)	20 mL	sodium sulfite (Analytical Grade, Fisher Scientific, Pittsburgh, PA, USA)
Dissolved Inorganic Iodine (Iodate and Iodide)	Dionex Ion Chromatography - Electroconductivity Detector (IC-ED)	20 mL	ethylenediaminetetraacetic acid (EDTA) (Analytical Grade, Fisher Scientific, Pittsburgh, PA, USA)
Total Dissolved Organic Halides (TDOX)	Rosemount-Dohrmann DX-2000 Organic Halide (TOX) Analyzer	100 mL	sodium sulfite
Total Dissolved Organic Iodine (TDOI)	TOX Analyzer, ICP-MS and IC-ED	100 mL	sodium sulfite
Dissolved Organic Carbon (DOC)/ Dissolved Nitrogen (DN)	Shimadzu TOC-V_{CPH} and TOC-V_{CPN} Analyzer	50 mL	sodium sulfite
Excitation Emission Matrices Fluorescence Spectroscopy (EEM)	Horiba-Jobin Yvon Fluorolog-321	10 mL	sodium sulfite
UV Absorbance	Hewlett Packard 8452A Diode Array Spectrophotometer	10 mL	sodium sulfite
Conductivity	Accumet 13-620-160	10 mL	EDTA
Non-Target Analysis	Agilent 6520 Liquid Chromatography Accurate Mass Quadrupole/Time-of-Flight (LC-Q/TOF)	250 mL	ascorbic acid (Analytical Grade, Fisher Scientific, Pittsburgh, PA, USA)
Trihalomethanes (THMs)	HP 6890 Gas Chromatograph micro Electron Capture Detector (µGC-ECD)	60 mL	ascorbic acid

Ten trihalomethanes (THM10, trichloro- (TCM), tribromo- (TBM), triiodo- (TIM), bromodichloro- (BDCM), dichloroiodo- (DCIM), dibromochloro- (DBCM), dibromoiodo- (DBIM), bromochloroiodo- (BCIM), chlorodiiodo- (CDIM), and bromodiiodo-methane (BDIM)) were liquid-liquid extracted using a concentration factor of 10 with methyl tert-butyl ether (MtBE) and analyzed on a gas chromatograph with a ZB-1 capillary column (30 m length, 0.25 mm inner diameter, 1.0-µm film thickness) (Phenomenex, Torrance, CA) and a ^{63}Ni micro electron capture detector (µGC-ECD) (Weinberg et al., 2002). THM standards were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA) and Orchid Cellmark (New Westminster, BC, Canada). All samples were analyzed in duplicate and 1,2-dibromopropane was used as an internal standard. The practical quantification limit (PQL) for each of the 10 THMs was 0.1 µg/L.

TDI of the filtered aqueous sample was determined by targeting the m/z 127 ion on the ICP-MS in no-gas mode with a 100 µg/L tellurium (m/z 125) internal standard (Takaku et al., 1995). Samples were made basic at a 5% ammonium hydroxide (Fisher Scientific, Pittsburgh, PA, USA) concentration to increase iodine signal stability. Samples were filtered prior to fractionation of TDOX into TDOI used the method of adsorption, pyrolysis, and collection (Hua and Reckhow, 2006) using the TOX analyzer followed by quantitation with ICP-MS. DOC and DN were measured following Standard Method 5310 (APHA et al., 1999). Specific UV absorbance at 254 nm was calculated by the EPA Method 415.3 (Potter and Wimsatt, 2009) and conductivity was measured using a conductivity electrode.

Further analysis of the organic iodine in the wastewater discharge and drinking water intake was conducted using non-target high resolution mass spectrometry analysis to qualitatively assess the organic iodine species and to quantify parent ICMs. The non-target

analysis used solid-phase extraction with Strata-X 60 mg cartridges (Phenomenex, Torrance, CA) in which the water sample was filtered after pH adjustment to 2 using H_2SO_4 to ensure protonation of target species for maximum recovery. Following the method previously used for measuring ICM parent compounds and biotransformation products in water (Pereira, 2005), 500 mL aliquots of each sample were adsorbed onto the cartridges then eluted into 10 mL of MtBE. Analytes were chromatographed on a Waters Acquity® LC CSH-C18 column (Milford, Massachusetts) using a 0.1% formic acid in water:0.1% formic acid in acetonitrile mobile phase with gradient elution (See Appendix A Table A-2) using an Agilent 6520 LC-Q/TOF high-resolution mass spectrometer (Santa Clara, California). The non-target analytical method consisted of triplicate full scans (50 to 950 m/z) to determine the parent mass and the associated isotope patterns. A semi-targeted LC-Q/TOF method was also used to identify parent ICMs by insource ionization for m/z 127 fragmentation.

A non-targeted screening workflow for iodinated organics was developed using the multiple Agilent software packages MassHunter Qualitative Analysis, ID Browser, Profinder and Mass Profiler Professional (Agilent Technologies, Santa Clara, CA, USA). The following steps for non-target analysis provide a basis for data reduction and feature-finding to deconvolute a full scan into individual chemical peaks:

1) Profinder was used to compare triplicates and remove molecular features that were not found in all three runs. Using the batch recursive extraction, molecular features were extracted independently from all three runs, compared against each other, and merged based-on retention times and m/z. In Profinder, the molecular feature extraction (MFE) tool for qualitative analysis matched the ions, isotopes, and adducts at retention times to establish if a peak is present.

2) After the triplicates were merged, they were compared to the merged blank triplicates to further confirm the molecular features were unique to the sample and not artifacts of the method. Mass Profiler Professional also looked for the "fold change" or the statistical significance of each individual mass feature and any feature shared with the blank were removed from the entity lists.

3) Further quality control was conducted on each triplicate using principle component analysis plots to determine if any samples were significantly different from the others. This option also served as a check for incorrectly assigned parameters during the previous data reduction steps.

4) Once the data reduction and quality control checks were completed, the ID Browser analysis was used to identify molecular features using the personal compound database with the exact mass and molecular formula for over 60 organic iodine species and known transformation products of iodinated contrast agents not listed in the library, and generating formulae for molecular features based on theoretical exact mass and isotope matches. The generating formulae option was also constrained for specific elemental compositions to focus on iodine containing compounds.

5) For further analysis between samples, multiple entities were created for each molecular feature list. A multiple-entity analysis was used to compare possible shared iodine-containing compounds for TDOI source tracking through "fingerprints" observed for each extracted sample.

3.3. Results

3.3.1. Surface Water Quality Data

This study first confirmed that the source of the organic iodine in the surface water was in the effluent discharged from WWTPs 2 and 3, since the concentrations were the highest in these and the post-WWTP stream concentrations were higher than those in the receiving stream prior to discharge. WWTPs 2 and 3 were known to serve large regional hospitals where iodinated medical waste would originate from in-patient procedures; WWTP 1 does not serve an area hospital and its effluent shows a lower but measurable contribution to the organic iodine load. Since iodinated contrast agents can be used for in- and out-patient procedures, ICM elimination from the body can take place away from the medical facility and contribute to source diffusion from septic tank leachate and WWTP discharges with no known medical facilities in the collection area. Appendix A Tables A-3 to A-6 show the surface water quality data for each sampling event.

Table 3-4 shows the TDI and TDOI concentrations from the water quality data for the March sampling event in the WWTP effluents, pre and post WWTP effluent streams, surface water, and the DWTP intake. The sampling locations listed in Table 3-4 are presented in sequence; for example, WWTP 3 is upstream of Location B which are both upstream of the DWTP intake. Iodate (PQL=20 µg/L) and iodide (PQL=10 µg/L) were not detected in the surface water and wastewater samples.

Table 3-4: Iodine analysis (N=2) for wastewater discharges and impacted surface waters for the March 2017 sampling event (average between duplicate analyses ± difference between average and duplicates). See Figure 3-3 for sample locations.

Sample Locations	Total Dissolved Iodine (µg as I/L)	Total Dissolved Organic Iodine (µg as I/L)
Pre-WWTP 1	39 ± 1	22 ± 3
WWTP 1	25 ± 1	17 ± 3
Post-WWTP 1	29 ± 1	17 ± 2
Pre-WWTP 2	19 ± 1	15 ± 2
WWTP 2	135 ± 1	113 ± 7
Post-WWTP 2	54 ± 2	37 ± 6
Pre-WWTP 3	14 ± 1	8 ± 2
WWTP 3	95 ± 1	98 ± 5
Post-WWTP 3	57± 1	53 ± 4
Location A	36 ± 1	27 ± 2
Location B	34 ± 2	28 ± 5
Location C	31 ± 1	25 ± 2
Location D	29 ± 1	23 ± 2
DWTP Intake	28 ± 1	15 ± 2
Location E	27 ± 1	18 ± 2
Location F	26 ± 1	18 ± 2
River	22 ± 1	23 ± 2
Location G	26 ± 1	18 ± 2
Location H	25 ± 1	17 ± 4
Location I	25 ± 1	18 ± 2

As seen from Table 3-4 and Appendix A Tables A-3 to A-6, organic iodine was detected consistently throughout the reservoir with the higher concentrations of iodine located at the north end closest to the sources of discharge (Location A) from the WWTPs 2 and 3 compared to the river input in the south (Location I). The decrease in TDI and TDOI concentrations from the wastewater discharge points across the reservoir to the drinking water intake are likely due to dilution effects from stream and runoff inputs and possibly adsorption to sediments (Peng et al., 2016).

3.3.2. Organic Iodine in the Drinking Water Treatment Plant

Table 3-5 show the TDOI and I-THM6 concentrations (sum of triiodo-, dichloroiodo-, dibromoiodo-, bromochloroiodo-, chlorodiiodo-, and bromodiiodo-methane) for the DWTP intake and finished water during the 2017 sampling events. This assessment of the organic iodine from the DWTP samples shows variability in TDOI concentration and I-THM formation between the four sampling events. However, organic iodine is still detected during each collection event and I-THMs are in measurable quantities in the finished water.

Table 3-5: Organic iodine in the drinking water treatment plant (DWTP) intake and finished water for each 2017 sampling event (see Figure 3-3 for schematic) (average between duplicate analyses ± difference between average and duplicates).

Sampling Event	Location	Average TDOI (µg as I/L)	Average I-THM6 (µg as I/L)
February	Intake	18.3 ± 0.3	*<0.1
	Finished Water	4.5 ± 0.4	0.9 ±0.3
March	Intake	15.6 ±0.3	<0.1
	Finished Water	2.4 ±0.3	1.6 ±0.2
June	Intake	14.8 ± 0.3	<0.1
	Finished Water	5.0 ± 0.5	3.8 ±0.2
July	Intake	9.0 ± 0.2	<0.1
	Finished Water	4.6 ± 0.4	3.4 ± 0.2

*- the concentration of each species was <0.1 µg/L

In general, chlorinated and brominated I-THM species were present in each of the sampling events, but TIM was not quantifiable. Overall, the I-THMs were present at lower concentrations during the February and March events compared to the June and July events, even though the influent TDOI concentrations were higher in February and March. Table 3-6 shows the speciation concentration as iodine observed for the I-THMs during each sampling event.

Table 3-6: Concentration as iodine for each individual I-THM.

Sampling Event	Finished Water Concentrations (μg/L as I)					
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM
February	<0.1	<0.1	0.6	0.1	0.2	<0.1
March	0.8	<0.1	0.6	0.1	0.1	<0.1
June	0.8	0.9	0.4	0.8	0.9	<0.1
July	1.1	1.0	<0.1	<0.1	1.2	<0.1

The organic iodine that enters the DWTP is partially removed or transformed during treatment into I-THMs. This transformation of chemical structure is evidenced by an absence of I-THMs in the DWTP intake and the presence of I-THMs in the finished water while the TDOI decreases. If unit process treatments were optimized for TDOI removal within the DWTP, then I-THM formation could be mitigated before distribution to the consumers.

3.3.3. Non-Target Analysis for Iodine Source Tracking

Qualitative analysis was used to identify the LC-Q/TOF chromatographed compounds that included iodine from the WWTP discharges and the DWTP intake to determine the source of the I-THM precursors. Thousands of molecular features were isolated from WWTP 1, 2, and 3 and DWTP intake extracts, ranging from 4947 in the DWTP intake to 6206 features in the WWTP 2 discharge. A multi-variate analysis data-reduction was performed to further elucidate which compounds were likely to contain either one, two, or three iodine atoms in the proposed molecular formula generated by exact mass, retention time, and isotope patterns with relative abundance of each m/z. Based on the molecular feature variables used in the MFE tool algorithm, each assignment of a molecular formula was given a score as to the goodness of fit by the ID Browser software, where a score of >70 out of 100 was needed for a tentative identification.

The presence of iohexol was confirmed in the WWTP 2 extract using a standard spiked at 25 µg/L as I into the extract enhancing the chromatographic peak already present which matched the retention time, fragmentation pattern, and exact mass of the peak for the unspiked sample (Appendix A Figure A-1). Using the known spiked iohexol concentration and the peak area, its approximate concentration in the original extract was determined to be 0.17 µg/L as I. Based on the concentration factor used for the sample extraction and a 50 % analyte recovery through the method this would translate to an approximate concentration of 6.8 ng/L in the effluent discharge. This chromatogram also shows an ICM or a biotransformed ICM at retention time of 5 min and due to the broad peak shape it could be attributed to two poorly resolved iohexol tautomers (Kormos et al., 2009). Tentative identification by using the non-target screening work flow for organic iodine is not able to distinguish between tautomers unless they are well resolved and can be associated with unique retention times.

Previous studies have observed >80% biotransformation of parent ICMs iohexol and iopromide to other products that still had the tri-iodinated ring structure intact; however, iopamidol only had a 35% biotransformation from the WWTP intake to the final effluent (Kormos et al., 2011). However, based on the findings from this case study, the low concentration of iohexol in WWTP effluent would suggest that parent ICMs may only contribute minimally to the TDOI concentration and the biotransformation products may represent a larger fraction.

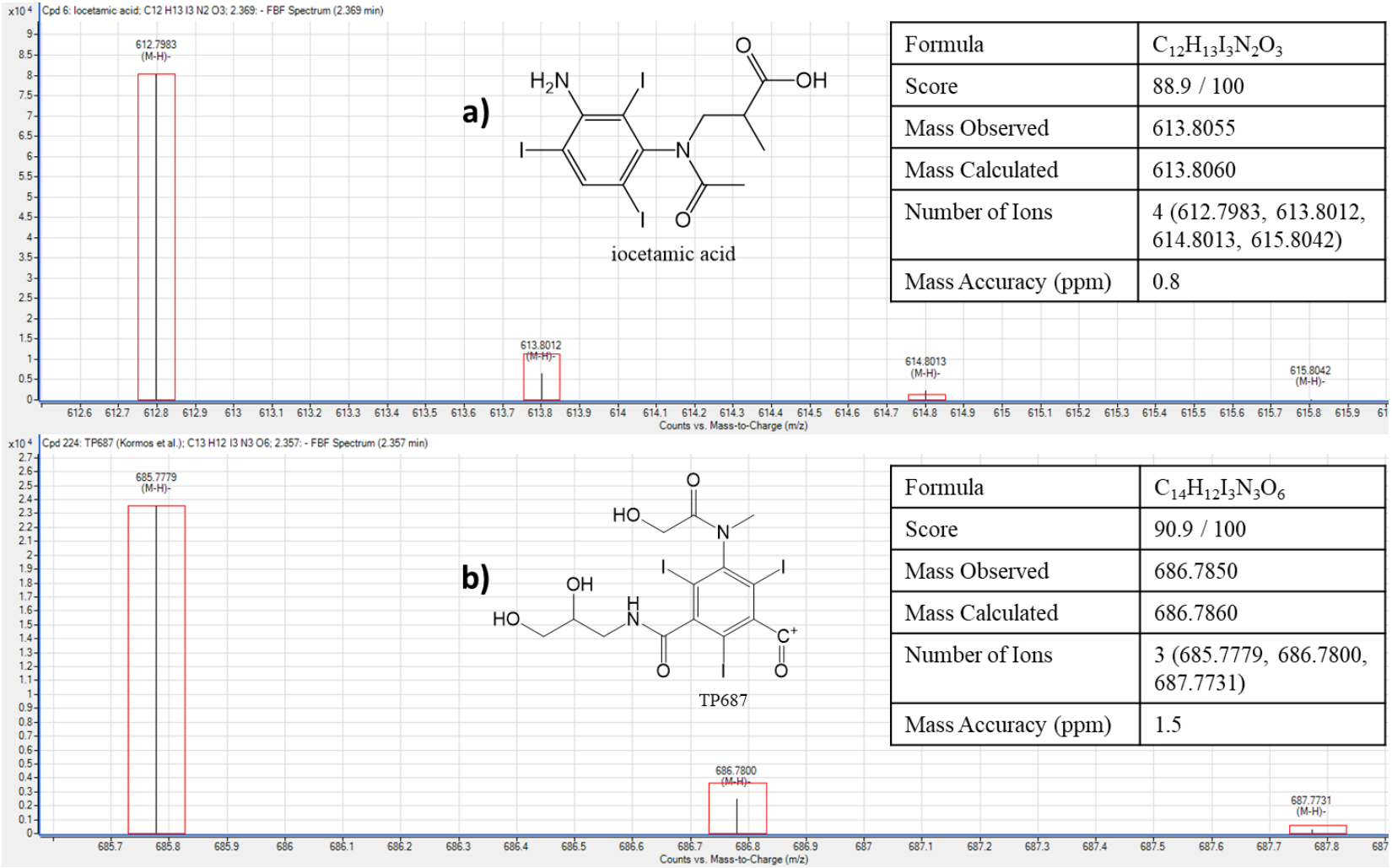
The compound lists for named transformation products identified by previous researchers and parent ICMs are presented for each WWTP effluent extract and DWTP intake extract in Appendix A Tables A-7 to A-12. The lists for each sampling event show the identification score, chemical formula, calculated mass, observed mass, mass accuracy, retention time, and number of

ions used for identification for each identified compound. Each chemical formula on the lists could be associated with different isomers, but based on the predominant use of iohexol in the upstream hospitals it is more plausible that these compounds are related to that parent ICM. Figure 3-5 shows the ID Browser tentative identification variables for two of the transformation products, iocetamic acid and iohexol TP687A/TP687B.

In all extracts tested, iocetamic acid ($C_{12}H_{13}I_3N_2O_3$) and iohexol TP687 ($C_{14}H_{12}I_3N_3O_6$) were present (Figure 3-5). From Kormos et al. (2011) identification of TP687, this iohexol ($C_{19}H_{26}I_3N_3O_9$) transformation byproduct is a Phase II (two-steps needed from parent ICM) transformation product previously measured in WWTP pre-denitrification and nitrification, post-denitrification and nitrification, and in the final effluent at concentrations <2 nmol.

WWTP 2 extracts also contained compounds tentatively identified as iohexol for all four sampling events, with the March and July extracts also containing iohexol TP787, iohexol TP835, iohexol TP773, and iohexol TP863. The March extract for WWTP 2 and June extract for WWTP 3 had a compound identified as iohexol. The June WWTP 3 extract also included compounds identified as iohexol TP787, iohexol TP817, iohexol TP819, iohexol TP773, iohexol TP835, iohexol TP863, and iohexol TP849.

The variability of iodinated compounds within each WWTP and between WWTPs shows that monitoring a single ICM or transformed ICM chemical rather than an aggregate measurement of TDOI will underestimate the iodine precursor load that could enter the downstream DWTP. Since analytical standards are not available for the majority of the biotransformed ICMs, there is a limited ability to monitor their concentrations other than using the TDOI analysis.



Formula	$C_{12}H_{13}I_3N_2O_3$
Score	88.9 / 100
Mass Observed	613.8055
Mass Calculated	613.8060
Number of Ions	4 (612.7983, 613.8012, 614.8013, 615.8042)
Mass Accuracy (ppm)	0.8

Formula	$C_{14}H_{12}I_3N_3O_6$
Score	90.9 / 100
Mass Observed	686.7850
Mass Calculated	686.7860
Number of Ions	3 (685.7779, 686.7800, 687.7731)
Mass Accuracy (ppm)	1.5

Figure 3-5: ID Browser identification tools for calculated isotope fraction relative abundance (red outlines) and observed isotope fraction abundance (black centroid lines). Tables include identification variables and scores for a) iocetamic acid and b) iohexol TP687.

3.4. Discussion

3.4.1. Correlation Analysis for TDOI

Using the water quality data presented for TDOI, TDI, TDOX, DOC, DN, SUVA, EEMs Peak T, and conductivity in Appendix A Tables A-3, A-4, A-5, and A-6, a correlation matrix graphing each parameter of water quality data was built (Appendix A Table A-13) to determine if trends existed within the chemical analysis. All surface water data were included in the correlation analysis for TDOI since all locations had detectable levels, including the pre-wastewater samples. Table 3-7 displays Pearson's correlation coefficients for each of the water quality parameters compared to TDOI. Correlation between water quality variables was determined using R Statistical Software version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria). Since TDOI is a component of TDI and TDOX, the strong correlations were expected. The DOC and SUVA, which is calculated from DOC, did not show a correlation with TDOI suggesting the iodine incorporation in the DOC is insignificant when compared on a concentration basis. DN, Peak T, and conductivity are water quality parameters often associated with wastewater discharge and since the TDOI originates from the wastewater discharge there is a significant correlation between these variables.

Table 3-7: Pearson's correlation coefficients (r) for reservoir water quality parameters (N=68 for each parameter).

Water Quality Parameter	TDOI
Total Dissolved Iodine (TDI)	0.969 ($p < 0.001$)
Total Dissolved Organic Halogens (TDOX)	0.767 ($p < 0.001$)
Dissolved Organic Carbon (DOC)	-0.055 ($p < 0.7$)
Dissolved Nitrogen (DN)	0.696 ($p < 0.001$)
SUVA	-0.353 ($p < 0.002$)
Fluorescence Excitation Emission Matrix Peak T (Tryptophan-like)	0.666 ($p < 0.001$)
Conductivity	0.534 ($p < 0.001$)

As seen in the Appendix A Tables A-3 to A-6, Peak T for each post-wastewater sampling point was elevated when compared to the pre-discharge sample. Since Peak T is an indicator of biologically derived or protein-like organic matter, the elevated levels of Peak T seen in the wastewater discharge and post-wastewater samples are consistent with previous observations (Gabor et al., 2014; Hudson et al., 2007; Ma, 2001). The positive correlation of TDOI with DN ($r=0.696$, $p< 0.001$) confirm a link between TDOI and the wastewater discharge since they originate from the same wastewater effluent. Since WWTPs are required to monitor nitrogen in their discharge according to the EPA National Pollutant Discharge and Elimination System (NPDES) permit, there is continuous monitoring of DN which could be used to predict possible ICM-impacted waters when medical imaging facilities are within the collection area.

In this case study, organic iodine discharged from two WWTPs that receive waste from large hospital facilities reaches a downstream drinking water intake. TDOI concentration in the three wastewater effluents is compared to that in the downstream finished drinking water in Table 3-8. The quantitative transformation of influent TDOI to I-THMs in the finished water does not show a significant linear relationship ($r=0.50$, $p< 0.5$, Table 3-8).

Table 3-8: Total dissolved organic iodine (TDOI, µg/L as I) in wastewater effluent and in finished water leaving the drinking water treatment plant (DWTP).

	TDOI (µg/L as I)			
Sample Collection	**WWTP1**	**WWTP2**	**WWTP3**	**DWTP Finished Water**
February	20	164	65	4.5
March	17	113	98	2.4
June	27	129	63	5.0
July	13	92	50	4.6

To further assess the impact of TDOI on the I-THM formation, iodine-incorporation factors were calculated for each sampling event and compared to the DWTP intake TDOI concentration. Equation 1 was used to calculate the iodine incorporation for the I-THMs:

$$Iodine\ Incorporation\ = \frac{\sum molar\ conc. \times \#Iodide}{\sum(molar\ conc.)\times(\#halogens)} \times 100 \qquad (1)$$

where the molar concentration of the iodinated species and the total number of halogenated species are used to determine the iodine incorporation factor. Appendix A Tables A-14 and A-15 show the I-THM6 and THM4 molar concentrations for each sampling event, respectively, with an example calculation for iodide incorporation into THMs shown in Appendix A Equation 1.

February had the lowest iodine incorporation of 2.4%, followed by both June and July with incorporation factors of 2.6%. March had the lowest finished drinking water TDOI at 2.4 µg/L as I but the highest iodine incorporation at 5.8%. Since the iodine incorporation percentage does not correlate to the TDOI concentration, variability in organic iodine speciation and removal/transformation efficiencies within the DWTP may play a significant role in the control of I-THM formation. Specifically, the free chlorine contact time prior to the formation of chloramines in the finished water could alter I-THM formation since previous literature suggests iodide forms iodate in the presence of free chlorine (Bichsel and von Gunten, 1999). Chapter 4 explores the changes to TDI and formation of I-THMs in each unit process throughout the same DWTP used in this case study (Figure 3-3).

With this correlation analysis and consideration of DWTP pre-treatment options, the medical waste residues may need to be treated at source rather than by the wastewater or drinking water treatment plants to ensure complete removal from the waste stream. Even with the advanced treatment used in all three WWTPs there was insufficient iodine remediation and

the treatment processes need to be further optimized for TDOI removal prior to discharge so that I-THM formation is limited in downstream drinking water plants.

3.4.2. Theoretical Cytotoxicity Changes Due to I-THM Formation

Based on the formation of I-THMs in the four sampling events as shown in Table 3-6, the theoretical cytotoxicity evaluation associated with the I-THMs was calculated using a method by Smith et al. (2010) and cytotoxicity data (Plewa et al., 2017; Wagner and Plewa, 2017). The calculated relative cytotoxicity technique is a powerful theoretical tool that uses the LC_{50} value and molar concentration for each chemical to predict the toxicity of DBP mixtures. This calculation will change based on the individual species present, particularly for those iodine-containing DBPs which have been as labeled forcing factors due to their relatively high toxicity compared to chlorine- and/or bromine-containing DBPs even at low concentrations (Allard et al., 2015; Wendel et al., 2014; Yang et al., 2014). Equation 2 was used to calculate the theoretical toxicity of a DBP mixture and assumes an additive interaction:

$$Total\ Relative\ Toxicity = \sum([DBP] \times (LC_{50})^{-1} \times 10^{6}) \quad (2)$$

where the total toxicity is the sum of the individual DBP concentrations in M times the reciprocal of the LC_{50} for cytotoxicity in units of molarity (M) determined for individual DBPs from the CHO cell assay with an adjustment factor of 10^6 to bring the calculation into whole numbers (Allard et al., 2015; Plewa et al., 2017; Smith et al., 2010). The molar concentrations for the individual I-THMs, theoretical toxicity LC_{50} values for individual THMs, and a sample calculation are shown in the Appendix A Equation 2.

To evaluate the total relative cytotoxicity increase associated with I-THM formation, the quantitative data from the GC-ECD was used to calculate the theoretical cytotoxicity associated

with the I-THMs and then compared to the theoretical cytotoxicity using the same concentrations as if they were attributed to TBM, the most toxic of the regulated THMs. THMs will still form without TDOI present, since NOM and HOCl residual would still be present in the finished water. When the relative cytotoxicity was calculated for the I-THMs detected in each sampling event and compared to the same concentrations attributed to TBM, the increase in total relative toxicity based on percent difference ranged from 38% (March) to 73% (February). This translates to a 1.5 to 2.1-fold increase ($p<0.001$, t-Test for paired two sample for means) in relative toxicity for this sub-group of DBP concentrations. Even with low levels of iodine incorporation (<6%) in the THM sub-group, the total relative cytotoxicity was significantly increased, which can have public health consequences with prolonged exposure.

3.4.3. Non-Target Analysis for Iodine-Source Tracking

Using the non-targeted screening technique with high-resolution mass spectrometry, at least a portion of the TDOI was shown to be associated with ICMs based on the biotransformation products identified and the presence of three iodine atoms in the proposed formula. Based on the named compounds tentatively identified in the extracts and the proposed molecular formulae of the remaining molecular features containing iodine, there is a direct connection to ICMs and their biotransformation products, which are being discharged from wastewater treatment plants receiving medical waste either from large hospitals or from individual patients. These compounds, ICMs and their transformation products such as iocetamic acid and iohexol TP687 that have the triiodinated benzene ring intact, are also entering the downstream DWTP intake where they can be removed by physical processes or further transformed by chemical reactions. If it was assumed that ozone did not alter the chemical

moieties of the TDOI compounds observed in the DWTP intake, then dehalogenation reactions can be proposed explaining possible pathways for the release of HOI that could then engage dissolved organic matter to produce low molecular weight DBPs such as I-THMs (Figure 3-6).

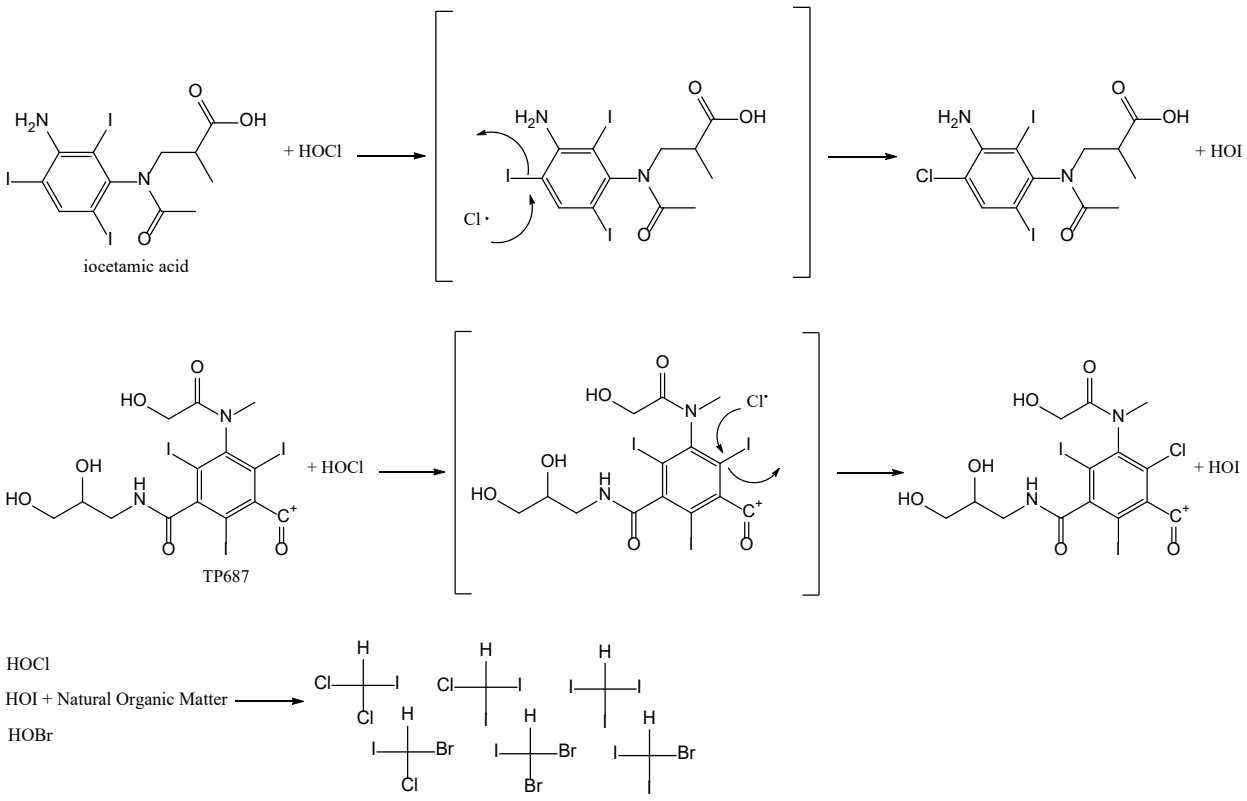


Figure 3-6: Proposed dehalogenation reactions of identified TDOI compounds found in the drinking water intake extracts and HOCl to form HOI then I-THMs.

3.5. Conclusions

This research highlights that wastewater plant discharges into surface waters that are downstream sources of drinking water can introduce persistent iodinated chemicals, at least some of which are from medical waste and can remain and become iodine precursors for I-THMs. Even with multiple unit processes in the DWTP studied here, the organic iodine was able to

persist through the treatment plant and produce I-THMs in the finished drinking water. Further investigation is necessary to determine if mitigation of organic iodine can be achieved by drinking water treatment processes or whether this should be the responsibilities of wastewater treatment strategies.

This case study supported the hypothesis that surface waters receiving treated hospital waste effluent contained levels of iodine that downstream drinking water plants using chloramine disinfection could convert into iodinated DBPs. Non-target chemical analysis has confirmed that one particular ICM, iohexol, may be responsible for at least a portion of the total organic iodine load in the drinking water source. Further identification of the iodinated organic species should be continued for additional confirmation of the iodine source. Correlation analysis suggests that dissolved nitrogen or Peak T from EEMs analysis could be used for surrogate iodine analysis in wastewater-impacted surface waters containing iodine. However, this correlation analysis needs to be strengthened through more surface water quality observations over seasonal changes and across watersheds.

This research highlights the need for increased drinking water source protection when upstream WWTP effluents contain organic iodine, such as from ICMs or their derivatives, due to the persistence of these I-THM precursors. I-THM formation in drinking water shows an increased theoretical cytotoxicity that could lead to adverse human health affects due to chronic exposure. With the increased need for water-reuse, the continual discharge of organic iodine in wastewater effluent containing residues of medical waste, and the widespread use of chloramines for disinfection, there is an increased risk of I-DBP formation in finished drinking water.

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CHAPTER 4 : TRACKING THE TRANSFORMATION OF TOTAL DISSOLVED IODINE WITHIN A DRINKING WATER TREATMENT PLANT: CAN PAC BE USED TO LIMIT I-THM FORMATION?

4.1 Introduction

Iodinated disinfection byproducts formed during drinking water treatment are a subgroup of DBPs that are also contaminants of emerging health concern due to their relative increased toxicity compared to the regulated DBPs (Richardson et al., 2008). Studies finding the 50% lethal concentration (LC_{50}) of a single compound with the Chinese Hamster Ovary (CHO) assay shows the cytotoxicity of the unregulated dichloroiodomethane (DCIM) is 2.3 times higher than the US Environmental Protection Agency (EPA) regulated trichloromethane (TCM) (Wagner and Plewa, 2017).

Based on the formation and stability of the iodinating agent HOI in drinking water conditions, I-DBPs are more likely to form in the presence of monochloramine than free chlorine due to the faster reaction rates with the latter ($k_{HOCl+HOI}$= 8.2 M^{-1} s^{-1}; $k_{NH2Cl+HOI}$=2x10^{-3} M^{-1} s^{-1}) (Bichsel and von Gunten, 1999). Chlorination of iodine-precursors also forms HOI but chlorine then rapidly oxidizes HOI to iodate, which greatly reduces iodine incorporation into organic DBPs (Bichsel and von Gunten, 1999).

Both inorganic and organic iodine precursors for I-DBPs have been measured in drinking water sources that also show a formation of iodinated trihalomethanes (I-THMs) and haloacetic acids (I-HAAs) in the finished water (Duirk et al., 2011; Richardson et al., 2008). These previous studies have not shown the transformation of organic iodine precursors within a drinking water

treatment plant or provided a treatment option for total dissolved iodine (TDI) removal. One subgroup of TDI that has been observed in surface waters receiving treated medical waste is iodinated contrast media (ICM), such as iohexol ($C_{19}H_{26}I_3N_3O_9$) (Duirk et al., 2011; Chapter 3). ICMs and their biotransformed derivatives can enter a drinking water treatment plant and become the iodine source for I-DBPs, but most studies do not investigate the changes to the ICM or biotransformed derivative within the treatment plant where the large molecular weight tri-iodinated structure is chemically changed to a small molecular weight I-THM or I-HAA.

Conventional drinking water treatment does not have targeted removal mechanisms for inorganic or organic iodine. A conventional drinking water treatment includes coagulation/flocculation, sedimentation, filtration, and disinfection with the main goal of pathogen inactivation/removal (Crittenden et al., 2012). In order to determine which treatment option to optimize for TDI removal, the changes in concentration and species needs to be determined for each unit process within a treatment plant. Occurrence data on I-THMs focuses primarily on the influent iodine concentrations and the finished water DBPs, with very little information of the distribution system concentrations other than one study that looked at one point in the system rather than multiple points throughout (Tan et al., 2016). Additional information on TDI will help with targeted removal before it can transform into cytotoxic I-DBPs and decrease consumer exposure through drinking water.

Previous studies have utilized powdered activated carbon (PAC) for micropollutant removal from soils and waters, utilizing the physical removal by adsorption mechanism. Within drinking water treatment, PAC is typically added for the removal of taste and odor compounds associated with algal blooms, which have limited health consequences and are typically public perception problems (Najm et al., 1991). PAC has also been used for targeted micropollutant

removal from water such as pesticides (Kearns et al., 2014; Thompson et al., 2016), per-/poly-fluoroalkyl substances (PFAS) (Hansen et al., 2010), pharmaceuticals (Kovalova et al., 2013), and personal care products (Tursi et al., 2018). Kovalova et al. (2013) found that the ICM diatrizoate was recalcitrant during PAC treatment of hospital wastewater but iomeprol, iopamidol, and iopromide could be physically removed up to 65%. Studies on the removal of organic iodine by PAC in a drinking water matrix have not been published.

PAC can be added at multiple stages in a water treatment plant as a dry powder or a pre-wetted slurry (Najm et al., 1991). PAC carbon sources originate from coal, wood, coconut shells, bamboo and other carbonaceous materials that are chemically or physically activated to increase surface area for maximum adsorption potential. PAC has a high ratio of area to volume and is designed to pass through an 80-mesh sieve or smaller (ASTM, 2019).

Since there are no US EPA drinking water regulations on taste and odor compounds, many treatment plants develop their own optimized dose procedures based on in-house jar testing for geosmin and 2-methyl isoborneol (MIB), which are the natural organics responsible for earthy and musty odors, respectively. These in-house dose response curves are highly dependent on water quality changes such as increased dissolved organic carbon (DOC) that can alter adsorption potential through competition and interference. The adsorption models for a given contaminant will inform PAC dosing needs for optimized removal based on water quality characteristics.

The objective of this study was to identify and investigate a treatment strategy for TDI removal in a drinking water treatment plant case study and to determine the changes to TDI speciation between unit processes that lead to I-DBP formation in the finished water.

4.2 Methods

4.2.1. Materials

Laboratory grade water (LGW) was prepared in-house from a Dracor system (Durham, NC, USA), which pre-filters inlet 7 MΩ-cm deionized water to 1 μm, quenches residual disinfectants, reduces total organic carbon to less than 0.2 mg as C/L with a activated carbon, and removes ions to 18 MΩ-cm with mixed bed ion-exchange resins. Free chlorine was prepared in LGW using an NaOCl stock solution (Fisher Scientific, Pittsburgh, PA, USA). Preformed monochloramine was produced by adding ammonium sulfate (Fisher Scientific, Pittsburgh, PA, USA) to free chlorine at a Cl_2/N weight ratio of 3/1. Ascorbic acid and sodium sulfite (Fisher Scientific, Pittsburgh, PA, USA) were used as quenching agents to remove residual chloramines or free chlorine. Sodium phosphate buffer was prepared at pH 7.4 using anhydrous monobasic sodium phosphate (Na_2HPO_4) and anhydrous dibasic sodium phosphate (Fisher Scientific, Pittsburgh, PA, USA). The PAC materials were Norit® PAC 20BF (Cabot Corporation, Boston, MA, USA), Aqua Nuchar 81283 (Ingevity, North Charleston, S.C., USA), and AquaSorb CP1, PAC-F (Jacobi Carbons, Columbus, OH, USA). Section 2.2.1 of Chapter 2 presents a full list of all other materials and chemicals needed for sample preparation and analysis in this chapter.

4.2.2. Drinking Water Treatment Plant and Distribution Sampling

The drinking water treatment plant in this case-study draws its source from a reservoir that is designated “highly-impacted” due to three wastewater discharge points upstream (North Carolina Division of Water Resources, 2017). This treatment plant serves a population of approximately 160,000 with an average plant flow of 24 MGD using free chlorine as their primary disinfectant with ammonia added to the finished water to form monochloramine for

residual within the distribution system. Water samples were collected from 7 locations within the drinking water treatment plant and 8 distribution samples chosen based on a water-age survey conducted by the plant. Figure 4-1 shows the drinking water treatment plant unit process steps and the stars represent the sampling locations, namely source water, after PAC addition, after ozone (O_3), before filter, after multi-media (sand/anthracite coal) filter, clearwell (HOCl only), and finished water (NH_2Cl). Chapter 3 showed the presence of TDI from ICMs and their biotransformation products in the plant's source water as a result of upstream treated wastewater discharge. Within the treatment plant, samples were collected for TDI, THM, I-THMs, DOC, dissolved nitrogen (DN), and non-target analysis of organic iodine. All samples were quenched with ascorbic acid, except for those for DOC and DN analysis that were quenched with sodium sulfite. The target DBPs were the regulated THM4 and the 6 unregulated I-THMs. I-THMs are good surrogates for I-DBPs since their formation demonstrates that HOI is reacting with NOM even if the formation pathways are different to those of other I-DBPs.

At the times of collection on May 14th, 2019 and August 14th, 2019, the PAC dose used at the plant was 20 mg/L Norit® 20BF, Coal-Based with a contact time of 2.5 hrs and the ozone dose was 6 mg/L as O_3. The PAC dose was determined by the in-house standard operating procedures based on the required removal of taste and odor compounds observed by the operators and is monitored multiple times a day.

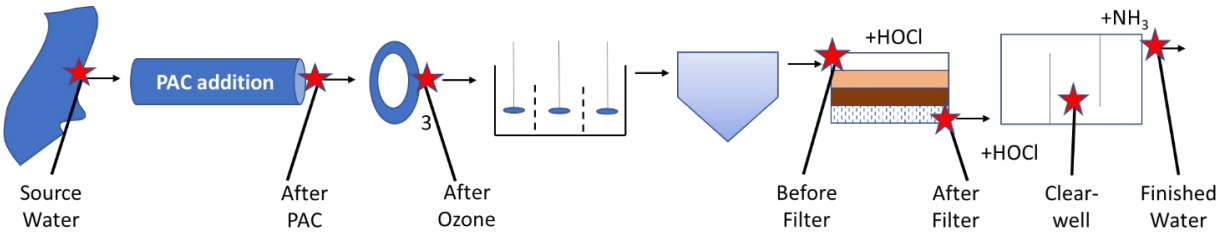


Figure 4-1: Drinking water treatment plant with sampling locations.

The distribution system samples, identified as locations A to H (Appendix B Table B-7), were chosen based on the quarterly monitoring locations used by the treatment plant for regulatory collection. The average water age within the distribution system samples were between 36 and 253 hrs, based on a 2006 tracer study (Personal Communication, 2019). Samples were collected in the field by a treatment plant operator for THM4 and TDI analysis. Total chlorine, pH, temperature, and conductivity were measured in the field using Hach colorimeter kits (Loveland, CO, USA). Distribution Location H, with an average water age of 253 hrs, was identified by the utility as the maximum residence time (MRT) location and is located within a school which could have significant differences in retention time depending on the time of year and whether school is in session.

A large sample of source water was also collected for matrix concentration by reverse-osmosis (RO) to be used in bench-scale experiments (see section 2.2.2 in Chapter 2).
The DOC and total dissolved organic iodine (TDOI) values for the source water at time of collection were 5.0 mg/L as C and 24 μg/L as I, and the levels in the resulting RO concentrate were 127 mg/L as C and 0.5 mg/L as I, respectively showing similar concentration factors of 25 and 21 to represent the source water. Inorganic iodide was below detection levels (< 10 μg/L) for the source water and RO concentrate. A comparison of the fluorescence excitation-emissions matrix (see section 3.2.4 in Chapter 3 for methods and Appendix E for procedures) of the source water and the RO concentrate showed similar concentration factors of 20 to 24 over the 3 peak regions, demonstrating the preservation of the organic matter matrix. Appendix B Table B-1 shows the source water characteristics on the day of sampling and the RO concentrate characteristics.

4.2.3. I-THM Degradation by Free Chlorine and Monochloramine Bench-Scale Experiments

I-THMs, as surrogate I-DBPs used within the drinking water treatment plant and distribution sampling outline above, were also studied to determine the impact of residual disinfectants on their continued formation in the distribution system. Iodoacid stability tests from section 2.4.1 in Chapter 2 show that an unquenched chloraminated matrix can impact the analyte concentrations, which could explain I-THM changes observed in the distribution system. A bench-scale experiment was designed to evaluate the stability of I-THMs in a distribution system using samples from the clearwell and the finished water within the drinking water treatment plant (Figure 4-1) just prior to entering the distribution system. The collected clearwell samples had a residual 4.0 mg/L as Cl_2 free chlorine (HOCl) while the finished water had a residual 4.0 mg/L as Cl_2 monochloramine (NH_2Cl) concentration at the time of collection. The waters were poured headspace-free into 250 mL acid-washed amber bottles with Teflon lined caps alongside an LGW control. Samples were used as is and with a 50 µg/L spike of each of the 6 I-THMs. clearwell The LGW with I-THM spike was treated with either HOCl or preformed NH_2Cl both at 4.0 mg/L as Cl_2 with disinfectant concentrations confirmed by a Hach colorimeter (Loveland, CO, USA) using the indophenol method 10171 (Hach MonoChlorF) for monochloramine and Hach DPD method 8021 for free chlorine. The pH for the LGW matrix was adjusted to 7.4 to match the pH of the finished water collected using a 20 mM phosphate buffer. The disinfectant contact times for each matrix were 0, 24 hrs, 72 hrs, 7 days, and 14 days with the samples continually agitated on an orbital shaker table (Lab Line Instruments, Melrose Park, IL, USA) (1000 rpm) at room temperature (21 ± 1 ºC).

4.2.4. PAC Adsorption of TDI Bench-Scale Experiments

Three types of PAC with their characteristics shown in Table 4-1 were selected for TDI removal experiments in both the LGW control and RO concentrate samples. The three PACs represent three different source materials with different surface porosities indicated by the Iodine Number. The Iodine Number (ASTM D4607-94) approximates the surface area of the PAC but does not represent iodine adsorption potentials in environmental samples. Since the PAC is added on a mass/volume basis, the apparent density could impact the absorption potential when the dose is the same for all three types. The PAC adsorption experiments were conducted in acid-washed 250 mL amber borosilicate bottles with Teflon-lined caps filled with 100 mL of LGW or RO concentrate with and without iohexol spiked at 1 mg/L as I. This concentration was chosen to allow for its measurement after adsorption with the practical quantitation limit (PQL) for its detection at 0.5 µg/L as I. A 10,000 mg/L slurry of each PAC was prepared in LGW and stirred with a magnetic stir bar for at least 30 minutes before use. The high slurry concentration reduced the dilution effects from dosing across a wide range.

Table 4-1: Powdered activated carbon (PAC) properties.

Parameter	**Coal-Based PAC**	**Wood-Based PAC**	**Coconut Shell-Based PAC**
Company	Cabot Corporation	Ingevity	Jacobi Carbons
Commercial name	Norit PAC 20BF	Aqua Nuchar 81283	AquaSorb CP1, PAC-F
Activation method	Steam	Chemical	Steam
Iodine number (mg/g)	800 minimum	900 minimum	1000 minimum
Apparent density (g/cm^3)	0.51	0.21 to 0.37	0.51
Ash content (%)	≤ 10	≤ 10	≤ 5
Passing 100 mesh (%)	99	≥ 99	≥ 99
Passing 200 mesh (%)	95	≥ 95	≥ 99
Passing 325 mesh (%)	≥ 90	≥ 90	≥ 95

The range of PAC doses were selected for the iohexol-spiked LGW based on the current dose (20 mg/L) used in the drinking water treatment plant and the maximum plant dose of 100 mg/L. The dose range for the iohexol-spiked RO concentrate samples was determined by using the same range as the iohexol-spiked samples initially and then extending until a significant (>90%) iohexol removal was observed. Aliquots of the PAC slurry were added to sample bottles using an adjustable volume pipette (Fisher Scientific, Pittsburgh, PA, USA) sufficient to provide 0, 15, 30, 50, 80, 100 mg/L of carbon for the LGW matrix and 0, 50, 100, 200, 300, 400, 500, 600 mg/L of carbon for the RO concentrate matrix after which the samples were agitated on an orbital shaker (Lab Line Instruments, Melrose Park, IL, USA) table at 2000 rpm for 2.5 hrs at room temperature (21 ± 1 ^{o}C). Duplicate aliquots of each sample were subsequently filtered through a 0.45 μm nylon membrane syringe disk filter (Fisher Scientific, Pittsburgh, PA, USA) prior to analysis. The controls for this experiment were LGW dosed with each PAC type at 100 mg/L, iohexol-spiked LGW without PAC, RO concentrate without PAC, and iohexol-spiked RO concentrate without PAC from which the initial TDI concentration for each batch of samples was determined. Split-dosing, or dosing the same sample twice with contact time for each dose, was not tested for TDI removal due to previous research showing only marginal improvements on geosmin and MIB removal (Graham et al., 2000). While it may help with TDI removal, split-dosing is not feasible for most drinking water treatment plants and was not explored in this study.

4.2.5. I-THM Formation Potential Following PAC Treatment Bench-Scale Experiments

PAC treatment was investigated further using a two-step process similar to a full-scale drinking water treatment plant. If PAC were introduced into a full-scale plant, it would be

followed at some stage by disinfection which establishes the importance of determining the I-THM formation potentials in the PAC treated samples after chlorination. The PAC concentrations that removed 50% of the iohexol from the iohexol-spiked RO concentrate were added to 100 mL RO concentrate and iohexol-spiked RO concentrate samples at a contact time of 2.5 hrs and filtered using a 0.45 μm nylon filter prior to chlorination with a dose of 240 mg/L as Cl_2 and a contact time of 24 hrs. Total PAC treatment volume was 800 mL from eight 250 mL bottles containing 100 mL in each, then mixed after filtration to have sufficient sample for both PAC-treated and PAC-treated then chlorinated samples. Samples were chlorinated headspace-free in 250 mL amber bottles in duplicate. The LGW pH was 6.5 and the RO concentrate pH was 7.5. The pH was not adjusted in these chlorination reactions so as not to interfere with the PAC adsorption treatment. The PAC treatment and chlorination were conducted at room temperature, 21.7 ± 1.0°C. After the desired contact time, samples were quenched of free chlorine with ascorbic acid for I-THM, TDI, and non-target analysis. Samples for iohexol and DOC analysis were quenched with sodium sulfite. These quenching agents were selected based on previous stability and recovery studies that showed minimal interference with the target analytes (Weinberg et al. 2002).

4.2.6. Analytical Methods

TDI of the filtered aqueous sample was determined by inductively coupled plasma – mass spectrometer (ICP-MS) as described in section 2.2.5 of Chapter 2. Analysis of hypochlorite solution from the drinking water treatment plant feed tanks was also analyzed for TDI after a solution was diluted by 1:10,000 with LGW, quenched with ascorbic acid, then filtered prior to ICP-MS analysis.

Ten THMs were liquid-liquid extracted from quenched disinfected samples using a concentration factor of 10 with methyl tert-butyl ether (MtBE). The 10 THMs were analyzed on a gas chromatograph with a ZB-1 capillary column (30 m length, 0.25 mm inner diameter, 1.0-μm film thickness) (Phenomenex, Torrance, CA, USA) and a ^{63}Ni micro electron capture detector (μGC-ECD) according to the method by Weinberg et al. (2002). THM standards were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA) and Orchid Cellmark (New Westminster, BC, Canada). All samples were analyzed in duplicate and 1,2-dibromopropane at 100 μg/L was used as an internal standard. The PQL for all 10 THMs were 0.1 μg/L. RO concentrate samples with high organic matter were diluted 1:2 with LGW to decrease emulsion formation during LLE and the PQL for I-THMs and THM4 in the concentrated matrix after the dilution was 1.0 μg/L.

Additional organic iodine analysis on each drinking water unit process and on the laboratory PAC-treated samples was conducted using non-target high resolution mass spectrometry analysis to qualitatively assess the organic iodine species and parent ICMs. The non-target analysis used solid-phase extraction with Strata-X 60 mg cartridges (Phenomenex, Torrance, CA, USA) in which the water sample was filtered after pH adjustment to 2 using H_2SO_4 to ensure protonation of target species for maximum recovery. Following the method previously used for measuring ICM parent compounds and biotransformation products in water (Pereira, 2005), aliquots of each sample were adsorbed onto the cartridges and then eluted into MtBE. The treatment plant samples and the MRT distribution sample were analyzed with SPE concentration factors of 50 while the higher DOC-containing source water and RO concentrate samples used a SPE concentration factor of 10.

SPE analytes were chromatographed on a Waters Acquity® LC CSH-C18 column (Milford, MA, USA) using a 0.1% formic acid in water:0.1% formic acid in acetonitrile mobile phase with gradient elution (see Appendix B Table B-2) and the column eluent flowed into an Agilent 6520 LC-Q/TOF high-resolution mass spectrometer (Santa Clara, CA, USA). A Breeze QS high-performance liquid chromatography (HPLC) system with a dual-wavelength UV absorbance detector (Waters, Milford, MA, USA) was used to determine the iohexol recovery rate in the RO concentrate matrix as 80% (see Appendix B Table B-3f or HPLC method details). The same HPLC method was used to confirm a correlation between the TDI and iohexol concentrations in the PAC experiments.

Additional water quality parameters measured for the drinking water treatment plant included DOC and DN after the samples were filtered with a 0.45 µm nylon filter and acidified to pH 2.5 following Standard Method 5310 (APHA (American Public Health Association) et al., 1999) using a Shimadzu TOC-V_{CPH} and TOC-V_{CPN} Analyzer; as well as iodide and bromide using a Dionex (Sunnyvale, CA, USA) ion chromatography-electroconductivity detector (IC-ED). DOC of the filtered PAC bench-scale experiments using a Shimadzu TOC-V_{CPH} was measured after a 1:20 dilution with LGW prior to acidification at pH 2.5.

4.3 Results

4.3.1. Drinking Water Treatment Plant Sampling

The source water contained measurable levels of TDI on both May and August 2019 sampling dates, which a portion is attributed to organic iodine based on non-target analysis. Even though analysis of the source water showed no detection of iodide and iodate (PQL < 10 µg/L) inorganic iodide could still be present as part of the TDI concentration. Table 4-2 shows the

concentration changes in total I-THMs as well as the individual species together with TDI between each treatment unit during the May and August 2019 sampling events. I-THMs begin to form before the filter where NaOCl (forming HOCl when combined with water) is added to control biological growth. I-THMs continue to increase in formation until the clearwell where the contact time with free chlorine is maximized for pathogen control. The major I-THM species observed were dichloroiodomethane (DCIM) and bromochloroiodomethane (BCIM) with the finished water concentration of 1.4 and 5.4 µg/L for August, respectively. The trend from before the filter to the finished water shows a significant increase in DCIM with the addition of HOCl.

In spite of removing taste and odor compounds sufficiently, the PAC dose of 20 mg/L with a contact time of 2.5hrs in the transmission line from the source water to the plant only removed 39% of the influent TDI and 34% of the influent TDI in May and August, respectively. The PAC was also responsible for a 12% DOC and 33% DN decrease in May and a 24% DOC and 17% DN decrease in August (Appendix B Table B-4)

Overall, the decrease in TDI across the plant was 36% for May and 63% for August. Due to the increase in the clearwell TDI for the May sampling, two sodium hypochlorite stock tanks at the drinking water treatment plant were measured for TDI. Both tanks had measurable levels (Tank 1 TDI of 93 µg/L as I and Tank 2 TDI of 970 µg/L as I), but even with significantly higher Tank 2 TDI concentration, the sodium hypochlorite (60,000 mg/L as Cl_2) would only contribute 0.065 µg/L as I with a dose of 4.0 mg/L as Cl_2. This shows that iodine is being introduced within the treatment plant, but does not fully explain the increase in the clearwell TDI. Additional water treatment chemicals may have iodine contamination.

To further investigate the increase in TDI within the treatment plant, samples were collected from the filter backwash. Significant concentrations of TDI (11.1 µg/L as I), I-THMs

(7.6 μg/L as I), and THM4 (39.9 μg/L) were observed in the filter backwash during the May sampling event, which suggests some adsorption removal from the filter media and the filter may be leaching TDI that can be precursors to I-THMs in the clearwell depending on the timing of the filter backwash and sample collection.

Table 4-2: Sampling within the drinking water treatment plant for total dissolved iodine (TDI) and total iodinated trihalomethanes (I-THM) in May and August 2019. I-THMs included dichloroiodomethane (DCIM), bromochloroiodomethane (BCIM), dibromoiodomethane (DBIM), chlorodiiodomethane (CDIM), bromodiiodomethane (BDIM), and triiodomethane (TIM). TIM was < 0.1 µg/L.

Average duplicates (N=2) ± range	Sampling Month	TDI (µg/L as I)	Total I-THM (µg/L as I)	Total I-THM (µg/L)	DCIM (µg/L)	BCIM (µg/L)	DBIM (µg/L)	CDIM (µg/L)	BDIM (µg/L)
Finished water	May	11.3 ± 0.6	11.4 ± 0.4	19.6 ± 1.2	15.3 ± 0.9	4.1 ± 0.2	< 0.1	< 0.1	0.2 ± 0.1
	August	4.3 ± 0.3	3.7 ± 0.3	7.0 ± 0.9	1.4 ± 0.1	5.4 ± 0.3	< 0.1	< 0.1	0.3 ± 0.1
Clearwell	May	12.9 ± 0.8	12.0 ± 0.6	19.8 ± 1.3	12.4 ± 1.1	3.3 ± 0.6	1.4 ± 0.3	2.7 ± 0.2	< 0.1
	August	4.9 ± 0.1	4.7 ± 0.4	7.8 ± 0.7	2.1 ± 0.3	6.2 ± 0.5	< 0.1	< 0.1	0.4 ± 0.1
After filter	May	10.0 ± 0.3	5.4 ± 0.8	9.8 ± 3.1	4.1 ± 1.3	2.1 ± 0.4	2.6 ± 0.8	< 0.1	1.0 ± 0.4
	August	4.5 ± 0.2	4.5 ± 0.1	8.5 ± 0.1	3.0 ± 0.1	2.1 ± 0.1	2.9 ± 0.1	0.4 ± 0.1	< 0.1
Before filter	May	8.9 ± 0.7	4.3 ± 0.6	7.5 ± 2.0	5.5 ± 1.2	2.0 ± 0.2	< 0.1	< 0.1	< 0.1
	August	4.9 ± 0.2	1.6 ± 0.2	2.6 ± 0.1	1.8 ± 0.1	0.3 ± 0.1	< 0.1	0.5 ± 0.1	< 0.1
After ozone	May	10.0 ± 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	August	7.8 ± 0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
After PAC	May	10.6 ± 0.4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	August	7.5 ± 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Source water	May	17.5 ± 0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	August	11.4 ± 0.4	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

From the DWTP intake, there was a decrease in TDI due to the addition of PAC and potential altering of TDI due to ozonation pre-filter followed by another decrease in TDI due to the coagulation/flocculation/sedimentation processes due to TDI incorporation or adsorption onto particulates and floc (Crittenden et al., 2012). Based on the I-THMs observed before the filter, after the filter, in the clearwell, and in the finished water, some form of organic iodine was still present prior to the addition of HOCl. The ozonation unit process, with a dose of 6 mg/L as O_3, showed minimal effect on the TDI concentration through the oxidation of iodide released from organic iodine transforming to iodate which may be due to the greater DOC concentration in comparison to TDI concentration and ozone selectivity for the reaction-rich sites of the NOM.

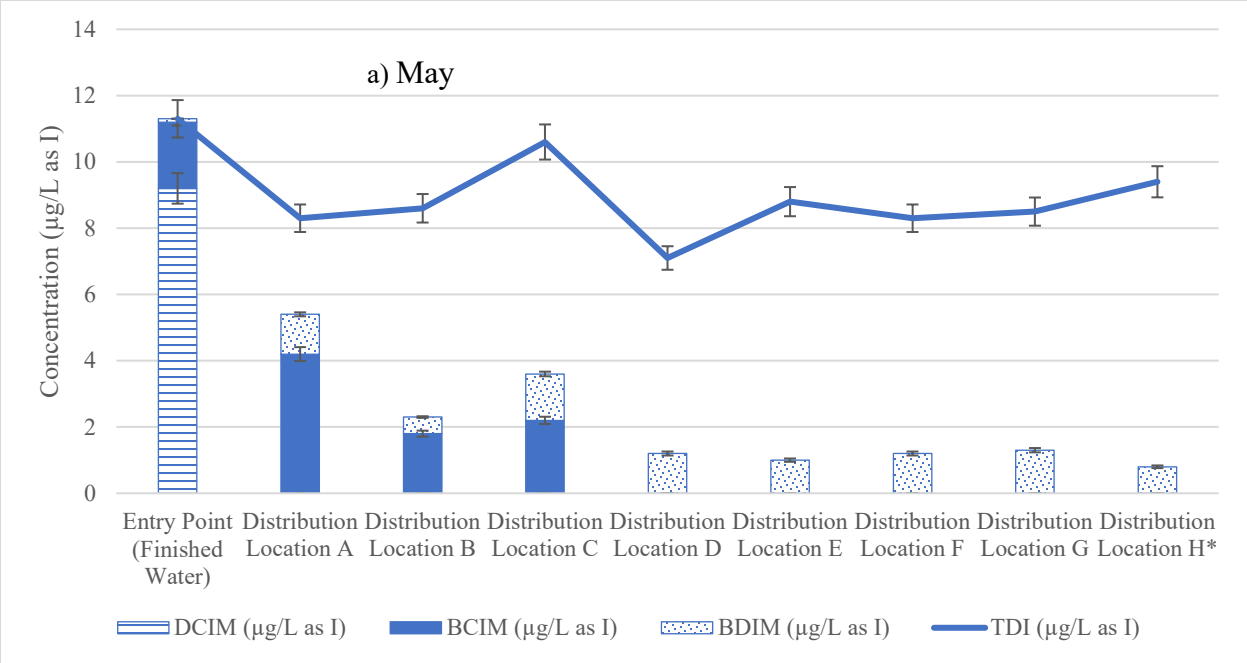
4.3.2. Drinking Water Distribution Sampling

The total chlorine disinfectant residual, pH, temperature, conductivity, and estimated water age for each sampling location in the distribution system are listed in Appendix B Table B-7 for May and August 2019 are Appendix B Table B-8. All locations show adequate chlorine residual for pathogen control, but speciation of the total chlorine was not available. The finished water (entry point) analysis measured a total chlorine residual of 3.50 mg/L as Cl_2, with 0.01 mg/L as free chlorine, 3.20 mg/L as monochloramine, and 0.05 mg/L as free ammonia. Based on the pH range observed and the chemical stability of NH_2Cl, monochloramine should be the predominant oxidative species. The MRT location does not have the lowest total chlorine residual, which may suggest increased water use at that location or increased disinfectant demand (biological activity) within the distribution system at locations such as Distribution Location B in May and Distribution Location D in August. There is only a weak correlation of

total chlorine residual to water age for August (R^2=0.24, $p<0.09$) and May data showed no correlation (R^2=0.024, $p<0.4$).

Overall, there was a decrease in I-THMs from the entry point to and throughout the distribution system. Figure 4-2 shows the changes in TDI and I-THM speciation for the May and August sampling events. TDI appears to increase from 4.3 µg/L at the entry point to the maximum of 5.8 µg/L at Distribution Location F, which may be due to biofilm releasing iodine back into the water. Iodinated THMs are still measurable within the distribution system and the decrease in I-THM concentration compared to the entry point (finished water) could also be due to decomposition of I-THMs from biological activity along the pipes or abiotic degradation from chemicals within the pipes or distribution system. There is evidence of brominated DBPs undergoing hydrolysis, aerobic biodegradation, and/abiotic reductive dehalogenation within distribution systems as previously described by Hozalski et al. (2008). THM4 for the distribution system samples were under the US EPA regulation limit of 80 µg/L (Appendix B Tables B-10 and B-11.

Changes in TDI from the entry point (finished water) to each distribution collection site could be due to water age at the location or dilution effects from the system, where previous water entered with less TDI and lower I-THM concentrations compared to the finished water at the time of sampling. There is a weak but significant correlation of water age to I-THM concentration for August (R^2=0.54, $p<0.0005$), which is similar to the May data (R^2=0.51, $p<0.001$), where the older water has lower I-THMs.



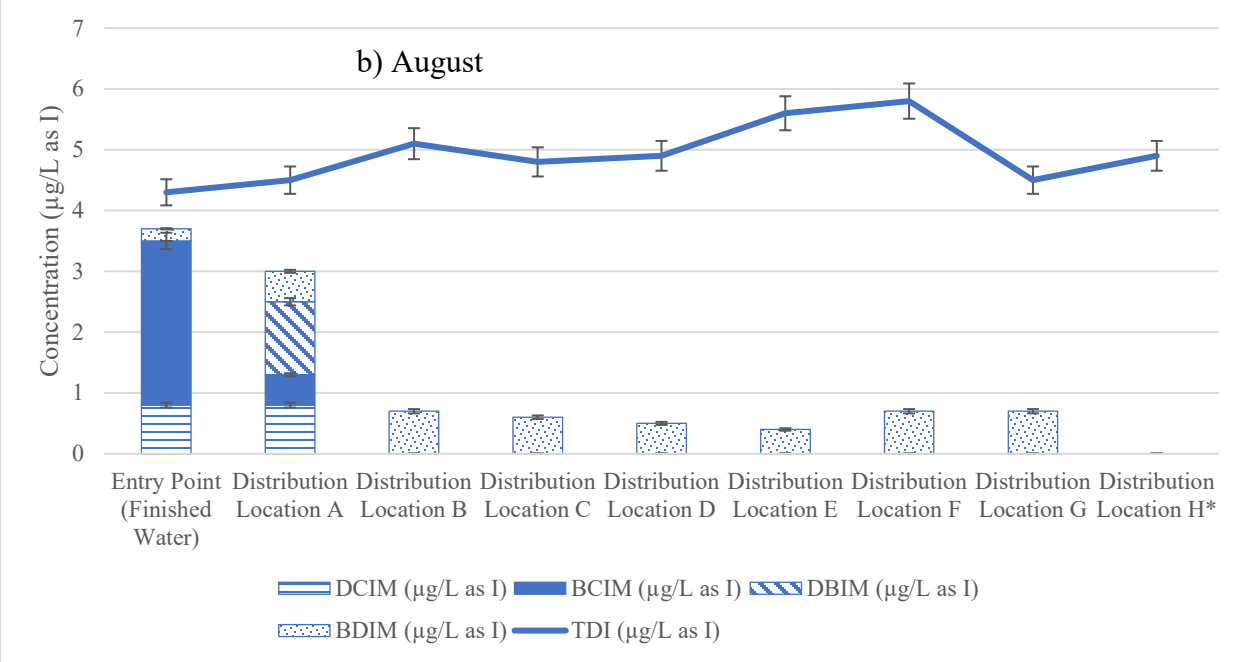


Figure 4-2: I-THM speciation and TDI for distribution system locations for a) May and b) August 2019 sampling events. Distribution locations are presented in order of increasing water age from left to right with Location H as the MRT.

4.3.3. Distribution Simulation for the Degradation of I-THM by Disinfectant

I-THM degradation/stability tests were conducted over 14 days with the initial residual for the treatment plant samples and the dose of HOCl or NH_2Cl at 4.0 mg/L as Cl_2 for the ITHM-spiked LGW samples. Residual disinfectant was present throughout the 14-day contact period for clearwell (3.9 mg/L as Cl_2 at the time of collection and 0.8 mg/L as Cl_2 after 14 days) and finished water (4.0 mg/L as Cl_2 at collection and 1.6 mg/L as Cl_2 after 14 days) samples. Overall, the longer contact time with both free chlorine and monochloramine showed a decrease in I-THM, which is the same trend observed in the actual distribution system. Table 4-3 shows the initial I-THM concentration for the finished water and clearwell and the subsequent changes to those concentrations over the 14-day contact time. Appendix B Tables B-11 and B-12 show the LGW+I-THM and I-THM spiked clearwell and finished water results for the I-THM degradation by disinfectant.

Table 4-3: Clearwell and finished water I-THM degradation due to time and exposure to free chlorine (HOCl) or monochloramine (NH_2Cl).

Contact time (N=2 ± range)	**Total I-THM (µg/L)**	**I-THM % Decrease**	**Total Chlorine (mg/L as Cl_2)**	**Total Chlorine % Decrease**
Initial Finished Water	6.7 ± 1	--	4.0	--
24 hrs	3.3 ± 1	50	3.8	5
72 hrs	1.4 ± 1	79	3.4	15
7 days	0.4 ± 0	94	2.2	45
14 days	0.4 ± 1	94	1.6	60
Initial Clearwell	7.3 ± 1	--	3.9	--
24 hrs	3.5 ± 1	52	2.4	38
72 hrs	0.9 ± 1	88	2.2	44
7 days	0.4 ± 1	94	1.0	74
14 days	0.4 ± 1	95	0.8	79

Half of the initial I-THM concentrations were lost after a 24 hr contact time that increased to ≥94% after 14 days for both finished and clearwell water. With I-THM spiked LGW, the HOCl sample showed more I-THM loss over the 14-day exposure with an 88% decrease compared to the 32% decrease observed in the NH_2Cl sample over the 14-day exposure. The clearwell and finished water spikes did not follow the same pattern as observed in the LGW spiked experiments. The clearwell with residual HOCl showed a lesser decrease over the 14-day exposure with a 56% decrease compared to the 82% decrease observed in the NH_2Cl 14-day exposure. The loss of I-THMs due to residual HOCl and NH_2Cl enforces the need for quenching agent even when the hold time between collection and extraction is 24 hrs.

4.3.4. Non-Target Analysis of Organic Iodine in the DWTP

Non-target analysis was used to follow the TDI throughout the treatment plant and to see qualitatively the transformation of ICMs and their biodegradation products into I-THMs. Physical removal of TDI occurred during PAC treatment, coagulation/flocculation/ sedimentation, and filtration (Table 4-2). Chemical transformation products were observed from ozonation and chlorination for the two sample events (Figures 4-3 and 4-4), but with the current data set it is not possible to confirm their structure due to decreased sensitivity of the instrument detector and decreased confidence in peak identification due to insufficient sample runs for recursive false-peak filtering. For the After PAC samples for both May and August, the overall number of organic iodine entities decreased compared to the source water intake but the comparison of tentative entity identification shows a change in organic iodine speciation which may be due to false peaks since ICMs are chemically inert (Lusic and Grinstaff, 2013) unless a reactive species like free chlorine is introduced (Wendel et al. 2016). With the current data set,

the qualitative analysis has tentatively identified compounds containing chlorine and iodine that supports the dehalogenation hypothesis for the release of HOI to form I-DBPs resulting in the chlorinated ring. Figure 4-3 shows 15 chlorinated organic iodine compounds observed in the drinking water treatment plant in May and Figure 4-4 shows 12 chlorinated organic iodine compounds in the August sampling. These chlorinated species should start to appear in the Before Filter sample where chlorine is first introduced in the treatment process. The non-target analysis of the Distribution Location H (MRT) shows multiple organic iodine compounds present that could be an I-DBP source in the distribution system. If the organic iodine is still present, it shows that chloramines do not have sufficient oxidative strength to initiate the dehalogenation reaction needed to form I-DBPs from organic iodine.

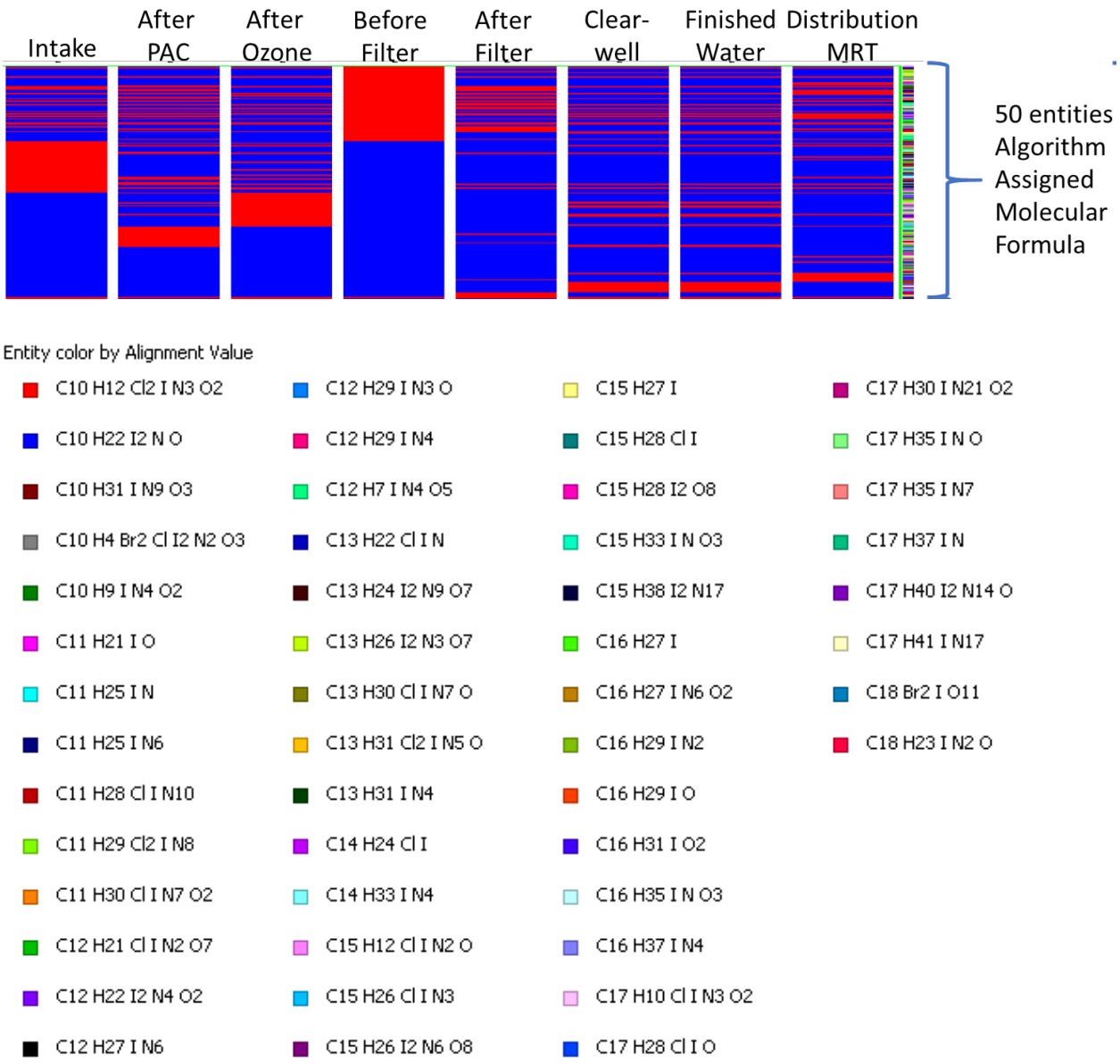


Figure 4-3: Heatmap of the presence (red) and absence (blue) of organic iodine based on the non-target analysis of May 2019 samples within the drinking water treatment plant and the distribution MRT. Shows the relationship across unit processes of tentatively identified organic iodine species from ESI+ Q/TOF.

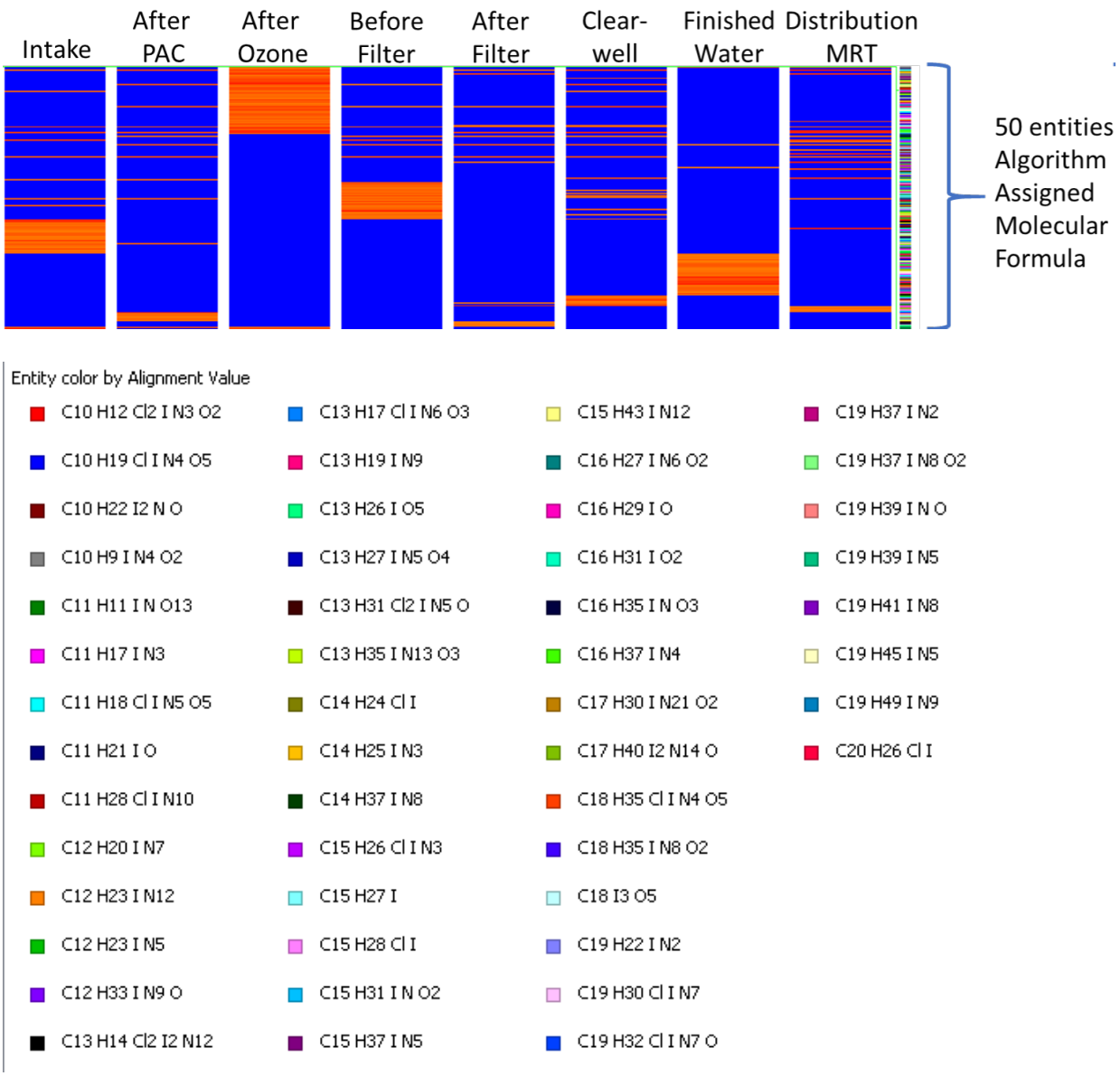


Figure 4-4: Heatmap of the presence (red) and absence (blue) of organic iodine based on the non-target analysis of August 2019 samples within the drinking water treatment plant and the distribution MRT. Shows the relationship across unit processes of tentatively identified organic iodine species from ESI+ Q/TOF.

4.3.5. PAC Removal of TDI in Bench Scale Experiments

Overall, the coconut-based PAC at 2.5 hours contact time was the most efficient at removing iohexol from iohexol-spiked LGW and iohexol-spiked RO concentrate followed by the

coal-based PAC which performed better than the wood-based PAC. Figure 4-3 shows the dose-removal curves based on the removal of TDI by variable PAC doses. For the unspiked RO concentrate, all 3 PAC types showed minimal TDI removal. However, for the unspiked RO concentrate, the wood-based PAC outperformed the coal-based PAC. The RO concentrate did contain organic iodine, (Appendix B Table B-15) but was not specifically iohexol which was established by LC-Q/TOF analysis (section 3.2.4 in Chapter 3). The TDI concentration decrease was observed with all three PAC types was very low for RO only, meaning the PAC showed little impact on TDI concentration over the PAC range selected (Figure 4-5). When iohexol was spiked into the RO concentrate (Appendix B Table B-17), the PAC removal efficiency by all PAC types increased significantly, where the theoretical PAC dose for 50% removal was less than half of that calculated for RO only. This difference in removal ability may be related to the adsorption ability of the PAC for iohexol compared to the other organic iodine chemicals that make up the RO mixture.

An increase in DOC was observed in LGW when PAC is introduced (Appendix B Table B14). A significant difference was not observed between the 0.20 µm and 0.45 µm filter DOC results. Minimal differences were also observed between the iohexol and TDI samples filtered with 0.20 µm and 0.45 µm filters. The contributions of carbon to the DOC from the PAC are approximately 0.7 mg/L as C with PAC 100 mg/L dose when filtered using a 0.45 µm nylon filter for both wood-based and coal-based PACs after 2.5 hrs of contact. The coconut-based PAC contributed less than 0.2 mg/L as C in LGW for the same 100 mg/L dose, which is expected based on the particle size associated with PAC. For the RO concentrate samples, DOC decreases with increasing PAC dose when added to RO concentrate, thus PAC is removing more DOC than it is contributing (Appendix B Table B-16).

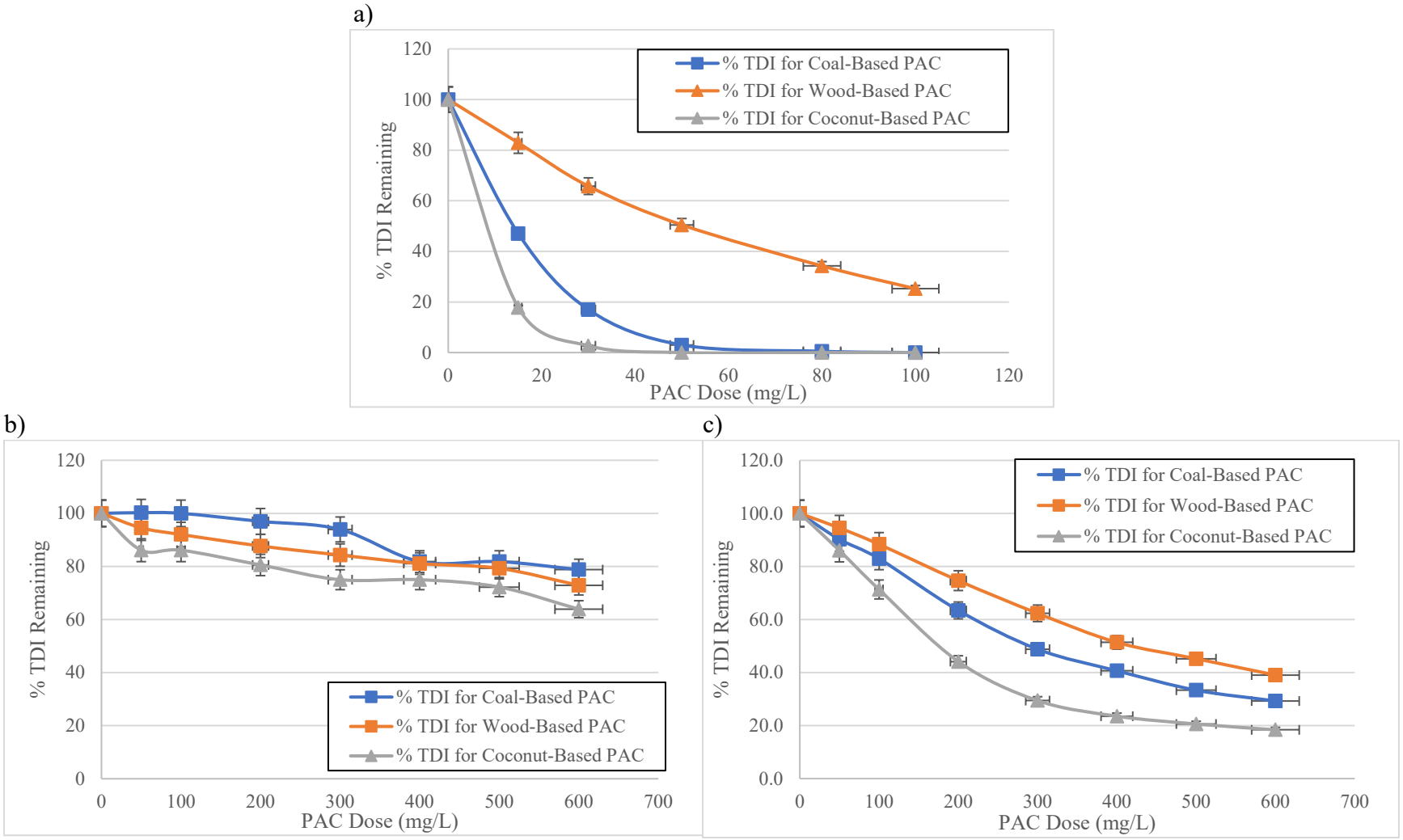


Figure 4-5: Dose-removal curves for TDI from PAC in a) iohexol-spiked LGW (1 mg/L as I) samples b) RO concentrate samples c) iohexol-spiked RO concentrate (1 mg/L as I) samples (N=2).

4.3.6. Non-Target Analysis of Organic Iodine in the PAC Experiments

Non-target analysis using an Agilent 6520 liquid chromatography accurate mass quadrupole/Time-of-Flight (LC-Q/TOF) (detailed explanation in section 3.2.4 of Chapter 3 Methods) was used to determine why TDI in the RO concentrate was not easily removed by PAC but iohexol in the same RO concentrate matrix was able to be removed using the PAC (Figure 4-3). This data is only preliminary due to the single injection instead of the 3 replicate injections needed for recursive quality control that can remove false peaks. The blank chromatograms were subtracted from each sample chromatogram but artifacts could remain due to the single injection data. Additionally, analysis of the same RO concentration from 2018 showed >5000 total entities instead of the <500 total entities seen with these samples which points to a decrease in Q/TOF detector sensitivity since the DOC and TDI analysis is not significantly different in the samples.

Comparing the entities lists for each sample shows that there are differences between types in the PAC removal of TDI as shown in Figure 4-6. RO concentrate without PAC treatment has 157 total compounds with an assigned molecular weight or a chemical formula that includes iodine and 124 of those compounds are not observed in the PAC- treated samples. There were only 3 compounds observed in all 4 samples (RO, RO+coal-based PAC, RO+wood-based PAC, RO+coconut-based PAC) and were tentatively identified as $C_{10}H_9IN_4O_2$, $C_{28}H_{59}IH_6$, and $C_{19}H_{49}IN_9$ by ID Browser (Agilent Technologies, Santa Clara, CA, USA).

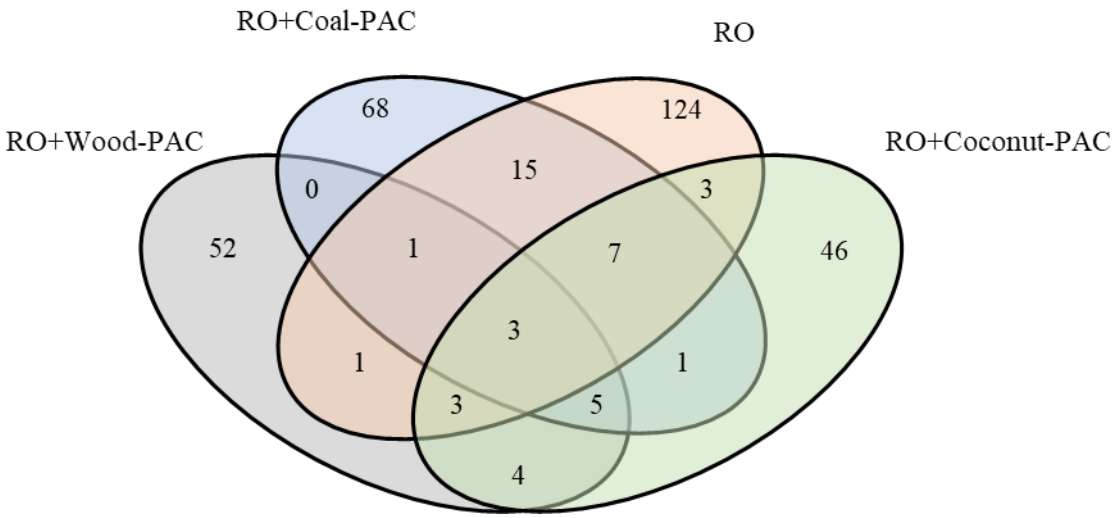


Figure 4-6: Comparison of tentatively identified organic iodine species from ESI+ Q/TOF for before and after PAC treatment of RO concentrate.

Figure 4-7 shows that differences are also observed between the coal-based, wood-based, and coconut-based PAC types in the removal of TDI in iohexol-spiked RO concentrate (1 mg/L as I). Without PAC treatment, the iohexol-spiked RO sample has 202 total compounds with an assigned molecular weight or a chemical formula that includes iodine and 169 of those compounds are not observed in the PAC treated samples. There were only 5 compounds observed in all 4 samples and were tentatively identified as $C_9H_{21}IN$, $C_{16}H_{35}INO_3$, $C_{10}H_9IN_4O_2$, $C_{19}H_{49}IN_9$, and $C_{19}H_{39}INO$ by ID Browser (Agilent Technologies, Santa Clara, CA, USA).

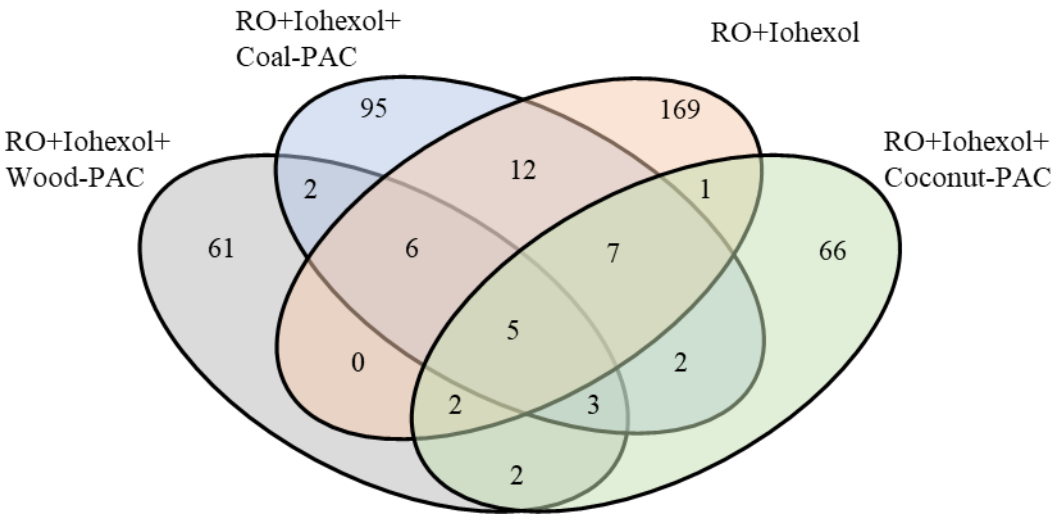


Figure 4-7: Qualitative analysis of non-target data using ESI+ for the tentative identification of individual iodine species before and after PAC treatment of iohexol-spiked RO concentrate (1 mg/L as I).

Iohexol was not impeded from adsorption in the iohexol-spiked RO concentrate matrix, but the TDI in the same RO matrix did not show the same adsorption potential. This suggests that DOC interference or competition for adsorption sites was not the primary reason for differences in TDI removal, rather the TDI species and chemical properties affected the adsorption.

4.3.7. Chlorination of PAC Treated Samples with I-THM Formation Potential

The PAC doses for the I-THM formation potential tests were based on approximately 50% removal of TDI in iohexol-spiked RO concentrate experiments, with the coal-based PAC 380 mg/L dose; wood-based 470 mg/L dose; and coconut-based 280 mg/L dose. TDI removal/loss was observed (from PAC treatment and chlorination disinfection. LGW samples showed filtering through 0.45 μm filters had minimal effect on the TDI concentration when large molecular weight organic iodine is present. When I-THMs are filtered prior to TDI analysis,

there is an 80% loss due to the filtration, which may account for TDI decrease after chlorination (Appendix Table 4-22).

All samples had measurable residuals for free chlorine and total chlorine. PAC removed significant amounts of chlorine reactants as seen by the decrease in DOC (Appendix B Table B-13) and increase in residual (Appendix B Table B-18) treated with PAC prior to chlorination. Even when PAC contributes DOC for some chlorine demand, in the RO samples this is negligible compared to the concentration of the RO concentrate DOC.

DCIM, DBIM, CDIM, and BDIM were present in all chlorinated samples and BCIM was detectable in the iohexol-spiked wood-based and coconut-based chlorinated samples. This may point to a difference in TDI species removal due to PAC type and the non-target organic iodine qualitative analysis supports the hypothesis that different species of TDI remained after each PAC treatment. When the I-THMs are normalized to the DOC concentrations after PAC-treatment the I-THMs formed from RO concentrate and the iohexol-spiked RO concentrate were not statistically different ($p<0.07$). Figure 4-8 shows the I-THM speciation normalized to remaining DOC for RO concentrate and iohexol-spiked RO concentrate for each PAC type. The chlorination of iohexol-spiked LGW did not produce detectable levels of I-THMs (PQL of 0.1 μg/L in LGW) which supports previous literature that shows a carbon source is needed for I-DBP formation from ICMs (Duirk et al., 2011).

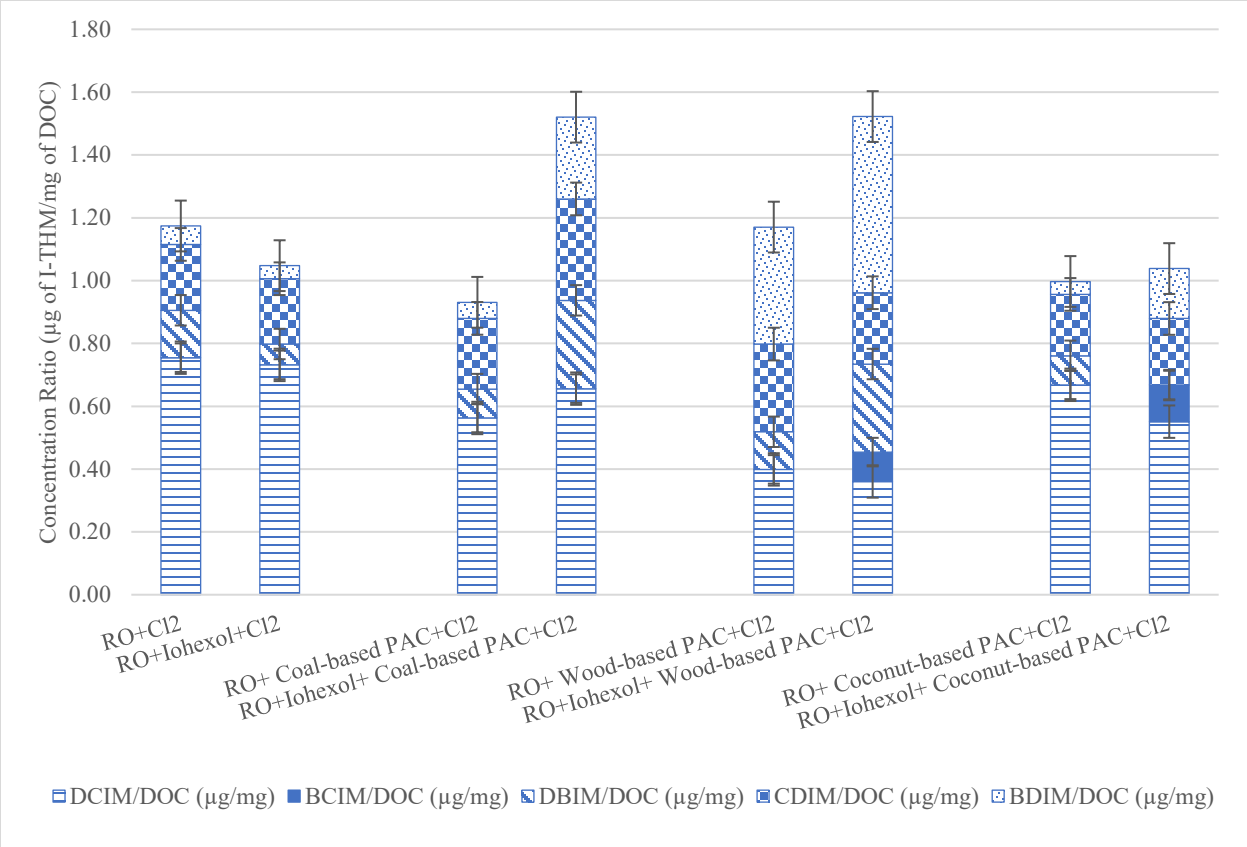


Figure 4-8: Normalized I-THM formation to DOC for RO concentrate and iohexol-spiked RO concentrate samples after chlorination (N=2). TIM was below the PQL of 1.0 µg/L. Appendix B Table B-19 shows the I-THM species concentration.

Preliminary non-target analysis of the chlorinated PAC-treated samples confirms that large molecular weight organic iodine compounds have reacted with chlorine. Figures 4-9 and 4-10 compare the entity lists for chlorinated, PAC-treated RO concentrate and chlorinated, PAC-treated RO concentrate with an iohexol spike at 1 mg/L as I, respectively. Of the 17 compounds identified from all 4 chlorinated RO concentrate samples, two were chlorinated species tentatively identified by ID Browser as $C_{40}H_{43}ClIO$ and $C_{41}H_{45}ClI$. These compounds contain over double the amount of carbon atoms seen in iohexol ($C_{19}H_{26}I_3N_3O_9$) which could suggest a non-covalent dimer formation due to the positive electrospray ionization (Pan, 2008).

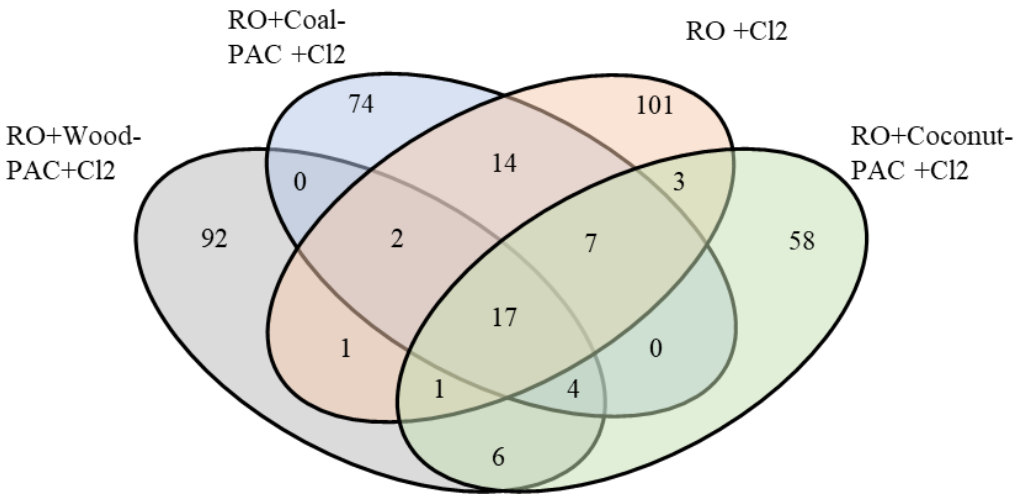


Figure 4-9: Qualitative analysis of non-target data using ESI+ for the identification of organic iodine species in chlorinated RO concentrate and chlorinated PAC-treated RO concentrate.

Of the 16 compounds identified in all 4 chlorinated RO concentrate with iohexol spiked samples, only one was a chlorinated species tentatively identified by ID Browser as $C_3Br_3Cl_3IN_2O_2$. This tentative compound identification points to bromination and chlorination of an organic iodine compounds, but more analysis is needed to confirm this. Bromide is present in the RO concentrate (Appendix B Table B-1) and brominated THMs were also observed in the DBP analysis (Figure 4-8).

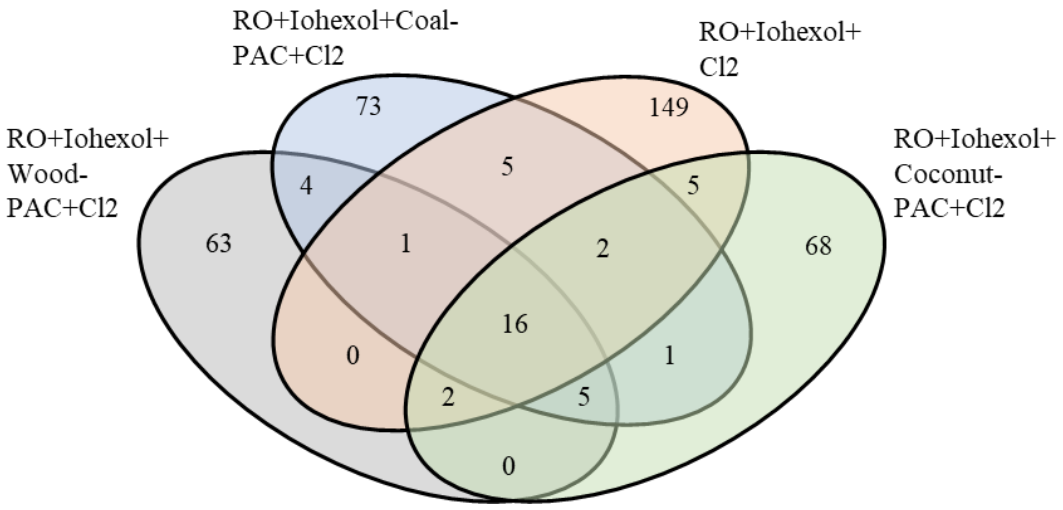


Figure 4-10: Qualitative analysis of non-target data using ESI+ for the identification of organic iodine species in chlorinated RO concentrate and chlorinated PAC-treated RO concentrate both with an iohexol spike (1 mg/L as I).

4.4 Discussion

4.4.1. I-THM and TDI in the Plant and Distribution System

The organic iodine that enters the drinking water treatment plant can be partially removed by the PAC or transformed during chlorination producing I-THMs. This transformation of the chemical structure is evidenced by an absence of I-THMs in the source water and the presence of I-THMs before the filter where chlorine is first introduced to the system.

For TDI removal, the unit process with the greatest decrease was the PAC addition. If this unit process were optimized for TDI removal within the DWTP, then I-THM formation could be further mitigated before distribution of the water to consumers. Larger amounts of the currently used coal-based PAC can be added at this plant (up to 80 mg/L) based on the current plant infrastructure in order to increase removal of TDI. However, based on the PAC dose response curves for TDI removal, a coconut-based PAC will increase the removal of TDI with the same dose. Based on the plant DOC concentrations, the PAC addition will also help decrease

organic carbon DBP precursors and increase the effectiveness of coagulation (Graham et al., 2000).

Within the treatment plant, I-THMs began to form after the first introduction of free chlorine showing that organic iodine precursors do not share the same pathway or reaction rates as inorganic iodide for HOI formation. Contrary to the previous research on inorganic iodide (Bichsel and von Gunten, 1999), chloramines appear to have little to no effect on the formation of I-THM within the drinking water treatment plant. Comparing inorganic versus organic iodine precursors in the presence of free chlorine, iodide rapidly forms HOI then iodate compared to organic iodine that must undergo a dehalogenation reaction (see Figure 3-6 in Chapter 3) before the formation of HOI. The difference in reaction steps and the requirement of strong oxidative power for the dehalogenation reaction explains the differences in I-THM formation observed. With the degradation of I-THMs in the presence of residual disinfection (see section 4.3.2), chloramines may play a significant role in I-DBP formation within the distribution system if iodide is released back into the water and able to form HOI.

By tracking the I-THMs through each drinking water treatment unit process, the favorable conditions for I-THM formation can be determined and the TDI precursor removal can be focused on an optimal location for maximum effect. In the drinking water treatment plant samples, equation 4-1 was used to calculate the iodine incorporation percentage for the THMs (Obolensky and Singer, 2005):

$$Iodine\ Incorporation\ Percentage = \frac{\sum molar\ conc. \times \#Iodide}{\sum(molar\ conc.)\times(\#halogens)} \times 100 \qquad (4\text{-}1)$$

where the molar concentration of the iodinated species and the total number of halogenated species are used to determine the iodine incorporation factor. An iodine incorporation sample calculation is shown in Appendix B Equation 1 and the molar concentrations of the I-THMs and THM4 are listed in Appendix B Tables B-20 to B-24. Table 4-4 shows the iodine incorporation percentages for the drinking water treatment plant by unit process where I-THMs were present.

For both the May and August sampling events, iodine incorporation was the highest before the filter where THM4 concentrations were lower, then decreased incorporation in the finished water due to the increase of the THM4. This change in iodine incorporation along with the tentative identification of the organic iodine (Figures 4-3 and 4-4) showing numerous speciation changes between unit processes shows that the organic iodine precursors are directly affecting the incorporation rates. The May iodine incorporation in the finished water was higher than in the August samples due to the higher source water TDI concentration and lower DOC concentration to compete for the same adsorption sites.

Table 4-4: Iodine incorporation factors for May and August 2019 sampling events.

Sample	May Incorporation Factor (%)	August Incorporation Factor (%)
Finished water	9.0	1.9
Clearwell	9.7	2.3
After filter	9.1	4.0
Before filter	28.5	38.2

To compare the I-THM speciation changes between each unit process in the drinking water treatment plant and to further compare those samples to the absence of I-THMs in the same matrix, equation 4-2 was used to calculate the theoretical toxicity of a DBP mixture and assumes an additive interaction:

$$Total\ Relative\ Toxicity = \sum([DBP] \times (LC_{50})^{-1} \times 10^{6}) \quad (4\text{-}2)$$

where the total relative toxicity is the sum of the individual DBP molar concentrations times the reciprocal of the LC_{50} for cytotoxicity in units of molarity (M) (Appendix B Table B-25) determined for individual DBPs from the CHO cell assay with an adjustment factor of 10^6 to bring the calculation into whole numbers (Allard et al., 2015; Plewa et al., 2017; Wagner and Plewa, 2017). A total relative toxicity sample calculation is shown in Appendix B Equation 2 and the relative toxicities for each species are shown in Appendix B Tables B-26 and B-27. To evaluate the total relative cytotoxicity associated with the iodine incorporation, the same quantitative data from the I-THMs were used but the concentrations were attributed to TBM, the most toxic regulated THM4 (Appendix B Tables B-28 and B-29).

The t-test (Data Analysis, Excel, Microsoft Office) shows a significant difference in the relative toxicity increase from I-THMs formation compared to assuming all TBM formation ($p<0.001$) in the May and August sampling events. Tables 4-5 and 4-6 show factors of relative theoretical toxicity change after each unit process where I-THMs were formed, ranging from 1.1 to 1.5. This analysis shows that the formation of I-THMs within the drinking water treatment plant significantly increase the cytotoxicity. Plant 7 from Chapter 2 is the same drinking water treatment plant used in this case study and showed the formation of iodoacetic acid (27 ± 1.2 ng/L) and diiodoacetic acid (8.7 ± 0.6 ng/L) in the finished water during a separate sample collection. If I-THMs are surrogates for other I-DBPs such as iodinated haloacetic acids, then the actual toxicity would increase even at low concentrations in comparison to the most-cytotoxic regulated THM4, TBM.

Table 4-5: Relative toxicity increase comparison for the May 2019 sampling event.

Location	Toxicity Increase from I-THM Contribution	Toxicity Increase assuming all TBM Contribution	% Difference	Factor of Toxicity Increase from I-THMs
Finished water	24.6	22.5	9	1.1
Clearwell	25.7	21.6	18	1.2
After filter	14.7	9.9	39	1.5
Before filter	9.6	8.6	11	1.1

Table 4-6: Relative toxicity increase comparison for the August 2019 sampling event.

Location	Toxicity Increase from I-THM Contribution	Toxicity Increase assuming all TBM Contribution	% Difference	Factor of Toxicity Increase from I-THMs
Finished water	11.0	7.2	41	1.5
Clearwell	13.3	8.9	39	1.5
After filter	12.5	8.4	38	1.5
Before filter	3.2	2.9	12	1.1

The decrease in iodine incorporation % and theoretical cytotoxicity between the clearwell and finished water suggests that I-THM decomposition from disinfectant residual is occurring within the treatment plant in addition to the distribution system.

There are no discernable trends for I-THM speciation within the distribution system based on the data available (water age, residual, pH, conductivity). This distribution system sampling scheme did not represent a plug flow view of the system, but rather a dynamic system with interactions with residual disinfectant, biofilm, and "old" water. The I-THMs measured in the distribution system were less than the THM4 and the DBP subgroups did have a correlation (May Pearson correlation coefficient r^2=0.66, $p<0.001$ and August Pearson correlation

coefficient r^2=0.63, $p<0.001$) between the analytes. Since the TDI concentrations are variable throughout the distribution system, it is unlikely that additional I-DBPs are forming from organic iodine due to their continued presence observed in Distribution Location H (MRT) based on the non-target analysis. Additionally, the stability tests showed the degradation of I-THMs when residual disinfectant is present, but it is not known if the iodine is incorporated into new I-DBPs.

4.4.2. PAC Isotherms and TDI Removal Ability

Even though the DOC concentration in the RO concentrate was significantly higher than the TDI concentration in the concentrate and from the added iohexol and competed for adsorption sites, each PAC type was able to remove TDI from the complex mixtures. If the PAC doses from the dose-removal curves are normalized to the DOC concentration (120 mg/L as DOC for RO, DOC of 5.0 for the source water), then the PAC doses are realistic for the DWTP operation and can provide increased TDI removal. Based on the observed iohexol removal, PAC addition is a good removal process. With the observed differences in the iohexol spike versus RO-concentrated source water organic iodine removal, the iodinated precursors that are unaffected by PAC treatment will be determined by their structure, surface volume, and partial-charge distribution.

Based on the iodine numbers, which are used to characterize activated carbon performance with higher numbers indicating higher degree of porosity and removal ability (ASTM, 2014), the coconut-based PAC was the best at removing iohexol and had the greater iodine number of 1000 mg/g. For the other PAC types, the wood-based PAC should show better iohexol removal ((mg/g) 800 minimum versus (mg/g) 900 minimum) than the coal-based PAC,

but that outcome was only observed in the RO concentrate matrix. Data from these three PACs suggest that the iodine number does not reflect the removal ability of organic iodine specifically.

To further determine the TDI adsorption abilities of the three carbon types, three isotherm models, Linear, Langmuir, and Freundlich, were compared for the PAC experiments using the TDI data and the variable PAC dose. The linear isotherm was calculated using Equation 4-3:

$$q_E = K_{linear} C_E \tag{4-3}$$

where C_E is the liquid-phase concentration of the trace contaminant (mg/L), q_E is the amount of trace contaminant adsorbed in the solid-phase in equilibrium with the liquid-phase concentration (mg/g), and K_{linear} is the linear equilibrium constant.

The Langmuir isotherm was calculated using Equation 4-4:

$$q_E = q_{max} (K_L C_E/(1+K_L C_E)) \tag{4-4}$$

where C_E is the liquid-phase concentration of the trace contaminant (mg/L), q_E is the amount of trace contaminant adsorbed in the solid-phase in equilibrium with the liquid-phase concentration (mg/g), q_{max} is the maximum adsorption capacity for forming a single layer, and K_L is the Langmuir equilibrium constant with units of L/mg.

The Freundlich isotherm was calculated using Equation 4-5:

$$q_E = K_f C_E^{\ 1/n} \tag{4-5}$$

where C_E is the liquid-phase concentration of the trace contaminant (mg/L), q_E is the amount of trace contaminant adsorbed in the solid-phase in equilibrium with the liquid-phase concentration (mg/g), n is the Freundlich equilibrium parameter, and K_f is the Freundlich adsorption capacity parameter $(mg/g)(mg/L)^{1/n}$. A larger K_f represents a higher adsorption affinity, whereas a larger n demonstrates a more homogeneous surface of the adsorbent.

The Langmuir isotherm best explained the iohexol-spiked RO concentrate, observing single layer isotherm as the best fit which may be due to steric hindrance from molecular structure and the uncharged state of iohexol. The adsorption potential appears different for iohexol and remaining organic iodine in the mixture, with iohexol having an increased adsorption ability over the RO concentrate TDI. Recalcitrant iodine in the RO concentrate may be due to molecular structural constraints in adsorption onto PAC. Organic iodine in the RO concentrate-only sample are smaller chemicals based on the non-target analysis and single-layer theory may not offer the full explanation when a mixture of TDI is present. Specific organo-iodine species in the RO concentrate could also have multi-layer adsorption with the Freundlich isotherm that could be impeded by other species following the Langmuir isotherm model. Table 4-7 shows the calculated isotherm model components for each matrix based on organic iodine removal for three different PACs.

Isotherm model information on the PAC treated of iohexol-spiked LGW is in Appendix B Table B-30. The observed q_{max} in iohexol-spiked LGW was higher than calculated q_{max}, which suggests more than just a monolayer for organic iodine removal when adsorption site competition with DOC is removed (Appendix B Table B-31).

Table 4-7: Linear, Langmuir, and Freundlich isotherms for TDI removal from RO concentrate and iohexol-spiked RO concentrate (1 mg/L as I) based on doses of coal-, wood-, and coconut-based PAC.

	Linear Isotherm	Langmuir Isotherms	Freundlich Isotherms
RO+ Coal-based PAC	Slope=K_{linear}= -2.22 R^2 = 0.72	Slope=0.0056 R^2=0.70 K_L=-0.31 q_{max}=0.021 mg/g	Slope=-3.2 R^2=0.74 K_f=0.002 n=-0.31
RO+ Wood-based PAC	Slope=K_{linear}=2.70 R^2 = 0.77	Slope=0.098 R^2=0.69 K_L=-0.54 q_{max}=0.011 mg/g	Slope=3.4 R^2=0.79 K_f=7.1 n=0.3
RO+ Coconut-based PAC	Slope=K_{linear}=4.13 R^2 = 0.12	Slope= 0.0056 R^2= 0.76 K_L=-0.31 q_{max}= 0.019 mg/g	Slope= 2.3 R^2= 0.13 K_f=0.002 n=0.44
RO+Iohexol + Coal-based PAC	Slope=K_{linear}=0.73 R^2 = 0.62	Slope=0.38 R^2=0.94 K_L=0.235 q_{max}=2.7 mg/g	Slope=0.30 R^2=0.72 K_f=2.2 n=3.3
RO+Iohexol + Wood-based PAC	Slope=K_{linear}=0.19 R^2 = 0.18	Slope=0.54 R^2=0.96 K_L=0.068 q_{max}=1.8 mg/g	Slope=0.13 R^2=0.28 K_f=1.7 n=7.7
RO+Iohexol + Coconut-based PAC	Slope=K_{linear}=0.97 R^2 = 0.73	Slope=0.19 R^2=0.94 K_L=0.326 q_{max}=5.1 mg/g	Slope=0.37 R^2= 0.81 K_f= 2.2 n=2.7

Based on the best-fit model, removal of organic iodine for each of the PAC types follows a single layer model shown in Figure 4-10 (Langmuir isotherms). This may be due to steric hindrance from the large molecular structure of the iodinated organics (as indicated by the non-target analysis tentative identification) and the iohexol is not charged, unlike iodide which used to generate the iodine number. To further test the single layer theory, a higher iohexol concentration (10 mg/L as I) was treated with a PAC dose of 100 mg/L for each carbon type. All

three PAC types exceeded the calculated q_{max}, which suggests more than a mono-layer for organic iodine removal.

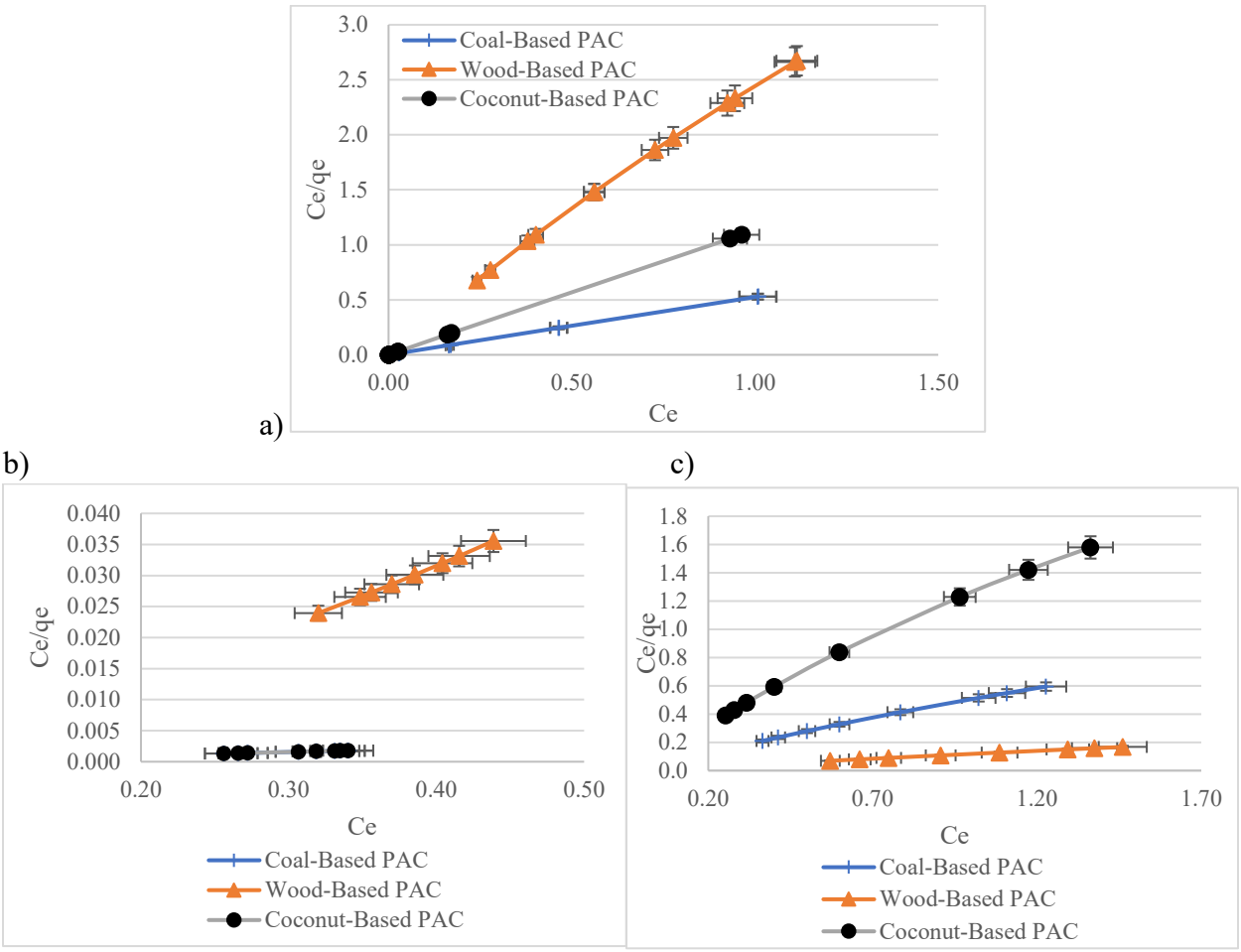


Figure 4-11: Langmuir isotherm linearization model for a) iohexol-spiked LGW, b) RO concentrate, and c) iohexol-spiked RO concentrate.

Intermolecular forces, such as Van der Waals dipole-dipole interactions, may explain the differences in adsorption ability of ICMs such as iohexol and their metabolites or degradation products such as iocetamic acid and iohexol TP687 identified in this study. While the compounds are not charged, they have localized partial negative and positive charges and these charge locations and magnitude are altered based on different chemical moieties. Dipole-dipole

interactions (intermolecular attraction—attractions between two molecules) may dictate the chemical's ability to adsorb to PAC and whether it will adsorb when other chemicals (with different dipoles) have previously been adsorbed. When the charges are calculated (Extended Huckel Charges, Chem3D, PerkinElmer) across the compound for each atom, differences in charge location and magnitude are observed for iohexol, iocetamic acid, and iohexol TP687. For iohexol, the charge on the iodine atoms ranged from 0.018 to 1.6, with the greatest partial positive charge located on an iodine atom (1.6) and the greatest partial negative charge located on the oxygen in the carbonyl groups of which there are three (range -0.83 to -0.96). For iocetamic acid, the charge on the iodine atoms ranged from 0.045 to 0.071, with the greatest partial positive charge located on a carbon atom (0.52) and the greatest partial negative charge located on the oxygen in the carbonyl groups of which there are two (-0.55 and -0.74). For iohexol TP687, the charge on the iodine atoms ranged from 0.06 to 1.6, with the greatest partial positive charge located on an iodine atom (1.6) and the greatest partial negative charge located on the oxygen in the carbonyl groups of which there are three (range -0.84 to -0.97).

With the different location and strength of partial charges on each compound, the intermolecular forces and steric hindrance could explain the adsorption differences between the iohexol and the organic iodine in the RO concentrate (iocetamic acid and iohexol TP687). With the stronger partial charge located away from the tri-iodinated ring, the adsorption of the metabolites may be impeded by the ring structure where the iohexol may be able to form additional layers due to electrostatic interactions with the partial charges located on the carbonyl groups and the iodine atoms.

Environmental levels of iohexol are much lower than 1 mg/L as I; it has been previously measured in surface water at 0.12 μg/L (Duirk et al., 2011). Based on this occurrence data, the

TDI will not approach q_{max} concentrations in source waters and the PAC is more likely to be exhausted by high DOC levels if it is used on untreated source water. Performance of the PAC removal ability will be impacted with the presence of DOC with the competition for adsorption sites on the carbon.

When comparing the removal of DOC by PAC dose, the coconut-based shows an increased removal ability at the higher doses (>400 mg/L), but otherwise tracks with the coal- and wood-based PACs. Since the PAC is added to the matrix as a slurry and the contact time is 2.5 hrs, the reduced performance associated with the addition of dry wood-based PAC was not observed (Graham et al., 2000).

Comparing the chlorinated RO concentrate and iohexol-spiked RO concentrate samples after PAC treatment, the iohexol spike did not significantly change the iodine incorporation in the I-THM formation when normalized for DOC ($p<0.2$). The iodine incorporation for I-THMs in the PAC treated versus non-PAC was also not significant ($p<0.3$).

Since the TDI concentrations were lowered due to the PAC doses and the iodine incorporation percentages for THMs were low for all chlorinated samples, then the PAC with the best removal of TDI using the lowest dose should be the recommended for removing organic iodine from source drinking waters. The iodine incorporation was similar for all PAC types and the differences in I-THM speciation were minimal such that the relative toxicity was not significantly when comparing PAC samples ($p<0.1$ to 0.3). Table 4-8 shows the iodine incorporation and relative toxicities of the PAC-treated RO concentrate and iohexol-spiked RO concentrate after chlorination and normalized with mg/L DOC. Appendix Tables B-32 and B-33 show the molar concentrations for the I-THMs and THM4, respectively. Appendix B Table B-34 shows the relative total toxicities for each I-THM species per mg/L of DOC after PAC treatment.

Table 4-8: Relative toxicity increase in the RO concentrate and the iohexol-spiked RO concentrate matrices due to iodine incorporation in THMs.

PAC treated chlorination	Iodine Incorporation in RO Concentrate (%)	Relative Toxicity in RO Concentrate from I-THMs	Iodine Incorporation in iohexol-spiked RO Concentrate (%)	Relative Toxicity in iohexol-spiked RO concentrate
RO+Cl_2	0.99	1.5	0.54	1.3
RO+ Coal-based PAC+Cl_2	1.03	1.2	0.61	2.2
RO+ Wood-based PAC+Cl_2	0.55	1.8	0.61	2.5
RO+ Coconut-based PAC+Cl_2	0.46	1.3	0.52	1.4

Based on the PAC dose-removal curves and isotherms, the coconut-based PAC showed the greatest removal ability of TDI and the greatest predicted q_{max} of 5.1 mg TDI/g PAC. To target 90% removal of TDI in the source water with the coconut-based PAC, the treatment plant dose should be approximately 34 mg/L, based on Equation 4-4 and the coconut-based PAC Langmuir isotherm variables (Table 4-7) of q_{max} = 5.1 mg/g, K_L = 0.326, and C_E = 90% $[TDI]_0$ = 1.22 mg/L:

$$q_E = \frac{mg\ of\ TDI}{g\ of\ PAC} = \frac{q_{max}(K_L C_E)}{1 + K_L C_E}$$

PAC dose for iohexol-spiked RO for 90% TDI removal =

(1.22 mg/L) / ((5.1 mg/g)(1.22 mg/L)(0.326)/(1+(1.22 mg/L)(0.326))) = 0.836 g/L = 836 mg/L

PAC dose based on drinking water treatment plant DOC for 90% TDI removal =

(836 mg/L)(5.1 mg/L as C)/(127 mg/L as C) = 34 mg/L

when using the Langmuir adsorption model describing the iohexol-spiked RO concentrate matrix. In comparison, the wood-based PAC dose would be 365 mg/L and the coal-based PAC dose would be 88 mg/L to achieve the same 90% removal based on the Langmuir isotherm.

4.5 Conclusions

I-THM formation during chlorination is occurring within the drinking water treatment plant selected for this study whose source water is impacted by upstream treated medical wastewater discharge. When free chlorine is applied at any location in the treatment plant and TDI is present, I-THMs form and can continue to form in the finished water. Once in the distribution system and new sources of TDI are introduced, I-THMs degrade due to the presence of residual disinfectant which can create spatial variability of I-THMs in the water delivered to the consumers. For this drinking water treatment plant, PAC is the existing treatment option identified by this research that can be optimized for TDI removal, with the added benefit of DOC removal for DBP precursors. This treatment plant plans to replace their multi-media filters with granular activated carbon (GAC) in the future, which could impact TDI removal with increased adsorption potential.

All three PACs tested were able to remove TDI from the RO concentrated source water with minimal differences in iodine incorporation and relative toxicities calculated for the I-THM formation potential of PAC treated RO concentrate and iohexol-spiked RO concentrate. The recommendation for this treatment plant based on the TDI concentrations through each unit process is to change the PAC type from coal-based to coconut-based and increased the dose to 34 mg/L for approximately 90% TDI removal. Due to the NOM and other chemicals in the source water, it would not be feasible to target 100% removal of TDI and the remaining TDI may lead

to I-THM but with a decreased formation potential due to decreased precursors. Additionally, PAC costs can depend on many factors, but general pricing for coconut-based PAC ($0.70/lb) is twice the cost of wood ($0.50/lb) and coal-based ($0.35/lb) PACs (Research and Markets, 2017), which will increase the operating costs of the drinking water treatment plant. Additional PAC testing for geosmin and MIB using the coconut PAC is also needed to ensure a switch in PAC type does not fail to meet the plant's current taste and odor removal needs.

From a public health view, these I-THM formation results are concerning based on their increased biological activity compared to regulated THMs and continued research is needed to reduce the exposure risk for the public. Additional distribution system testing is also needed to determine the fate of iodine from the I-THMs and whether it can be incorporated into new I-DBPs.

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CHAPTER 5 : CONCLUSIONS AND IMPLICATIONS

5.1 Summary of Results

Collectively, the studies presented in this dissertation demonstrate that I-DBPs are more widespread in drinking water than previously understood. Previous iodine chemistry showed inorganic iodide in a chloraminated drinking water treatment plant as the predominant I-DBP formation pathway and that chlorination of iodide would form iodate. Most chloraminating drinking water treatment plants use chlorine as a primary disinfectant then add ammonia for chloramine formation prior to distribution, which would lead the inorganic iodide to form iodate and not incorporate into I-DBPs. However, this research has demonstrated that chlorine can react with organic iodine and form I-DBPs through a proposed dehalogenation reaction. Organic iodine should also be considered a precursor for I-DBPs in drinking water when chlorine is used as a disinfectant for any step within the treatment plant. This research has indeed shown that source drinking waters impacted by upstream wastewater treatment plants receiving medical waste have a strong likelihood of I-DBP formation during chlorination. These findings support the central hypothesis that organic iodine from medical waste can be precursors for I-DBPs in downstream drinking water treatment plants.

Chapter 2 demonstrated that iodoacids are formed in drinking water treatment plants and an analytical method was developed that could successfully extract them from a drinking water matrix that also contained bromine- and chlorine-containing haloacetic acids at orders of magnitude higher concentrations.

With a multi-step extraction process that utilizes LLE and SPE, iodoacetic acid, chloroiodoacetic acid, bromoiodoacetic acid, and diiodoacetic acid were concentrated from ng/L levels observed in drinking water treatment plants to µg/L levels that were esterified and then quantified using GC-MS ion trap positive electron impact for fragmentation and negative chemical ionization for confirmation of iodine at m/z 127. Using this extraction/concentration and analytical method, 13 HAA species were resolved from each other and the quantitation of the 4 IAs at orders of magnitude lower concentrations than HAA9 in drinking water was achieved. This method can now be used to monitor the currently unregulated iodinated HAAs.

In Chapter 3, source waters were identified in a North Carolina watershed that could be impacted by medical waste and the case study showed that organic iodine can be discharged from a wastewater treatment plant and enter a downstream drinking water treatment plant. Overall, the organic iodine source tracking indicated the presence of treated medical waste and that source diffusion away from hospital-impacted wastewater treatment plants increased the impact of medical waste across downstream communities. Non-target analysis on the total dissolved organic iodine showed the ICMs and their degradation products were only small contributors to the total organic iodine load. Even with multiple unit processes in the DWTP studied here, the organic iodine was able to persist through the treatment plant and produce I-THMs in the finished drinking water. Further investigation into optimizing the PAC unit process determined that mitigation of organic iodine could be achieved by an existing drinking water treatment process.

Results represented in Chapter 4 showed that I-THM formation in a drinking water treatment plant starts with the addition of free chlorine when organic iodine is present rather than after the generation of chloramines as suggested by previous researchers (Bichsel and von

Gunten, 2000). Additionally, sampling of I-THMs in the distribution system did not show a correlation with water age and lab-based tests showed a decrease in I-THMs when a disinfection residual was present. With the I-THM degradation rate of 16 μg/L I-THMs/day for the iohexol-spiked finished water, iodine might be re-released in the distribution system in a way that residual chloramine could form new I-DBPs. Non-target analysis showed that large molecular weight organic iodine was present at the maximum residence time in the distribution. By looking at each unit process within the case study drinking water treatment plant an existing treatment, namely use of PAC, appeared to reduce the organic iodine precursor in addition to the traditional taste and odor compounds it is used for. PAC can, therefore, be an effective multi-problem removal tool for TDI. All three PACs tested (coal-based, wood-based, and coconut-based) were able to remove organic iodine from the RO-concentrated source water with minimal differences in iodine incorporation and relative toxicities calculated for the I-THM formation potential of PAC-treated RO concentrate and iohexol-spiked RO concentrate. The recommendation based on the results of this study is to change the current coal-based PAC to coconut-based and increase the dose from its current level of 20 mg/L to 34 mg/L for approximately 90% TDI removal.

With the increased demand on water resources, the continual discharge into surface water of organic iodine in wastewater effluent containing residues of medical waste, and the widespread use of chlorine and chloramines for disinfection, there is an increased risk of I-DBP formation in finished drinking water. This research shows that increased removal of TDI could be achieved with a single unit process optimization within the drinking water treatment plant, but a pilot plant and/or full-scale testing is needed for confirmation and for wider implementation.

5.2 Examination of Individual Hypotheses

Hypothesis 1: A robust and reproducible analytical method for the quantification of iodoacids from drinking water can be developed using a multi-step extraction and analysis by gas chromatography mass spectrometry (GC/MS).

The iodoacid method has filled a gap in I-DBP research by developing a robust method for IA extraction and detection in drinking water. Shown in Chapter 2 and summarized in Figure 5-1, a new method for the extraction, detection, and quantification of iodoacids in drinking water with the presence of other HAAs was developed using LLE, SPE, and GC-MS. The four iodoacids were successfully resolved from co-extractants that are typically seen at orders of magnitude higher concentration. Since the detection limit for this method is in the ng/L range, it can be directly used for monitoring levels of four iodoacids in drinking water, which will allow for more occurrence data to be collected and subsequent evaluation of the health implications associated with chloramination of water containing inorganic iodide and chlorination of water containing organic iodine.

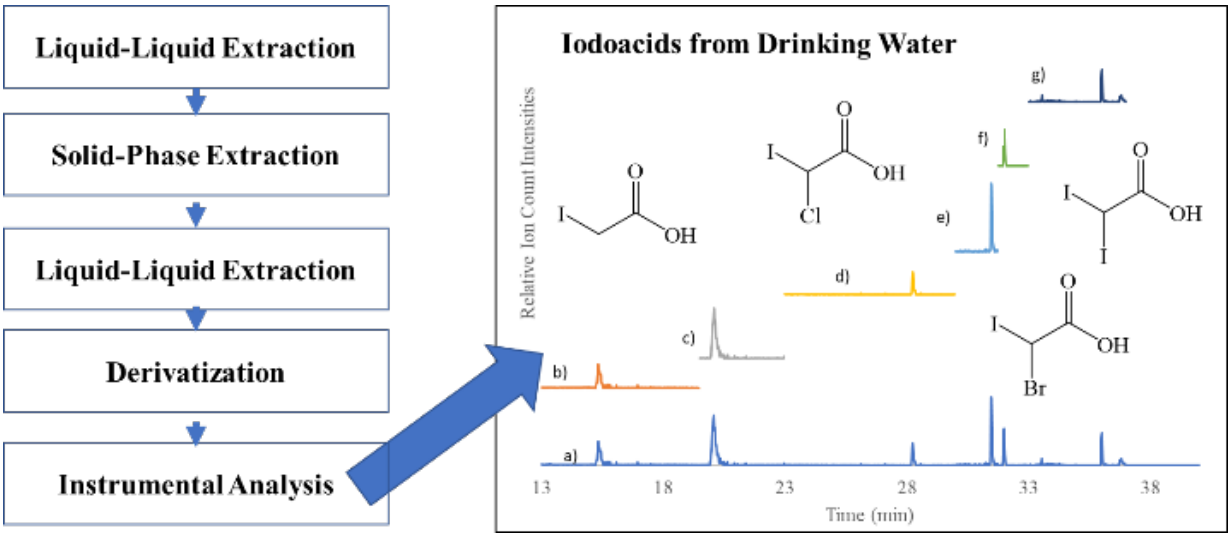


Figure 5-1: Summary of the iodoacid method and the chromatographic resolution of four iodoacids from nine haloacetic acids.

Hypothesis 2. Surface waters receiving treated hospital waste effluent will show elevated levels of iodine and drinking water plants using these impacted surface waters will generate I-DBPs.

Organic iodine from treated medical waste was tracked from the point of wastewater discharge to the downstream drinking water treatment plant where it became precursor material for I-DBP formation. This case study supported the hypothesis that surface waters receiving treated hospital waste effluent contained levels of iodine that downstream drinking water plants using chloramine disinfection could convert into I-DBPs. However, the I-THMs, a surrogate for the larger I-DBP group, formed when chlorine was added prior to the formation of chloramines in the finished water. Non-target chemical analysis has confirmed that one particular ICM, iohexol, and its biotransformed products are responsible for at least a portion of the total organic iodine load in the drinking water source. Identification of the iodinated organic species shows

that quantification of the ICMs only will underestimate the iodinated precursors that can enter a drinking water treatment plant that lead to the formation of I-THMs (and other I-DBPs) in the finished water. The organic iodine source tracking identified the treated medical waste from the upstream wastewater discharge as a prominent point source (Figure 5-2), but source diffusion was also observed. Correlation analysis suggests that dissolved nitrogen or Peak T from EEMs analysis could be used for surrogate iodine analysis in wastewater-impacted surface waters containing iodine since they are indicators of wastewater. However, this correlation analysis needs to be strengthened through more surface water quality observations over seasonal changes and across multiple watersheds.

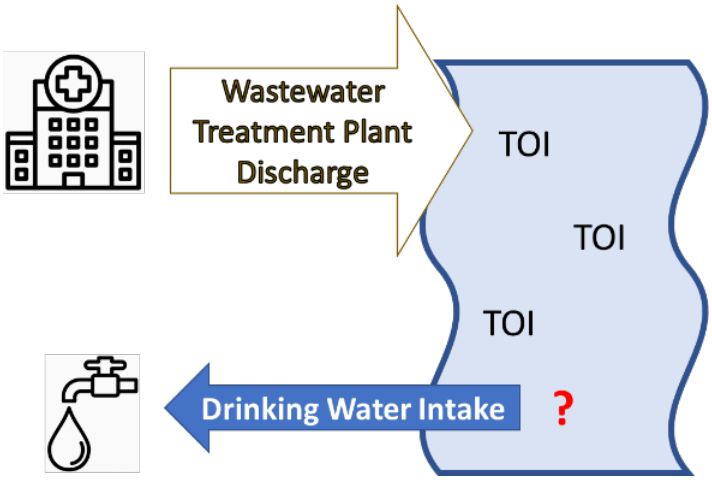


Figure 5-2: Connecting the total organic iodine source from treated hospital waste from upstream wastewater discharge to the downstream drinking water treatment plant.

Hypothesis 3. An existing unit process within the drinking water treatment plant can be optimized for iodine precursor removal to limit iodine incorporation into THMs and reduce the theoretical relative cytotoxicity in finished water.

I-THM formation occurred within the case-study drinking water treatment plant whose source water is impacted by upstream treated medical wastewater discharge. When free chlorine is applied in the treatment plant and TDI is present, I-THMs form and enter the distribution system but can be degraded due to disinfectant residuals (Figure 5-3). PAC is an existing treatment option identified by this research that can be optimized for TDI removal, with the added benefit of DOC removal for an additional decrease in DBP precursors. All three PACs tested were able to remove TDI from the RO-concentrated source water with minimal differences in iodine incorporation and relative toxicities calculated for the I-THM formation potential of PAC-treated RO concentrate and iohexol-spiked RO concentrate (Figure 5-4). Based on these findings, the HOI formation and reactivity diagram from Bischel and von Gunten (1999) has been updated to reflect the formation of I-DBPs from organic iodine in the presence of free chlorine (Figure 5-5). Even though the iodine incorporation is low, the presence of I-THMs are concerning based on their increased cytotoxicity compared to regulated THMs and continued research is needed to reduce the exposure risk for the public. The recommendation for this treatment plant based on the TDI concentrations through each unit process is to change the PAC type from coal-based to coconut-based and increase the dose to 34 mg/L for approximately 90% TDI removal.

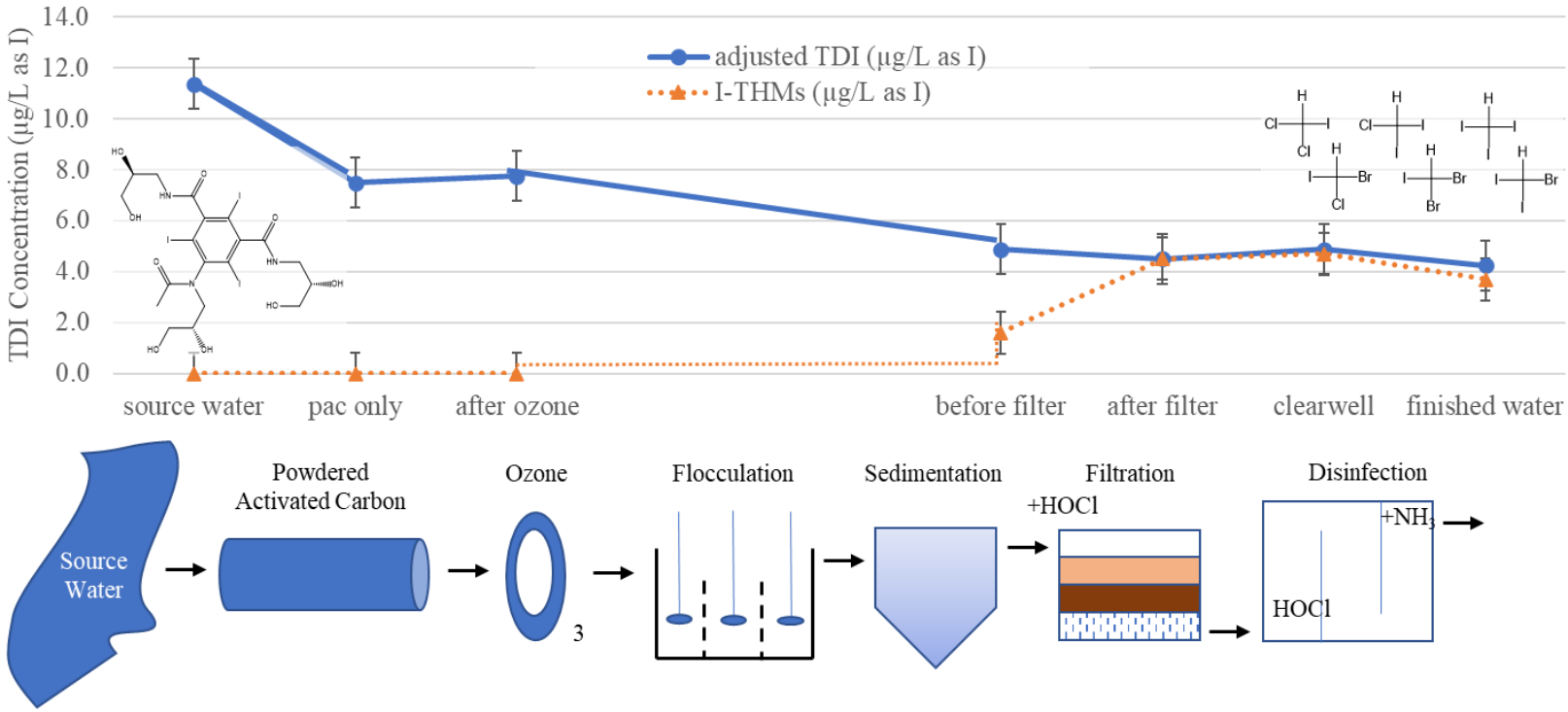
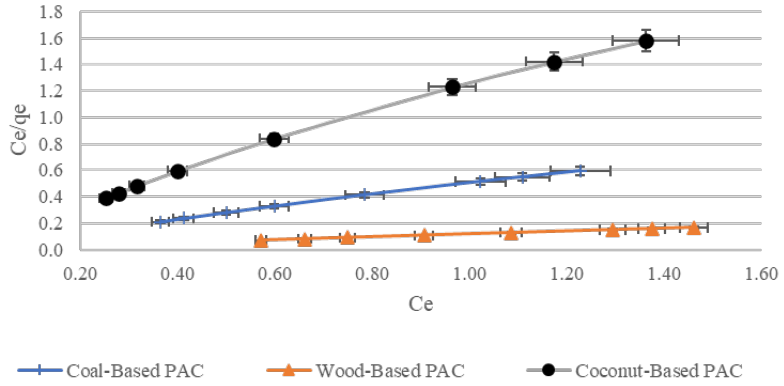


Figure 5-3: Total dissolved iodine removal and transformation within the case study drinking water treatment plant.

PAC removal of TDI from iohexol-spiked RO concentrate



Theoretical Toxicities after Chlorination of PAC-treated iohexol-spiked RO concentrate

	Iodine Incorporation in iohexol-spiked RO Concentrate (%)	Relative Toxicity in iohexol-spiked RO Concentrate
RO+Cl_2	0.54	1.3
RO+ Coal-based PAC+Cl_2	0.61	2.2
RO+ Wood-based PAC+Cl_2	0.61	2.5
RO+ Coconut-based PAC+Cl_2	0.52	1.4

Relative Iodine Incorporation and Theoretical Toxicity Increase Due to I-THM formation from the Chlorination of PAC-treated iohexol-spiked RO concentrate

Coconut-Based PAC < Coal-Based PAC < Wood-Based PAC

Figure 5-4: Calculated isotherms for powdered activated carbon treatment with theoretical toxicities and iodine incorporation following chlorination.

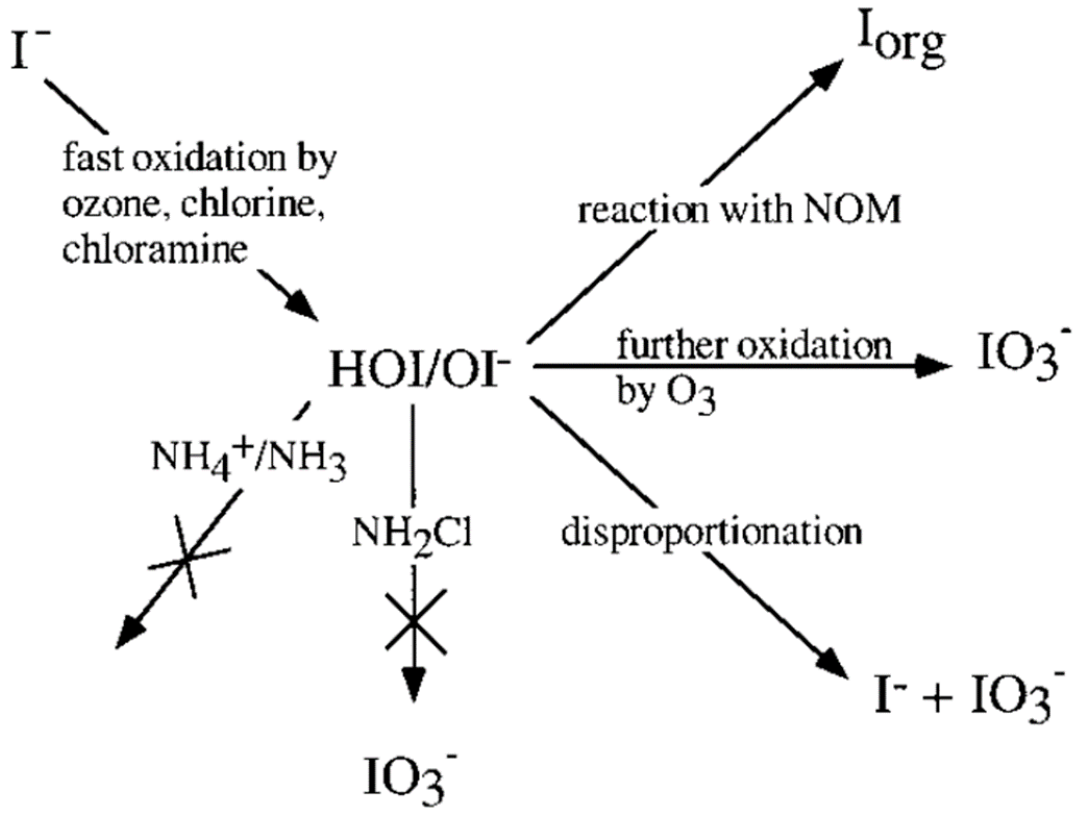


Figure 5-5: Formation and reactivity of HOI (updated from Bichsel and von Gunten, 1999).

5.3 Implications and Public Health Relevance

Due to the absence of or limited regulations on medical discharge to the sanitary sewer, many pharmaceutically active agents from hospitals and patients reach wastewater treatment plants which may not be able to adequately remove them. Discharging treated wastewater with anthropogenic pollutants intact into receiving surface waters will shift the burden of treatment to downstream communities that will need to upgrade their drinking water treatment systems to remove the contaminants.

One main difficulty in regulating ICMs and their biotransformed products, is that current research shows they are not biologically active when discharged from a wastewater treatment plant (Wendel et al., 2016). However, once those large iodinated organic compounds enter a

drinking water treatment plant and react with a strong oxidant, smaller, highly toxic, iodinated byproducts can form (Wendel et al., 2014). From the current regulatory perspective, since there are no limits on biologically inert ICMs, the burden to remove the contaminant falls solely on the downstream drinking water treatment plant.

Since I-DBPs are not currently regulated in drinking water, utilities do not monitor their occurrence or adjust their DBP reduction strategies. Using a multi-problem-solving treatment like PAC could prove beneficial for drinking water treatment plants in reducing the formation potential of all DBPs through DOC removal, with the added benefit of TDI removal and reduction in I-DBP formation potential. PAC addition can also help address the increasing amounts of anthropogenic contaminants such as pesticides, herbicides, PFAS, and endocrine disruptors that need to be removed from drinking water sources in addition to taste and odor compounds from algal blooms (Bansal and Goyal, 2005; Chu et al., 2011; Jasim et al., 2006; Kingsbury et al., 2008; Loos et al., 2013; Shimabuku et al., 2016). The PAC option may prove to be a cost-effective measure when multiple absorbable contaminants are present.

For process operation, the addition of a PAC by rapid mix would not need a large foot print compared to additional granular activated carbon filters or increased energy needs compared to membrane filtration (Crittenden et al., 2012). However, there will be increased waste volume from sedimentation tanks and a need for additional drying ponds.

The public health impacts of organic iodine appear to be in the jurisdiction of the drinking water treatment plant. However, there should be an increased accountability from upstream discharge locations for a more balanced approach to source water management when anthropogenic contaminants compromise water quality downstream. If a watershed approach was adopted, then equitable responsibility for all stakeholders could be determined such that the

total burden for removal is not placed on the downstream plants. Since organic iodine from ICMs and their biotransformed products are not limited to wastewater treatment plants due to source diffusion, it may not be appropriate to regulate only the hospital waste streams prior to the wastewater treatment plant.

Improved drinking water quality has numerous benefits including improved aesthetics and most importantly health. Compared to bottled water, municipal drinking water supplies contribute to a more environmentally and economically sustainable society. Drinking water plants demonstrating the capability to treat compromised source water sources will also be enabled to tap into additional water sources that could ultimately reduce the treatment requirements for non-sustainable solutions for water reuse.

5.4 Recommendations for Future Work

This research revealed a direct link between biotransformed ICMs in wastewater discharge and I-DBP formation in downstream drinking water treatment plants. Sampling at other locations with respect to medical-waste impacted wastewater discharge and surface water as a drinking water source, should be conducted to determine how widespread the organic iodine issue has become and the magnitude of the contaminant problem. With increasing issues of droughts and water shortages, as seen across the US (Chang and Bonnette, 2016), indirect potable water-reuse will become increasingly necessary and wastewater inputs will need to be viewed in a watershed approach to regulations.

Monitoring of organic iodine inputs to surface waters should also be coupled with I-DBP occurrence. Now that methods are available for measuring iodoacids and they have been found in drinking water, these unregulated I-DBPs should be added to future Contaminant Candidate

Lists (CCL) or Unregulated Contaminant Monitoring Rules (UCMR) to determine the occurrence of these toxic I-DBPs and to conduct epidemiological studies on their human health effects. Previous research focused on I-DBPs in drinking water treatment plants using chloramine for disinfection, but the results of the research in this thesis show that I-DBPs can be formed from organic iodine and in drinking water treatment plants using free chlorine as well. By monitoring I-THMs and TDI in the distribution system, this research has shown that I-THMs are not stable in the presence of residual disinfectant and new I-DBPs could be forming. With the increased formation potential of I-DBPs in medical waste impacted source waters, there is a renewed need for I-DBP surveillance.

Several approaches are necessary to further validate these conclusions and to strengthen the argument for addition of PAC treatment options to existing drinking water treatment plants for TDI removal. Optimized PAC type and dosing was shown to be an effective organic iodine removal process within a drinking water treatment plant. While PAC is a multi-problem-solving unit process, it can be costly to use as a year-round solution and the waste produced from the process still needs to be disposed properly so that these contaminants are not reintroduced into the environment. TDI tracking within drinking water treatment plants with different unit processes or with different source water characteristics could provide more insight for increased removal of iodine precursors.

Currently, the cost burden for the removal of these anthropogenic chemicals has been sent to downstream communities instead of addressed at the same locations from which they originated. Other treatment strategies within the wastewater treatment plant should continue to be explored since previous research shows significant reductions of >80% removal of parent ICMs across the plant by biotransformation (Kormos et al., 2011). Increased removal efficiency

by biological and chemical mechanisms (Jeong et al., 2010; Kormos et al., 2010; Moro et al., 2015; Onesios et al., 2009) could lead to lower TDI in wastewater effluents and lower iodinated precursors entering the downstream drinking water treatment plants.

These additional occurrence studies and adjustments to PAC treatment optimization along with the research presented in Chapters 2, 3, and 4 can provide utilities and regulatory agencies with targets for high priority precursor removal or adjustment of treatment strategies to mitigate DBP mixture toxicities due to organic iodine.

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APPENDIX A: SUPPORTING INFORMATION FOR CHAPTER 3

Appendix A Table A-1: Reservoir characteristics

Maximum Length	**26 km**
Maximum Width	8.0 km
Surface Area	56.4 km^2
Average Depth	4.5 m
Maximum Depth	43 m
Drainage Area	4,375 km^2
Storage Capacity	56 hm^3

*Data from Source Water Assessment Program Report (North Carolina Department of Environmental Quality, 2015)

Non-Target Analysis of Sampling of Wastewater Discharges and Drinking Water Intake

Sample extracts were concentrated by a factor of 50 with 5µL injections onto a C-18 column in positive and negative electrospray-ionization (ESI+ and ESI-) modes. The signal to noise ratio of 5 or above was used to confirm the presence of a peak.

Appendix A Table A-2: Settings for the LC mobile phase and Q/TOF mass spectrometer

Mobile Phase Program		
Time (min)	Mobile phase A 0.1%Formic Acid in water	Mobile phase B 0.1%Formic Acid in acetonitrile
0	95%	5%
1	95%	5%
8	10%	90%
8.1	0%	100%
15	0%	100%
15.1	95%	5%
18	95%	5%
MS-only mode		
Capillary Voltage (V)	3000	
Fragmentor Voltage (V)	360	
Skimmer voltage (V)	65	
Scan Range (m/z)	50 to 950	
Scan Rate (spectra/sec)	3	
Reference Masses (m/z)	121.05087300 922.00979800	

Appendix A Table A-3: Surface water quality parameters for the February 2017 sampling event.

Sampling Location	DOC (mg/L as C)	DN (mg/L as N)	TDI (µg/L as Cl)	TDOI (µg/L as I)	TDOX (µg/L as Cl)	SUVA (L/mg-M)	EEM Peak T	Conductivity (µS/cm)
Pre-WWTP 1	6.3	0.4	30.3	22	31	2.9	0.29	280
WWTP 1	5.3	4.5	22.4	20	96	2.4	0.58	447
Post-WWTP 1	5.4	3.3	29.3	22	73	2.5	0.43	455
Pre-WWTP 2	5.0	0.3	14.1	12	24	3.3	0.28	187
WWTP 2	6.7	5.9	146.8	144	143	2.4	0.88	425
Post-WWTP 2	5.8	1.2	36.5	32	30	3.1	0.39	222
Pre-WWTP 3	3.1	0.4	5.8	10	18	2.8	0.15	138
WWTP 3	5.0	5.2	63.2	65	70	2.5	0.66	467
Post-WWTP 3	4.1	3.2	34.4	33	65	2.5	0.35	279
Location A	5.4	0.5	25.6	32	24	2.5	0.23	199
Location B	5.8	0.6	26.7	23	28	2.8	0.27	203
Location C	5.7	0.6	25.7	23	19	2.5	0.24	196
Location D	5.6	0.5	21.4	20	23	2.4	0.23	152
DWTP Intake	5.1	0.5	18.4	18	54	3.1	0.29	135
Location E	5.6	0.6	19.9	20	24	2.4	0.24	141
Location F	5.5	0.6	19.6	20	23	2.7	0.31	138
River	4.4	1.5	16.4	15	22	3.0	0.43	206
Location G	4.7	1.1	15.5	15	26	2.8	0.27	190
Location H	4.9	1.0	14.8	15	25	2.9	0.25	179
Location I	5.1	0.9	16.2	17	26	3.2	0.44	164

Appendix A Table A-4: Surface water quality parameters for the March 2017 sampling event

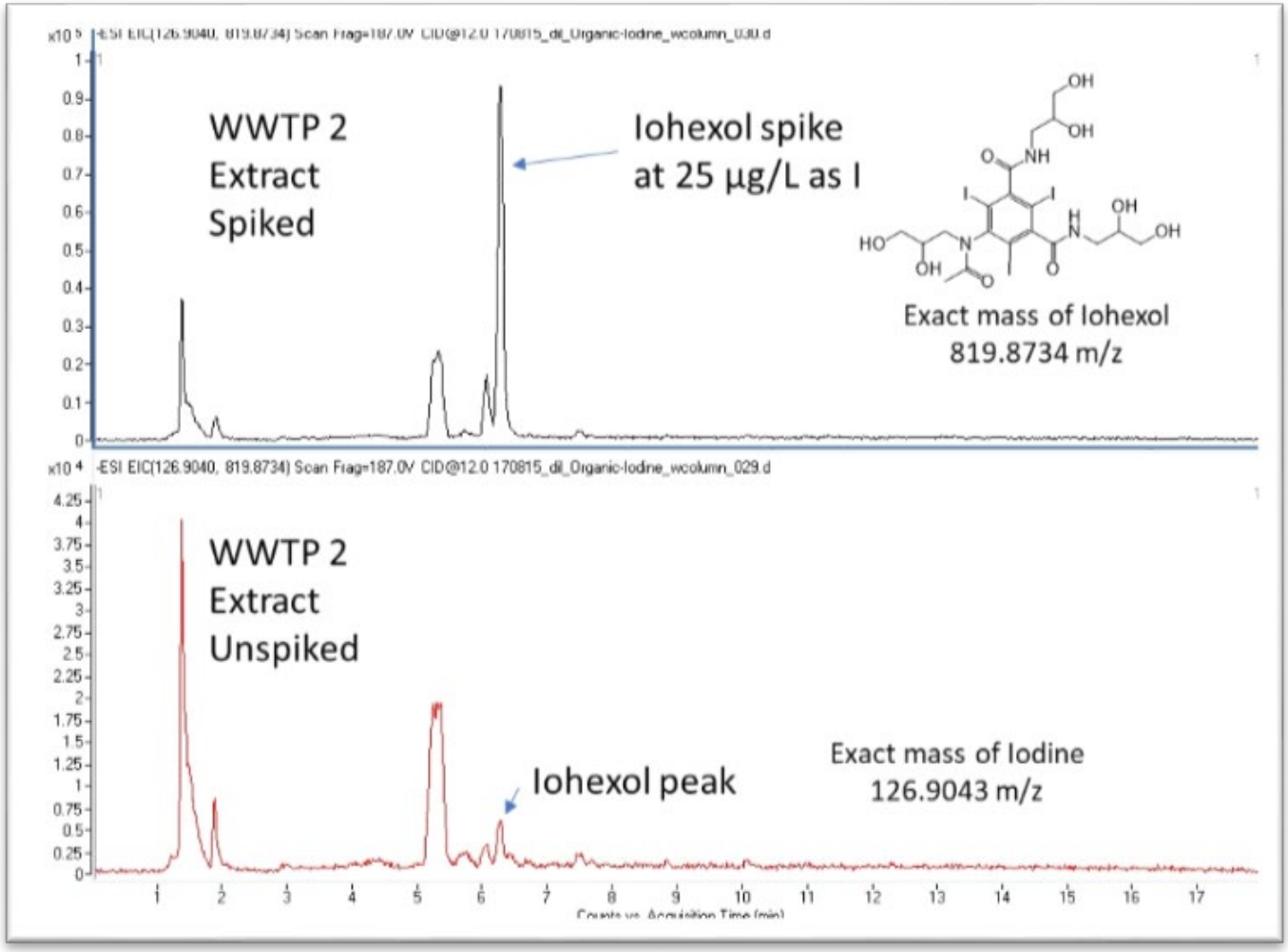
Sampling Location	DOC (mg/L as C)	DN (mg/L as N)	TDI (µg/L as Cl)	TDOI (µg/L as I)	TDOX (µg/L as Cl)	SUVA (L/mg-M)	EEM Peak T	Conductivity (µS/cm)
Pre-WWTP 1	6.2	0.2	39.1	22	33	3.5	0.33	265
WWTP 1	5.7	5.2	25.7	17	104	2.1	0.64	506
Post-WWTP 1	5.8	2.4	29.3	17	66	2.5	0.44	406
Pre-WWTP 2	3.8	0.2	18.9	15	25	4.1	0.21	179
WWTP 2	6.7	6.0	135.1	113	114	2.3	0.87	480
Post-WWTP 2	5.4	2.4	53.5	37	48	3.2	0.40	276
Pre-WWTP 3	2.8	0.3	14.3	8	16	3.0	0.12	154
WWTP 3	4.5	6.4	94.8	98	71	2.4	0.62	467
Post-WWTP 3	3.6	4.0	57.2	53	57	2.6	0.38	305
Location A	5.5	0.3	35.5	27	44	2.6	0.26	191
Location B	5.6	0.2	34.4	28	47	2.6	0.28	208
Location C	5.5	0.1	30.9	25	38	2.6	0.27	191
Location D	5.4	0.3	29.2	23	23	2.3	0.21	165
DWTP Intake	5.6	0.7	27.7	15	35	2.8	0.26	263
Location E	5.6	0.4	27.0	18	30	2.5	0.28	163
Location F	5.3	0.2	25.7	18	17	2.6	0.30	116
River	4.6	1.6	22.0	23	49	3.0	0.76	223
Location G	4.7	0.9	25.9	18	33	2.8	0.56	201
Location H	4.8	0.8	25.4	17	28	2.7	0.48	189
Location I	4.9	0.8	25.0	18	30	2.7	0.49	183

Appendix A Table A-5: Surface water quality parameters for the June 2017 sampling event

Sampling Location	DOC (mg/L as C)	DN (mg/L as N)	TDI (µg/L as Cl)	TDOI (µg/L as I)	TDOX (µg/L as Cl)	SUVA (L/mg-M)	EEM Peak T	Conductivity (µS/cm)
Pre-WWTP 1	16.8	0.9	16.4	12	34	3.8	0.57	106
WWTP 1	6.8	5.0	21.2	27	104	2.5	0.70	423
Post-WWTP 1	9.5	0.8	17.4	12	34	4.3	0.72	130
Pre-WWTP 2	11.2	0.6	8.8	5	24	3.8	0.38	71
WWTP 2	6.5	4.5	121.2	129	132	2.6	0.75	340
Post-WWTP 2	11.2	0.5	9.8	7	26	3.8	0.43	74
Pre-WWTP 3	5.7	0.5	10.3	10	19	3.3	0.28	95
WWTP 3	4.4	7.4	84.0	63	75	2.5	0.52	421
Post-WWTP 3	6.7	1.3	20.7	20	31	3.9	0.35	159
Location A	6.6	0.4	24.2	25	27	2.8	0.34	164
Location B	6.4	0.4	23.6	22	35	2.6	0.29	159
Location C	6.5	0.4	24.2	17	25	2.5	0.27	161
Location D	6.5	0.4	20.0	17	24	2.5	0.27	206
DWTP Intake	6.2	0.5	21.4	15	28	3.1	0.31	146
Location E	6.3	0.4	19.0	17	15	2.6	0.27	147
Location F	6.6	0.4	19.2	20	24	2.7	0.33	141
River	6.3	0.7	14.8	13	24	3.9	0.48	109
Location G	8.1	0.7	7.4	8	9	3.7	0.31	71
Location H	8.3	0.7	7.5	8	15	3.4	0.30	76
Location I	7.5	0.8	10.4	10	19	3.4	0.34	99

Appendix A Table A-6: Surface water quality parameters for the July 2017 sampling event

Sampling Location	DOC (mg/L as C)	DN (mg/L as N)	TDI (µg/L as Cl)	TDOI (µg/L as I)	TDOX (µg/L as Cl)	SUVA (L/mg-M)	EEM Peak T	Conductivity (µS/cm)
Pre-WWTP 1	4.0	0.6	8.1	5	18	2.4	0.22	160
WWTP 1	5.4	6.5	10.3	13	101	2.7	0.56	462
Post-WWTP 1	5.2	2.6	11.4	8	53	3.0	0.39	283
Pre-WWTP 2	4.7	0.4	10.6	8	15	4.3	0.32	150
WWTP 2	6.1	7.9	80.8	92	119	2.8	0.81	423
Post-WWTP 2	5.6	4.5	49.2	43	64	2.9	0.43	283
Pre-WWTP 3	3.4	0.8	7.7	7	17	3.4	0.22	173
WWTP 3	4.1	6.2	41.9	50	86	2.7	0.57	520
Post-WWTP 3	4.0	4.1	28.5	32	51	2.8	0.38	351
Location A	5.8	0.5	13.4	12	26	2.9	0.30	194
Location B	5.8	0.4	13.4	8	24	3.0	0.32	184
Location C	5.8	0.4	11.5	8	20	2.9	0.28	175
Location D	5.7	0.4	10.3	7	22	2.8	0.29	167
DWTP Intake	6.8	0.7	10.9	7	9	2.5	0.29	573
Location E	5.7	0.4	9.6	8	24	2.8	0.30	153
Location F	5.5	0.4	8.7	8	25	2.8	0.28	165
River	4.8	1.8	14.0	13	46	3.3	0.43	265
Location G	5.1	0.6	12.0	8	31	3.2	0.34	218
Location H	5.5	0.5	10.0	8	29	3.0	0.30	212
Location I	5.2	0.4	8.7	7	26	3.1	0.27	156



Appendix A Figure A-1: Extracted ion chromatograms for organic iodine in WWTP effluent at m/z =126.9043 with and without iohexol spike using in-source fragmentation.

Non-Target Qualitative Results of Dissolved Organic Iodine in Wastewater Discharges and Drinking Water Intake

Compound (Cpd) lists were generated from the four each sampling event extracts, and organic iodine compounds not observed in all four samples are listed separately. Compound match scores had to be >70 to be included on named lists.

Cpd number corresponds to a total named organic iodine compound count. Formula is the molecular formula based on fragmentation patterns and masses observed. Mass (Tgt) is the calculated (predicted) exact mass of the named compound. Diff (ppm) is the difference between the observed mass and the predicted mass. Ions are the number of ions in the fragmentation pattern that determined the molecular formula. RT is the chromatographic peak retention time.

Appendix A Table A-7: Drinking water treatment plant compound list

Cpd	Name	Score (Tgt)	Formula	Precursor	Mass	Mass (Tgt)	Mass Accuracy (ppm)	RT (min)	Ions
1	Iocetamic acid	88.47	C12 H13 I3 N2 O3	612.7991	613.8063	613.806	0.48	2.336	5
2	TP687 (Kormos et al.)	91.09	C13 H12 I3 N3 O6	685.7783	686.7843	686.786	-2.54	2.336	5

Appendix A Table A-8: WWTP 1 discharge compound list

Cpd	Name	Score (Tgt)	Formula	Precursor	Mass	Mass (Tgt)	Mass Accuracy (ppm)	RT (min)	Ions
1	Iocetamic acid	88.15	C12 H13 I3 N2 O3	612.7983	613.8056	613.806	-0.74	2.35	5
2	TP687 (Kormos et al.)	91.55	C13 H12 I3 N3 O6	685.7778	686.7839	686.786	-3.12	2.35	5

Appendix A Table A-9: WWTP 2 discharge compound list

Cpd	Name	Score (Tgt)	Formula	Precursor	Mass	Mass (Tgt)	Mass Accuracy (ppm)	RT (min)	Ions
1	Iocetamic acid	88.49	C12 H13 I3 N2 O3	612.7983	613.8056	613.8060	-0.71	2.378	4
2	TP687 (Kormos et al.)	93.06	C13 H12 I3 N3 O6	685.7778	686.7841	686.7860	-2.85	2.361	5
3	Iomeprol	97.17	C17 H22 I3 N3 O8	775.8468	776.854	776.8541	-0.14	5.330	3

Appendix A Table A-10: July and March only for WWTP 2 discharge compound list

Cpd	Name	Score (Tgt)	Formula	Precursor	Mass	Mass (Tgt)	Mass Accuracy (ppm)	RT	Ions
1	Iocetamic acid	89.28	C12 H13 I3 N2 O3	612.7988	613.8060	613.8060	-0.04	2.363	4
2	TP687 (Kormos et al.)	92.17	C13 H12 I3 N3 O6	685.7780	686.7852	686.7860	-1.17	2.363	3
3	Iomeprol	98.42	C17 H22 I3 N3 O8	775.8465	776.8537	776.8541	-0.48	5.332	3
4	TP773 (Kormos et al.)	73.60	C16 H14 I3 N3 O9	831.7979	772.7826	772.7864	-4.91	1.202	3
5	TP787 (Kormos et al.)	73.94	C17 H16 I3 N3 O9	831.7979	786.7968	786.8021	-6.64	1.351	6
6	TP817 (Kormos et al.)	91.06	C18 H18 I3 N3 O10	861.8099	816.8114	816.8126	-1.44	1.269	5
7	TP835 (Kormos et al.)	82.92	C19 H24 I3 N3 O10	833.8516	834.8577	834.8596	-2.22	1.866	3
8	TP863 (Kormos et al.)	91.82	C19 H20 I3 N3 O12	861.8104	862.8176	862.8181	-0.62	1.269	3
9*	Iohexol	83.66	C19 H26 I3 N3 O9	819.8728	820.8789	820.8803	-1.68	6.241	3

*seen in March WWTP2 sample extract only

Appendix A Table A-11: WWTP 3 discharge compound list

Cpd	Name	Score (Tgt)	Formula	Precursor	Mass	Mass (Tgt)	Mass Accuracy (ppm)	RT (min)	Ions
1	Iocetamic acid	87.49	C12 H13 I3 N2 O3	612.7979	613.8051	613.8060	-1.53	2.376	5
2	TP687 (Kormos et al.)	88.88	C13 H12 I3 N3 O6	685.7771	686.7831	686.7860	-4.17	2.376	5

Appendix A Table A-12: June only for WWTP 3 discharge compound list

Cpd	Name	Score (Tgt)	Formula	Precursor	Mass	Mass (Tgt)	Mass Accuracy (ppm)	RT (min)	Ions
1	Iocetamic acid	88.87	C12 H13 I3 N2 O3	612.7985	613.8057	613.8060	-0.49	2.368	5
2	TP687 (Kormos et al.)	91.18	C13 H12 I3 N3 O6	685.7784	686.7856	686.7860	-0.58	2.368	4
3	TP773 (Kormos et al.)	93.09	C16 H14 I3 N3 O9	831.7994	772.7861	772.7864	-0.39	1.273	5
4	TP787 (Kormos et al.)	91.70	C17 H16 I3 N3 O9	831.7993	786.8010	786.8021	-1.41	1.373	6
5	TP817 (Kormos et al.)	80.45	C18 H18 I3 N3 O10	861.8102	816.8125	816.8126	-0.13	1.373	4
6	TP835 (Kormos et al.)	98.85	C19 H24 I3 N3 O10	833.8524	834.8593	834.8596	-0.29	1.871	4
7	TP863 (Kormos et al.)	79.96	C19 H20 I3 N3 O12	861.8101	862.8174	862.8181	-0.83	1.207	2
8	Iohexol	95.63	C19 H26 I3 N3 O9	819.8742	820.8812	820.8803	1.13	6.266	4
9	TP819 (Kormos et al.)	76.54	C18 H20 I3 N3 O10	817.8187	818.8254	818.8283	-3.53	1.373	3
10	TP849 (Kormos et al.)	84.06	C19 H22 I3 N3 O11	847.8315	848.8377	848.8388	-1.4	1.373	5

Appendix A Table A-13: Pearson's correlation coefficients (r) for surface water quality parameters

	TDOI	TDI	TDOX	DOC	DN	SUVA	EEM Peak T	Conductivity
TDOI	--							
TDI	0.969	--						
TDOX	0.767	0.747	--					
DOC	-0.055	-0.052	-0.044	--				
DN	0.696	0.683	0.880	-0.126	--			
SUVA	-0.353	-0.364	-0.376	0.384	-0.346	--		
EEM Peak T	0.666	0.654	0.799	0.215	0.749	-0.081	--	
Conductivity	0.534	0.559	0.769	-0.235	0.819	-0.494	0.588	--

Iodine Incorporation Factor for I-THMs in Finished Drinking Water

Appendix A Table A-14: Molar concentration for each individual I-THM

Sampling Event	Finished Water Concentrations (μM)					
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM
February	<0.00005	<0.00004	0.0047	0.00046	0.00087	<0.00003
March	0.0066	<0.00004	0.0043	0.00020	0.00058	<0.00003
June	0.0062	0.0074	0.0030	0.00314	0.00346	<0.00003
July	0.0090	0.0082	<0.00003	<0.00003	0.0049	<0.00003

Appendix A Table A-15: Molar concentration for each individual THM4

Sampling Event	Finished Water Concentrations (μM)			
	TCM	DCBM	CDBM	TBM
February	0.054	0.041	0.0026	<0.00004
March	0.032	0.029	<0.00005	<0.00004
June	0.158	0.143	0.045	0.0073
July	0.167	0.125	0.033	<0.00004

(Appendix A Equation 1)

$$Trihalomethane\ Iodine\ Incorporation\ for\ February\ Finished\ Water = \left(\frac{(0.0047\ \mu M) + (0.00046\ \mu M\ \times 2) + (0.00087\ \mu M\ \times 2)}{(0.0047\ \mu M\ \times 3) + (0.00046\ \mu M\ \times 3) + (0.00087\ \mu M\ \times 3) + (0.054\ \mu M\ \times 3) + (0.041\ \mu M\ \times 3) + (0.0026\ \mu M\ \times 3)}\right) \times 100 = 2.4\%$$

Appendix A Table A-16: Iodine incorporation into THMs for 2017 sampling events

Sampling Event	% Iodine Incorporation
February	2.4
March	5.8
June	2.6
July	2.6

Theoretical Toxicity of I-THMs

The LC_{50} value is the concentration of each individual THM inducing 50% reduction in the density of Chinese Hamster Ovary (CHO) cells treated for 72hrs.

Appendix A Table A-17: LC_{50} values for each individual THM

Disinfection by-product	Acronym	LC_{50} (M)*
Chlorodiiodomethane	CDIM	2.41×10^{-3}
Triiodomethane	TIM	6.60×10^{-5}
Dibromoiodomethane	DBIM	1.91×10^{-3}
Bromochloroiodomethane	BCIM	2.42×10^{-3}
Tribromomethane	TBM	3.96×10^{-3}
Chlorodibromomethane	CDIM	5.36×10^{-3}
Trichloromethane	TCM	9.62×10^{-3}
Bromodichloromethane	BDCM	1.15×10^{-2}
Dichloroiodomethane	DCIM	4.13×10^{-3}
Bromodiiodomethane	BDIM	1.40×10^{-3}

*References: (Plewa and Wagner, 2009; Richardson et al., 2008)

Example calculation for the relative total toxicity due to I-THM formation from the February 2017 sampling event can be seen in Appendix A Equation 2:

(Appendix A Equation 2)

$$\begin{aligned} Relative\ Total\ Toxicity \\ &= ([0]_{DCIM} \times (4.13\mathrm{E}^{-3})^{-1}_{DCIM} \times 10^6) \\ &+ ([0]_{BCIM} \times (2.42\mathrm{E}^{-3})^{-1}_{BCIM} \times 10^6) \\ &+ ([0.0000000047]_{DBIM} \times (1.91\mathrm{E}^{-3})^{-1}_{DBIM} \times 10^6) \\ &+ ([0.00000000046]_{CDIM} \times (5.36\mathrm{E}^{-3})^{-1}_{CDIM} \times 10^6) \\ &+ ([0.00000000087]_{BDIM} \times (1.40\mathrm{E}^{-3})^{-1}_{BDIM} \times 10^6) \\ &+ ([0]_{TIM} \times (6.60\mathrm{E}^{-5})^{-1}_{TIM} \times 10^6) = 3.2 \end{aligned}$$

Appendix A Table A-18: Relative total toxicity increase due to I-THM formation

	Toxicity from Individual I-THMs						Total Toxicity
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM	Sum
February	NQ*	NQ	2.4	0.2	0.6	NQ	3.3
March	1.6	NQ	2.3	0.1	0.4	NQ	4.4
June	1.5	3.1	1.6	1.3	2.5	NQ	9.9
July	2.2	3.4	NQ	NQ	3.5	NQ	9.1

*NQ = not quantifiable

Appendix A Table A-19: Relative total toxicity increase assuming TBM (most toxic non-iodinated THM) formation instead of I-THM formation

	Toxicity from Individual I-THMs						Total Toxicity
	TBM	TBM	TBM	TBM	TBM	TBM	Sum
February	NQ*	NQ	1.2	0.1	0.2	NQ	1.5
March	1.7	NQ	1.1	0.1	0.1	NQ	3.0
June	1.6	1.9	0.8	0.8	0.9	NQ	5.9
July	2.3	2.1	NQ	NQ	1.2	NQ	5.6

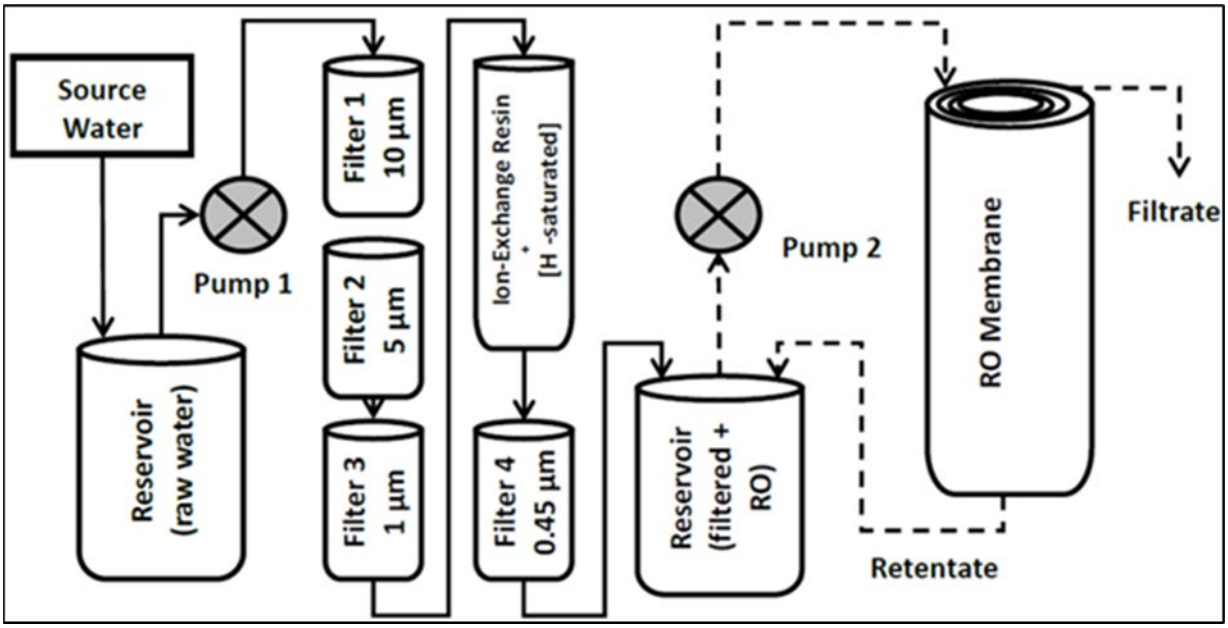
*NQ = not quantifiable

Appendix A Table A-20: Comparison of relative total toxicity increases from I-THMs versus assuming TBM formation

	Toxicity Increase from I-THM Contribution	Toxicity Increase assuming all TBM Contribution	% Difference	Factor of Toxicity Increase from I-THMs
February	3.3	1.5	73	2.1
March	4.4	3.0	38	1.5
June	9.9	5.9	51	1.7
July	9.1	5.6	48	1.6

APPENDIX B: SUPPORTING INFORMATION FOR CHAPTER 4

Appendix B Figure B--1: Reverse-osmosis system for concentrating source water matrix.



Appendix B Table B-1: Source water and source water reverse-osmosis (RO) concentrate water quality parameters.

Parameter	Source Water	Source Water Concentrate.	Concentration Factor
DOC (mg/L as C)	5.0	126.6	25
DN (mg/L as N)	0.6	< 0.25	0
TOX (µg/L as Cl)	33	657	20
TDOI (µg/L as I)	23.7	488	21
EEM Peak A	1.48	36.2	24
EEM Peak C	0.37	8.8	24
EEM Peak T	0.20	5.6	28
Chloride (mg/L as Cl)	4.8	70	15
Bromide (µg/L as Br)	59.5	1593	27
Iodide (µg/L as I)	< 10	< 10	--

Non-Target Analysis of Sampling of Wastewater Discharges and Drinking Water Intake

Sample extracts were concentrated by a factor of 50 with 5µL injections onto a C-18 column in positive and negative electrospray-ionization (ESI+ and ESI-) modes. The signal to noise ratio of 5 or above was used to confirm the presence of a peak.

Appendix B Table B-2: Settings for the LC mobile phase and Q/TOF mass spectrometer

Mobile Phase Program		
Time (min)	Mobile phase A 0.1%Formic Acid in water	Mobile phase B 0.1%Formic Acid in acetonitrile
0	95%	5%
1	95%	5%
8	10%	90%
8.1	0%	100%
15	0%	100%
15.1	95%	5%
18	95%	5%
MS-only mode		
Capillary Voltage (V)	3000	
Fragmentor Voltage (V)	360	
Skimmer voltage (V)	65	
Scan Range (m/z)	50 to 950	
Scan Rate (spectra/sec)	3	
Reference Masses (m/z)	121.05087300 922.00979800	

Appendix B Table B-3: HPLC Method for iohexol in SPE method extracts

Instrumentation	Waters Binary HPLC Pump 1525, Waters 717 plus Autosampler, Waters 2487 Dual-Wavelength UV Absorbance Detector Monitoring UV 254 nm and 270 nm, Waters Breeze Software
Mobile Phase:	70:30 Acetonitrile:LGW (isocratic)
Stationary Phase	Discovery C-18 HPLC column 25 cm x 4.6 mm, 5µm
Injection volume	15 µL
Run Time	15 min

Appendix B Table B-4: Dissolved organic carbon and dissolved nitrogen for drinking water treatment samples collected in May and August 2019 (PQL = 0.1mg/L as C/N).

Average duplicates	May 2019		August 2019	
	DOC (mg/L as C)	DN (mg/L as N)	DOC (mg/L as C)	DN (mg/L as N)
Finished water	1.1	1.7	1.3	0.9
Clearwell	1.2	1.5	0.4	0.3
After filter	1.1	1.6	0.4	0.3
Before filter	1.3	1.7	0.5	0.3
After ozone	3.8	5.2	0.5	0.5
After PAC	4.5	5.1	0.5	0.4
Source water	5.1	6.7	0.6	0.6

Appendix B Table B-5: Regulated trihalomethanes (THM4) in the drinking water treatment plant for the May 2019 sampling event (N=2).

Average duplicates ± range	THM4 (µg/L)	TCM (µg/L)	BDCM (µg/L)	DBCM (µg/L)	TBM (µg/L)
Finished water	38.6 ± 6.1	23.1 ± 4.4	< 0.1	15.5 ± 1.5	< 0.1
Clearwell	36.7 ± 1.6	24.0 ± 0.1	< 0.1	11.8 ± 1.7	0.9 ± 0.2
After filter	17.4 ± 1.6	12.1 ± 0.4	< 0.1	5.3 ± 1.8	< 0.1
Before filter	0.8 ± 0.1	0.8 ± 0.1	< 0.1	< 0.1	< 0.1
After ozone	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
After PAC	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Source water	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Finished water	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

Appendix B Table B-6: Regulated trihalomethanes (THM4) in the drinking water treatment plant for the August 2019 sampling event (N=2).

Average duplicates ± range	THM4 (µg/L)	TCM (µg/L)	BDCM (µg/L)	DBCM (µg/L)	TBM (µg/L)
Finished water	42.2 ± 4.3	11.1 ± 1.3	15.6 ± 1.6	12.6 ± 1.2	2.9 ± 0.3
Clearwell	44.6 ± 1.1	7.2 ± 0.3	16.4 ± 0.4	16.2 ± 1.0	4.8 ± 0.6
After filter	23.6 ± 1.5	2.8 ± 0.3	8.0 ± 0.2	9.2 ± 0.6	3.6 ± 0.1
Before filter	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
After ozone	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
After PAC	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Source water	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

Appendix B Table B-7: Water quality parameters for distribution sampling in May 2019.

Location	Average Water Age (hrs)	Total Chlorine (mg/L as Cl_2)	pH	Temp (^oC)	Conductivity
Entry Point (Finished Water)	0	3.71	7.8	24.6	201
Distribution Location A	24 -48	3.60	8.21	21.3	257
Distribution Location B	48 – 72	2.00	7.9	20.9	256
Distribution Location C	74	3.42	8.11	19.6	252
Distribution Location D	100	3.00	8.13	21.4	256
Distribution Location E	96 – 120	3.11	7.67	20.5	259
Distribution Location F	115	3.23	7.46	20.4	258
Distribution Location G	148	3.36	7.53	19.9	261
Distribution Location H	253	3.08	8.18	21.2	254

Appendix B Table B-8: Water quality parameters for distribution sampling in August 2019.

Location	Average Water Age (hrs)	Total Chlorine (mg/L as Cl_2)	pH	Temp (^oC)	Conductivity
Entry Point (Finished Water)	0	3.50	7.60	30.3	229
Distribution Location A	24 -48	3.43	8.21	27.7	232
Distribution Location B	48 – 72	2.71	7.80	27.8	235
Distribution Location C	74	2.88	8.45	27.6	237
Distribution Location D	100	2.40	8.01	28.1	241
Distribution Location E	96 – 120	2.60	8.15	27.8	239
Distribution Location F	115	2.78	7.68	27.8	236
Distribution Location G	148	2.93	7.93	27.1	234
Distribution Location H	253	2.81	8.09	26.4	234

Appendix B Table B-9: Regulated trihalomethanes (THM4) in the distribution system for the May 2019 sampling event (N=2).

Average duplicates ± range	THM4 (µg/L)	TCM (µg/L)	BDCM (µg/L)	DBCM (µg/L)	TBM (µg/L)
Entry Point (Finished Water)	38.6 ± 6.1	23.1 ± 4.4	< 0.1	15.5 ± 1.5	< 0.1
Distribution Location A	31.1 ± 0.4	24.0 ± 0.5	< 0.1	7.1 ± 0.8	< 0.1
Distribution Location B	24.2 ± 4.5	18.3 ± 4.5	< 0.1	5.9 ± 2.0	< 0.1
Distribution Location C	27.0 ± 2.4	20.5 ± 4.0	< 0.1	6.5 ± 1.7	< 0.1
Distribution Location D	25.7 ± 1.0	20.4 ± 1.7	< 0.1	5.3 ± 0.7	< 0.1
Distribution Location E	34.5 ± 1.7	28.0 ± 2.0	< 0.1	6.5 ± 0.3	< 0.1
Distribution Location F	22.7 ± 1.8	21.3 ± 0.3	< 0.1	5.8 ± 0.9	< 0.1
Distribution Location G	30.5 ± 2.0	22.6 ± 2.2	< 0.1	9.6 ± 0.8	< 0.1
Distribution Location H	28.6 ± 0.5	24.2 ± 0.3	< 0.1	4.4 ± 0.7	< 0.1

Appendix B Table B-10: Regulated trihalomethanes (THM4) in the distribution system for the August 2019 sampling event (N=2).

Average duplicates ± range	THM4 (µg/L)	TCM (µg/L)	BDCM (µg/L)	DBCM (µg/L)	TBM (µg/L)
Entry Point (Finished Water)	42.2 ± 4.3	11.1 ± 1.3	15.6 ± 1.6	12.6 ± 1.2	2.9 ± 0.3
Distribution Location A	28.4 ± 0.5	9.6 ± 0.3	10.6 ± 0.2	7.3 ± 0.4	1.0 ± 0.1
Distribution Location B	28.9 ± 0.6	9.5 ± 0.1	10.1 ± 0.0	7.8 ± 0.3	1.5 ± 0.0
Distribution Location C	30.0 ± 0.9	10.0 ± 0.1	10.7 ± 0.5	7.9 ± 0.4	1.3 ± 0.2
Distribution Location D	29.6 ± 2.6	9.4 ± 1.2	10.6 ± 0.9	8.0 ± 0.7	1.5 ± 0.4
Distribution Location E	32.1 ± 1.6	10.5 ± 0.7	11.0 ± 0.1	8.9 ± 0.3	1.7 ± 0.5
Distribution Location F	29.9 ± 4.2	9.1 ± 1.2	10.7 ± 1.6	8.4 ± 0.7	1.7 ± 0.6
Distribution Location G	24.6 ± 2.3	8.3 ± 0.8	9.1 ± 0.8	6.5 ± 0.7	0.8 ± 0.1
Distribution Location H	28.4 ± 2.2	9.4 ± 0.4	10.2 ± 0.9	7.6 ± 1.0	1.2 ± 0.3

Appendix B Table B-11: I-THM degradation in LGW by free chlorine and monochloramine residual. I-THMs were spiked at 50 µg/L for each of the 6 I-THM species and 100% recovery would be 300 µg/L.

Sample (N=2 ±range)	**Avg I-THM**	**%Decrease**
LGW+HOCl@ 7 days	<0.01	--
LGW+50 I-THMs	295 ± 11	--
LGW+50 I-THMs +HOCl@24hrs	241 ± 2	18
LGW+50 I-THMs +HOCl@72hrs	96 ± 3	67
LGW+50 I-THMs +HOCl@ 7days	83 ± 8	72
LGW+50 I-THMs +HOCl@ 14days	35 ± 1	88
Sample (N=2 ±range)	**Avg I-THM**	**%Decrease**
LGW+NH_2Cl@ 7days	<0.01	--
LGW+50 I-THMs +NH_2Cl@24hrs	303 ± 7	--
LGW+50 I-THMs +NH_2Cl@72hrs[*]	*352 ± 30*[*]	*-23**
LGW+50 I-THMs +NH_2Cl@ 7days	252 ± 1	15
LGW+50 I-THMs +NH_2Cl@ 14days	201 ± 5	32

[*]this sample shows a formation of I-THMs, which is not possible in the LGW spike. It may have been incorrectly spiked with I-THMs.

Appendix B Table B-12: I-THM degradation in I-THM spiked finished water and I-THM spiked clearwell by free chlorine and monochloramine residual. I-THMs were spiked at 50 µg/L for each of the 6 I-THM species and 100% recovery would be 300 µg/L.

Sample (N=2 ±range)	**Avg I-THM**	**%Decrease**
Finished water+50 I-THMs @24hrs	289 ± 7	--
Finished water+50 I-THMs @72hrs	232 ± 28	20
Finished water+50 I-THMs @ 7days	48 ± 2	83
Finished water+50 I-THMs @ 14days	53 ±8	82
Sample (N=2 ±range)	**Avg I-THM**	**%Decrease**
Clearwell+50 I-THMs @24hrs	297 ± 5	--
Clearwell+50 I-THMs @72hrs	213 ± 35	28
Clearwell+50 I-THMs @ 7days	149 ± 11	50
Clearwell+50 I-THMs @ 14days	131± 18	56

Appendix B Table B-13: PAC contributions to DOC in an LGW matrix.

	Coal-based	Wood-based	Coconut-based
PAC Dose (mg/L)	**DOC (mg/L as C)**	**DOC (mg/L as C)**	**DOC (mg/L as C)**
0	< 0.1	< 0.1	< 0.1
15	0.5	0.5	0.0
30	0.5	0.5	0.2
50	0.5	0.6	0.2
80	0.5	0.7	0.2
100	0.6	0.7	0.2

Appendix B Table B-14: Total dissolved iodine (mg/L as I) changes after PAC treatment and 24 hrs chlorination.

	After PAC	% Decrease from PAC	After Cl_2	% Decrease from Cl_2	Total % Decrease
LGW+Iohexol	2.23	--	1.93	13	13
LGW+Iohexol + Coal-based PAC	0.52	77	0.43	18	81
LGW+Iohexol + Wood-based PAC	0.09	96	0.06	29	97
LGW+Iohexol + Coconut-based PAC	1.15	48	0.87	24	61
RO only	0.50	--	0.38	27	27
RO + Coal-based PAC	0.44	16	0.29	34	45
RO +Wood-based PAC	0.35	33	0.25	28	52
RO +Coconut-based PAC	0.43	18	0.31	27	40
RO +Iohexol	1.52	--	0.91	40	40
RO +Iohexol + Coal-based PAC	0.54	65	0.44	18	71
RO +Iohexol + Wood-based PAC	0.22	86	0.16	26	89
RO +Iohexol + Coconut-based PAC	0.38	75	0.27	28	82

Appendix B Table B-15: DOC removal in RO concentrate samples by PAC.

	Coal-based	Wood-based	Coconut-based
PAC Dose (mg/L)	DOC (mg/L as C)	DOC (mg/L as C)	DOC (mg/L as C)
0	116	116	116
50	115	106	115
100	106	103	110
200	106	99	101
300	97	95	96
400	90	94	88
500	89	90	80
600	89	82	71

Appendix B Table B-16: TDI removal in RO concentrate samples by PAC.

PAC Dose (mg/L)	TDI (mg/L) for Coal-Based PAC	TDI (mg/L) for Wood-Based PAC	TDI (mg/L) for Coconut-Based PAC
0	0.36	0.36	0.36
50	0.36	0.34	0.31
100	0.36	0.33	0.31
200	0.35	0.32	0.29
300	0.34	0.30	0.27
400	0.29	0.29	0.27
500	0.29	0.29	0.26
600	0.28	0.26	0.23

Appendix B Table B-17: DOC removal in RO concentrate with iohexol spike at 1 mg/L as I samples by PAC.

	Coal-based	Wood-based	Coconut-based
PAC Dose (mg/L)	DOC (mg/L as C)	DOC (mg/L as C)	DOC (mg/L as C)
0	117	117	117
50	107	114	114
100	110	116	110
200	102	107	100
300	95	100	94
400	94	92	89
500	89	91	81
600	87	86	75

Appendix B Table B-18: TDI removal in iohexol-spiked RO concentrate samples by PAC.

PAC Dose (mg/L)	TDI (mg/L) for Coal-Based PAC	TDI (mg/L) for Wood-Based PAC	TDI (mg/L) for Coconut-Based PAC
0	1.36	1.36	1.36
50	1.23	1.28	1.17
100	1.13	1.20	0.97
200	0.86	1.01	0.6
300	0.66	0.85	0.4
400	0.55	0.70	0.32
500	0.45	0.61	0.28
600	0.40	0.53	0.25

Appendix B Table B-19: Total chlorine and free chlorine residuals (measurement*dilution factor)

	Free Chlorine (mg/L as Cl_2)	Total Chlorine (mg/L as Cl_2)
LGW+Cl_2	120 (0.60*200)	122 (0.61*200)
LGW+Iohexol+Cl_2	140 (100*1.40)	145 (100*1.45)
LGW+Iohexol+Coal-based PAC+Cl_2	152 (100*1.52)	155 (100*1.55)
LGW+Iohexol+Wood-based PAC+Cl_2	146 (100*1.46)	147 (100*1.47)
LGW+Iohexol+Coconut-based PAC+Cl_2	145 (100*1.45)	143 (100*1.43)
RO+Cl_2	22.5 (50*0.45)	23.0 (50*0.46)
RO+Iohexol+Cl_2	0.10 (1*0.10)	14.3 (10*1.43)
RO+Coal-based PAC+Cl_2	69.5 (50*1.39)	75.5 (50*1.51)
RO+Iohexol+Coal-based PAC+Cl_2	21.0 (50*0.42)	26.5 (50*0.53)
RO+Wood-based PAC+Cl_2	21.6 (10*2.16)	22.2 (10*2.22)
RO+Iohexol+Wood-based PAC+Cl_2	42.8 (25*1.71)	44.0 (25*1.76)
RO+Coconut-based PAC+Cl_2	44.5 (25*1.78)	53.3 (25*2.13)
RO+Iohexol+Coconut-based PAC+Cl_2	47.5 (25*1.90)	70.5 (50*1.41)

Appendix B Table B-20: I-THM formation from chlorination of PAC treated RO concentrate and iohexol-spiked RO concentrate samples. TIM was below the PQL of 1.0 µg/L.

	Total I-THMs (µg/L)	**DCIM (µg/L)**	**BCIM (µg/L)**	**DBIM (µg/L)**	**CDIM (µg/L)**	**BDIM (µg/L)**
RO+Cl_2	140 ± 10	90 ± 4	< 1.0	18 ± 3	25 ± 2	7 ± 2
RO+Iohexol+Cl_2	126 ± 5	88 ± 9	< 1.0	8 ± 1	25 ± 2	5 ± 2
RO+ Coal-based PAC+Cl_2	90 ± 8	55 ± 3	< 1.0	9 ± 3	22 ± 2	5 ± 1
RO+Iohexol+ Coal-based PAC+Cl_2	146 ± 14	63 ± 1	< 1.0	27 ± 7	31 ± 5	25 ± 3
RO+ Wood-based PAC+Cl_2	88 ± 1	30 ± 3	< 1.0	9 ± 3	21 ± 1	28 ± 1
RO+Iohexol+ Wood-based PAC+Cl_2	107 ± 6	27 ± 3	7 ± 1	21 ± 3	17 ± 4	42 ± 3
RO+ Coconut-based PAC+Cl_2	97 ± 2	65 ± 4	< 1.0	9 ± 1	19 ± 2	4 ± 1
RO+Iohexol+ Coconut-based PAC+Cl_2	87 ± 2	52 ± 3	11 ± 2	< 1.0	20 ± 2	15 ± 2

Drinking Water Treatment Plant Iodine Incorporation and Relative Toxicity for May and August 2019 Sampling Events

Appendix B Table B-21: Molar concentration for each individual I-THM during the May 2019 sampling event.

	May (μM)					
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM
Finished water	0.0726	0.0161	<0.00003	<0.00003	0.00058	<0.00003
Clearwell	0.0588	0.0129	0.0047	0.00893	<0.00003	<0.00003
After filter	0.0194	0.0082	0.0087	<0.00003	0.00288	<0.00003
Before filter	0.0261	0.0078	<0.00003	<0.00003	<0.00003	<0.00003

Appendix B Table B-22: Molar concentration for each individual THM4 during the May 2019 sampling event.

	May (μM)			
	TCM	BDCM	DBCM	TBM
Finished water	0.170	<0.00006	0.0745	<0.00004
Clearwell	0.177	<0.00006	0.057	0.003
After filter	0.090	<0.00006	0.025	<0.00004
Before filter	0.006	<0.00006	<0.00005	<0.00004

Example calculation for the iodine incorporation for I-THM formation from the May 2019 finished water can be seen in SI Equation 1:

(SI Equation 1)

$$Trihalomethane\ Iodine\ Incorporation\ for\ May\ Finished\ Water = \left(\frac{[0.0726\ \mu M] + [0.0161\ \mu M] + [0.00058\ \mu M \times 2]}{[0.0726\mu M \times 3] + [0.0161\mu M \times 3] + [0.00058\mu M \times 3] + [0.170\mu M \times 3] + [0.0745\mu M \times 3]}\right) \times 100 = 9.0\%$$

Appendix B Table B-23: Molar concentration for each individual I-THM during the August 2019 sampling event.

	August (μM)					
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM
Finished water	0.0066	0.0212	<0.00003	<0.00003	0.00087	<0.00003
Clearwell	0.0100	0.0243	<0.00003	<0.00003	0.00115	<0.00003
After filter	0.0142	0.0082	0.0097	0.00132	<0.00003	<0.00003
Before filter	0.0085	0.0012	<0.00003	0.00165	<0.00003	<0.00003

Appendix B Table B-24: Molar concentration for each individual THM4 during the August 2019 sampling event.

	August (μM)			
	TCM	BDCM	DBCM	TBM
Finished water	0.164	0.191	0.1207	0.0231
Clearwell	0.106	0.201	0.155	0.038
After filter	0.042	0.098	0.088	0.0282
Before filter	<0.00007	<0.00006	<0.00005	<0.00004

Appendix B Table B-25: LC_{50} values for each individual THMs

Disinfection by-product	**Acronym**	**LC_{50} (M)***
Chlorodiiodomethane	CDIM	2.41×10^{-3}
Triiodomethane	TIM	6.60×10^{-5}
Dibromoiodomethane	DBIM	1.91×10^{-3}
Bromochloroiodomethane	BCIM	2.42×10^{-3}
Tribromomethane	TBM	3.96×10^{-3}
Chlorodibromomethane	CDIM	5.36×10^{-3}
Trichloromethane	TCM	9.62×10^{-3}
Bromodichloromethane	BDCM	1.15×10^{-2}
Dichloroiodomethane	DCIM	4.13×10^{-3}
Bromodiiodomethane	BDIM	1.40×10^{-3}

*References: (Plewa and Wagner, 2009; Richardson et al., 2008)

Example calculation for the relative total toxicity due to I-THM formation from the May 2019 finished water can be seen in Appendix B Equation 2:

(Appendix B Equation 2)

$$\begin{aligned} &Relative\ Total\ Toxicity \\ &= ([0.0000726]_{DCIM} \times (4.13\mathrm{E}^{-3})_{DCIM}^{-1} \times 10^6) \\ &+ ([0.0000161]_{BCIM} \times (2.42\mathrm{E}^{-3})_{BCIM}^{-1} \times 10^6) \\ &+ ([0]_{DBIM} \times (1.91\mathrm{E}^{-3})_{DBIM}^{-1} \times 10^6) + ([0]_{CDIM} \times (5.36\mathrm{E}^{-3})_{CDIM}^{-1} \times 10^6) \\ &+ ([0.00000058]_{BDIM} \times (1.40\mathrm{E}^{-3})_{BDIM}^{-1} \times 10^6) \\ &+ ([0]_{TIM} \times (6.60\mathrm{E}^{-5})_{TIM}^{-1} \times 10^6) = 24.6 \end{aligned}$$

Appendix B Table B-26: Relative total relative toxicity increase due to May 2019 sampling event I-THM formation.

May Sampling Event	Toxicity from Individual I-THMs						Total Toxicity
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM	Sum
Finished water	17.6	6.6	NQ*	NQ	0.4	NQ	24.6
Clearwell	14.2	5.3	2.4	3.7	NQ	NQ	25.7
After filter	4.7	3.4	4.5	NQ	2.1	NQ	14.7
Before filter	6.3	3.2	NQ	NQ	0.0	NQ	9.6

*NQ = not quantifiable

Appendix B Table B-27: Relative total relative toxicity increase due to August 2019 sampling event I-THM formation.

August Sampling Event	Toxicity from Individual I-THMs						Total Toxicity
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM	Sum
Finished water	1.6	8.7	NQ*	0.0	0.6	NQ	11.0
Clearwell	2.4	10.0	0.0	0.0	0.8	NQ	13.3
After filter	3.4	3.4	5.1	0.5	0.0	NQ	12.5
Before filter	2.1	0.5	0.0	0.7	0.0	NQ	3.2

*NQ = not quantifiable

Appendix B Table B-28: Relative total toxicity increase assuming TBM (most toxic non-iodinated THM) formation instead of I-THM formation during the May 2019 sampling event.

May Sampling Event	Toxicity from Individual I-THMs						Total Toxicity
	TBM	TBM	TBM	TBM	TBM	TBM	Sum
Finished water	18.3	4.1	NQ*	NQ	0.1	NQ	22.5
Clearwell	14.9	3.3	1.2	2.3	NQ	NQ	21.6
After filter	4.9	2.1	2.2	NQ	0.7	NQ	9.9
Before filter	6.6	2.0	NQ	NQ	NQ	NQ	8.6

*NQ = not quantifiable

Appendix B Table B-29: Relative total toxicity increase assuming TBM (most toxic non-iodinated THM) formation instead of I-THM formation during the August 2019 sampling event.

August Sampling Event	Toxicity from Individual I-THMs						Total Toxicity
	TBM	TBM	TBM	TBM	TBM	TBM	Sum
Finished water	1.7	5.3	NQ*	NQ	0.2	NQ	7.2
Clearwell	2.5	6.1	NQ	NQ	0.3	NQ	8.9
After filter	3.6	2.1	2.4	0.3	NQ	NQ	8.4
Before filter	2.2	0.3	NQ	0.4	NQ	NQ	2.9

*NQ = not quantifiable

Appendix B Table B-30: Linear, Langmuir, and Freundlich isotherms for TDI removal from iohexol-spiked LGW based on doses of coal-, wood-, and coconut-based PAC.

	Linear Isotherm Slope and R^2 K_{linear}	Langmuir Isotherms Slope and R^2 K_L	Freundlich Isotherms Slope and R^2 K_f
LGW+Iohexol (1mg/L as I)+PAC (Coal-based)	Slope=K_{linear}=51.1 R^2 = 0.86	Slope=0.027 R^2=0.99 K_L=0.015 q_{max}=36.5 mg/g	Slope=0.2116 R^2=0.99 K_f=41.7 n=4.7
LGW+Iohexol (1mg/L as I)+PAC (Wood-based)	Slope=K_{linear}=5.6 R^2 = 0.76	Slope=0.068 R^2=0.96 K_L=0.20 q_{max}=14.7 mg/g	Slope=0.29 R^2=0.82 K_f=12.6 n=3.4
LGW+Iohexol (1mg/L as I)+PAC (Coconut-based)	Slope=K_{linear}=230 R^2 = 0.87	Slope= 0.019 R^2= 0.99 K_L= 0.022 q_{max}= 53.8 mg/g	Slope= 0.28 R^2= 0.99 K_f= 41.7 n= 3.6

Appendix B Table B-31: Qmax analysis from Langmuir Isotherms for TDI removal from iohexol-spiked LGW based on doses of coal-, wood-, and coconut-based PAC.

	Calculated Q_{max} with Iohexol spike at 1 mg/L as I	Observed Q_{max} with Iohexol spike at 9 mg/L as I	% Difference
LGW+Iohexol +PAC (Coal-based)	q_{max}=36.5 mg/g	q_{max}=48 mg/g	27
LGW+Iohexol +PAC (Wood-based)	q_{max}=14.7 mg/g	q_{max}=77 mg/g	135
LGW+Iohexol +PAC (Coconut-based)	q_{max}= 53.8 mg/g	q_{max}=73 mg/g	30

Appendix B Table B-32: Molar concentration for each individual I-THM from the chlorination of RO concentrate and iohexol-spiked RO concentrate after PAC treatment.

	I-THMs (μM)					
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM
RO+Cl_2	0.427	<0.0003	0.060	0.083	0.020	<0.0003
RO+Coal-based PAC+Cl_2	0.261	<0.0003	0.030	0.073	0.014	<0.0003
RO+Wood-based PAC+Cl_2	0.142	<0.0003	0.030	0.069	0.081	<0.0003
RO+Coconut-based PAC+Cl_2	0.308	<0.0003	0.030	0.063	0.012	<0.0003
RO+Iohexol+Cl_2	0.417	<0.0003	0.027	0.083	0.014	<0.0003
RO+Iohexol+ Coal-based PAC+Cl_2	0.299	<0.0003	0.090	0.103	0.072	<0.0003
RO+Iohexol+ Wood-based PAC+Cl_2	0.128	0.027	0.070	0.056	0.121	<0.0003
RO+Iohexol+ Coconut-based PAC+Cl_2	0.247	0.043	<0.0003	0.066	0.043	<0.0003

Appendix B Table B-33: Molar concentration for each individual THM4 from the chlorination of RO concentrate and iohexol-spiked RO concentrate after PAC treatment.

	THM4 (μM)			
	TCM	BDCM	DBCM	TBM
RO+Cl_2	20.0	2.6	0.18	0.05
RO+Coal-based PAC+Cl_2	12.7	1.8	0.12	0.01
RO+Wood-based PAC+Cl_2	23.7	4.0	0.48	0.01
RO+Coconut-based PAC+Cl_2	30.4	4.0	0.35	0.04
RO+Iohexol+Cl_2	34.3	4.3	0.38	0.10
RO+Iohexol+ Coal-based PAC+Cl_2	34.0	5.6	0.41	0.04
RO+Iohexol+ Wood-based PAC+Cl_2	26.3	4.3	0.50	<0.0003
RO+Iohexol+ Coconut-based PAC+Cl_2	28.0	3.9	0.42	0.02

Appendix B Table B-34: Relative total relative toxicity increase per mg/L of DOC due to I-THM formation potential in RO concentrate and iohexol-spiked RO concentrate after PAC treatment.

PAC treated chlorination	Toxicity from Individual I-THMs per initial mg/L of DOC						Total Toxicity
	DCIM	BCIM	DBIM	CDIM	BDIM	TIM	Sum
RO+Cl_2	0.87	NQ*	0.26	0.29	0.12	NQ	1.5
RO+ Coal-based PAC+Cl_2	0.65	NQ	0.16	0.31	0.11	NQ	1.2
RO+ Wood-based PAC+Cl_2	0.46	NQ	0.21	0.38	0.77	NQ	1.8
RO+ Coconut-based PAC+Cl_2	0.77	NQ	0.16	0.27	0.08	NQ	1.3
RO+Iohexol+Cl_2	0.84	NQ	0.12	0.29	0.09	NQ	1.3
RO+Iohexol+ Coal-based PAC+Cl_2	0.75	NQ	0.49	0.44	0.54	NQ	2.2
RO+Iohexol+ Wood-based PAC+Cl_2	0.41	0.15	0.49	0.31	1.16	NQ	2.5
RO+Iohexol+ Coconut-based PAC+Cl_2	0.63	0.19	0.00	0.29	0.33	NQ	1.4

*NQ = not quantifiable

APPENDIX C: REVERSE OSMOSIS (RO) CONCENTRATION STANDARD OPERATING PROCEDURES

NOMinator 3000

Prepared by: Bonnie Lyon, updated by Kirsten Studer

Materials:

- RO Concentration unit with tubing)
- Two big blue bins for collecting water
- Lances for holding tubing
- Yard stick, label tape and pen
- Graduated cylinder (500 mL or 1 L)
- Amber glass bottles for collecting final RO concentrate (4 L, acid washed, dried)
- 40 mL vials with caps for collecting samples throughout process
- Power strip with ground fault circuit interrupter

NOTES: ●Cover the pumps when accessing filter housing to reduce water on pumps

●If filter pump does not turn on, then need to replace the fuse

●Use the white lances to straighten and secure the tubing beneath the water line

●Store unit with all valves closed and don't change the bypass valve adjustment

Clean filters

1. Collect finished water in big blue bin labeled #1 using "Collection" tubing (blue and white). Collect about 1 ft of finished water for cleaning filters. Remove tubing and put aside.

2. Place one end of “Filter Inlet” tubing inside a lance into bin #1. Place end of “Filter Outlet” tubing into sink for waste.
3. Plug filter pump power cord into power strip and turn on filter pump by first setting the filter pump control speed dial to 0, then flip switch to “run” and turn dial to 100. Let waste from first 30 seconds of cleaning drain into sink, then put “Filter Outlet” tubing into another lance in bin #1 and let pump run ~ 5 minutes. *Be careful to keep pump inlet tubing submerged so no air is pulled through pump and filters*
4. To drain rinse water from bin before shutting off pump (since it is heavy when filled with water and hard to dump out), put outlet of filters into sink and pump almost all of water out of bin.
5. To turn pump off, set the filter pump control speed dial to 0, then flip switch away from “run”.
6. Empty remaining rinse water from bin #1 and wipe dry with paper towels.
7. If desired, collect some rinse water in 40 mL vials for analyses back at UNC.

Filter water to be RO concentrated

8. Collect water to be concentrated in bin #1 using “Collection” tubing (blue and white). Connect end of tubing without metal fitting to outlet of desired water spout and place other end into bin #1.
9. Fill bin until almost full – past 180L mark because some will leak during filtering.
10. Carefully remove tubing from water outlet and bin and drain excess water in tubing into sink.
11. Collect some of the water that will be concentrated in 40 mL vials for UNC analyses.

12. Put end of filter pump inlet tubing into bin #1 which is filled with raw or settled water (other end is still connected to filtering pump).
13. Put outlet of filters (filter discharge tube) into bin #2.
14. Turn on filtering pump and filter collected water until bin #2 is filled to 180 L mark. To turn on filter pump, turn speed to 0, flip switch to run, then turn speed to 100.
15. Turn off pump by turning speed to zero then switch off pump. Empty any remaining water from bin #1 into sink. Set bin #1 and filtering tubing aside. Unplug filtering pump from power strip.

RO concentration

16. Plug in RO pump cord to power strip.
17. Place RO pump inlet tubing that is connected to RO pump inlet into bin #2 which contains the filtered water to be RO concentrated. Also place RO pump outlet tubing that is connected to outlet of RO membrane into bin #2. *Be careful to keep pump inlet tubing submerged so no air is pulled through pump and membrane*
18. Move the bypass valve handle (black) to closed position (up) and orange shut off valve closed (perpendicular).
19. Place the RO pump bypass tubing into the same bin as the RO inlet tubing.
20. Prime the RO pump. Keep the filter lines in the drum with water. Fill the RO pump inlet tubing with water using discharge of filter system. Note: Inlet to filter needs to be submerged and be sure the line is full by shaking the tubing then plunging down under the water surface.

21. Open the bypass valve (black) handle then quickly turn the RO pump on. Water should now be exiting the permeate line and concentrate line.
22. Open orange valve next to inlet of RO membrane – turn parallel to tubing to open. During storage, valve should be in the perpendicular position which is closed.
23. To increase flow through the RO membrane, lift black handled bypass valve to change pressure on system up to 200 psi (system is safe guarded to 200 psi).
24. Place RO permeate tubing into sink.
25. Run RO pump until desired concentration factor is obtained – for example, until ~ 4 L remain.
26. Take periodic samples of RO concentrate and permeate for UNC analyses – record water height by measuring with yard stick.

To collect concentrate

27. Place outlet of RO concentrate into collection bottles (put bottles in secondary container to catch spilled water and be able to account for total final volume). Turn pump back on and collect RO concentrate. Again, be careful not to let air into RO pump tubing inlet. Turn pump off and measure any remaining water to account for final volume.

To stop pump

28. Slowly open the black handled RO bypass valve until completely open (parallel to pump).
29. Press stop on the RO pump control.

If draining completely

30. Put the concentrate line out to air

31. Place bypass line to drain
32. Open concentrate flow valve completely
33. May want to run through with clean filtered water if storing over longer periods of time. RO membrane should be stored in 2% sodium bisulfite for long time periods.
34. Leave unit with orange valve closed and bypass valve closed.

Clean filters

35. Collect finished water in bin #1 for rinsing filters and RO membrane.
36. Clean filters as done in steps 2 and 3. Turn off filter pump.
37. Put RO inlet tubing in bin #1, put outlet tube into sink. Run RO pump ~1 minute or until water leaving RO concentrate tubing is clear. Turn off RO pump.
38. Empty bins #1 and #2 and wipe dry with paper towels. Put black lids loosely on bins.

APPENDIX D: DIAZOMETHANE GENERATION

Prepared by Katja Weissbach; Updated by Kirsten Studer

1) Notes: **• Work in the hood and use double gloving for the reaction part of this operation!**

• All glassware used for this reaction needs to be placed in the NaOH bath for neutralization prior to washing and reusing!!!

2) Instructions:

• Reagent 1 takes time to dissolve, so prepare it first by placing a small stir bar into the vial labeled “Reagent 1” and adding approximately 3.3g of Diazald (use a pre-marked beaker), 5 mL Carbitol (use glass pipette) and 5 ml MtBE (use glass pipette) stir until most of the solid is dissolved

• Prepare 5 N NaOH bath in large beaker by adding 100 g NaOH pellets and dissolving in 500 mL tap water (stir right away, it will cake to the bottom of the beaker!)

• To the vial labeled “Reagent 2” add 6 mL LGW, 10 mL methanol, and 4 mL 45% potassium hydroxide. Swirl gently.

• Fill large beaker with ice

• Take the “bomb” from the freezer and empty out the contents of the previous diazomethane batch into the NaOH bath

• Add 5 mL MtBE to the “Collection vial” close it using the cap with the tubing AND vent; place it in the beaker filled with ice (make sure the tubing is immersed in the MtBE)

• Assemble the “Generation vial”, making sure the tubing is not going to be immersed in the reagents

• Pipette 6 mL of Reagent 1 into the “Generation Vial”

• Pipette 6 mL of Reagent 2 into the “Generation Vial” and immediately screw on the cap with the connector by holding the cap and turning the vial (Diazomethane will form in the headspace of the vial and will collect in the MtBE in the collection vial).

• Allow the reaction to continue for 2 to 3 minutes (make sure no bubbles in the tubing go back to the generation vial).

• Remove the cap from the generation vial and place it in the NaOH bath to neutralize.

• Remove the tubing and cap the collection vial and replace it with a regular open top cap with a Teflon lined septum; label the vial with content, your name and the date and place the vial in the “bomb” in the freezer before using it for derivatization (make sure to wipe off excess water from the outside of the collection vial before placing it in the freezer bomb)

• Let the NaOH bath neutralize the glassware for a couple of hours or overnight and then discard the liquid into a specially marked waste container

3) Glassware/Materials:

• Diazomethane Generation Apparatus (40 mL generation vial with connectors, 40 mL collection vial with tubing and vent)

• 2 clear 40 mL vials with open top caps and septa, labeled “Reagent 1” and “Reagent 2”

• Large glass or plastic beaker for 5N NaOH bath

• Spatula to stir NaOH bath

• Large beaker for ice bath

• Six 10 mL pipettes

• Pipette bulb

• Vial rack

• Vortexer or stir plate

• Small stir bar (to fit 40 mL vial)

• Premarked diazald beaker

• Stopwatch

Chemicals:

• Diazald (N-methyl-N-nitroso-p-toluenesulfonamide) Aldrich (Milwaukee, WI), 99%, catalog #2D, 800-D

• Carbitol (Di (ethylene glycol) ethyl ether) Aldrich (Milwaukee, WI), 99+%, catalog #E455-0

• MtBE Aldrich (Milwaukee, WI), 99+%, catalog #44,380-8

• Laboratory Grade Water

• Methanol Burdick & Jackson (Muskegan, MI) B&J Brand™ High Purity methanol (catalog #231-235)

• 45% Potassium Hydroxide (KOH)

• Sodium Hydroxide Pellets, ACS low carbonate grade

APPENDIX E: EXCITATION EMISSION MATRIX GENERATION USING A HORIBA-JOBIN YVON FLUOROLOG-321

1. Power up and shutting down sequence
2. Before getting started
    a. Sample Temperature, Instrument Logs, Data Storage and Organization, Cuvette Cleaning, and Storage
3. Daily Instrument Checks
    a. Lamp Scan (Excitation Check)
    b. Water Raman Scan (Emission Check)
    c. Cuvette Scan Check (Contamination Check)
4. Running your samples
5. Info about imaging spectrometer characterization & other info to gather
    a. Gain setting comparison
    b. Lamp warm up

Instrument Start Up

1. Press the big button to turn on fan on the lamp box
2. Press the smaller button to turn on the lamp on the lamp box (the lamp has to warm up for 20 min).
3. Turn on the computer and wait for it to boot up.
4. Turn on the power switch on the SpectraAcQ tower ("the brain"): wait for it to initialize: green lights go on, beeps, pause, then 2 beeps. After this sequence, it is ready for the next step.

5. Turn on the Fluorohub controller by pressing the power switch button: wait for it to initialize before moving to next step. It will say “ready for PC connection” when it is ready.
6. Turn on the imaging spectrometer by pressing the power switch button on, wait for it to initialize before moving to next step. Two green lights will come on and it will make two click sounds and a beep and then it is ready.
7. Turn on the Synapse detector power supply by pressing the power switch button.
8. The detector also needs to initialize/warm up wait for the detector temperature and power lights to turn green (may take a few minutes). The lights are located on the back of the detector.
    a. If the power light does not turn on promptly (within ~2min) you will need to turn off the (1) Synapse Power Supply and (2) imaging spectrometer off and back on (1) imaging spectrometer (2) Synapse Power Supply.
    b. The temperature light will initially be amber. You must wait for it to turn green. This indicates that the correct temperature has been achieved.
9. Once the detector temperature light is green, open up the FluorEssence Software by double clicking the FluorEssenceV3 icon on the desktop to activate the software
10. Click the ‘Experiment Menu’ found in the upper right corner of the toolbar
11. Wait for the system to initialize (normally takes about 1-3 minutes). All items must have a green check. If any have a red x and are not properly initialized you will need to power down and restart everything (see next page for power down instructions).

Instrument Power Down

1. Exit software and power down computer. Make sure to record how many hours the lamp was on.
2. Turn off Synapse power supply.
3. Turn off imaging spectrometer .
4. Turn off the Fluorohub controller.
5. Turn off the Spectra brain.
6. Turn off the lamp.
7. Leave the fan in the lamp box running for 20 minutes in order to cool the lamp compartment.

Before getting started…

Make sure all your samples and water (used for blanks) are at room temperature unless you need to run them all cold. If you want to compare data, samples must be run at the same temperature.

Instrument Logs

- Sign in to CoryLab_Fluorolog_LOGBOOK (file on desktop). Enter the given information which consists of the preliminary instrument checks (lamp scan, raman water scan, cuvette check).
- Open Fluorolog_Sample_Log and indicate the date, scientist, lab group, type of samples, and additional info in worksheet. Make sure your sample file name and absorbance name match the information you enter in the spreadsheet.

    - You MUST run a blank that corresponds EXACTLY to each variation of the scan you run. In other words, every time you change anything about the scan settings including integration time you MUST run a NEW blank with the EXACT SAME SETTINGS and make sure that you meticulously keep track of which blank corresponds to each sample in the sample log. If you do not your corrections will be wrong and your data intensities will be wrong.
- Make sure you have a quartz cuvette, all sides should be clear, no ground/frosted glass.

Data Storage & Organization

- FluorEssence (the software) stores multiple data objects in "projects" which are set up to act as folders with all scans run for a given "experiment". For now, we will use the project format to save all data run for a given "experiment". This means all samples run by one person in a given day will be saved as a project named by date and will include all samples and daily checks run by the scientist on that day. Projects will be saved in the specific lab group folder. Within a given date it is possible that multiple individuals will have projects, hence the initials are added to distinguish.
- Depending on the number of samples you are running you may need multiple projects. This is because the software seems to start running slowly and have more problems when a project contains more than about 30 scans. .

Cuvette Cleaning & Storage

For daily cuvette cleaning, rinse cuvette with lab water about 20x and store dry. Periodically, rinsing the lab grade water will not work to clean cuvette (a large peak will remain upon running

the cuvette check scan despite exhaustive rinsing). In this case, set up a mini-advanced oxidation treatment to clean the cuvette by adding a few drops of ~30% H2O2 into a beaker filled with lab water. Put the cuvette into the beaker with the peroxide solution and put it in sunlight (or brightest light possible) for ~20-30 min, repeat as necessary to till clean.

Daily Instrument Checks

- Lamp Scan (Excitation Check)
- Water Raman Scan (Emission Check)
- Cuvette Scan Check (Contamination Check)

Xenon Lamp Scan (Excitation Check)

- Click the 'Experiment Menu' then select spectra, excitation, and click next
- Load the lamp scan file.
- Load lamp.xml
- Check the Monos and Detector settings and confirm they agree with the settings in the images below.
- Make sure the sample chamber of the Fluorolog is empty and completely closed (and then click "run" to begin the scan.
- When the scan completes a prompt to save the project will appear.

- Next find the highest peak, zoom in on the region around the highest peak with the “zoom in” tool and check the wavelength and intensity with the “data reader” tool. The peak should be 467 +/- 0.5 nm. If it is out of this range, you must recalibrate for excitation.

Water Raman Scan (Emissions Check) for Photometer Detector

- Rinse the 4 mL quartz cuvette with lab water, fill the cuvette with the same water (to the neck of cuvette), wipe the cuvette clean with kimwipes, insert into the chamber, and close the lid completely. Always wear nitrile gloves! Fingerprints are not easily removed from the cuvette; therefore do not touch the cuvette with your fingers. Always insert the cuvette in the same position (with the small Q facing to the right)
- Click the ‘Experiment Menu’ in the toolbar and select emission
- In the experiment file box, click load, go up one folder level to scans, and select RamanS2.xml
- Parameters should be: emission scan, run over 365-450 nm at ex=350 nm, increment = 0.5 nm, 0.25s integration time, 5 nm slit widths, S2, S2c, S2c/R1c mode, dark offset turned ON, and corrections turned OFF, looking like the following in the Monos and Detectors boxes:
- When the scan is finished, click on the most prominent peak in the box labeled S2c/R1c (there should be 2 boxes for the 2 different scan modes, see image below). The Raman peak should appear at 397 +/- 0.5 nm.
- If the Raman peak is within the acceptable range, record the peak position, the intensity

Water Raman Scan (Emissions Check) for Spectrometer Detector

- Rinse the 4 mL quart cuvette with lab water, fill the cuvette with the same water (to the neck of cuvette), wipe the cuvette clean with kimwipes, insert into the chamber, and close the lid completely. Always insert the cuvette in the same position (with the small Q facing to the right)
- Click the 'Experiment Menu' in the toolbar and select emission
- In the experiment file box, click load, go up one folder level to scans, and select RamanS1.xml
- Parameters should be: emission scan, center wavelength 550nm, ex=350 nm, 0.2s integration time, 5 nm slit widths, S1c, S1c/R1c mode, dark offset turned ON, and corrections turned OFF.
- When the scan is finished, zoom in on the second peak from the left. The first peak is the Rayleigh scattering peak and it is much sharper and taller and should be located at the excitation wavelength (i.e., 350 nm). The Raman peak should appear at 397 +/- 0.5 nm.
- If the Raman peak is within the acceptable range, record the peak position and the intensity

Cuvette Check Scan (for contamination)

- The cuvette check using the CCD detector is an EEM with a very short integration time (0.25 seconds).
- If running a cuvette check right after a Raman check you can leave the cuvette in the chamber but if you haven't run the Raman scan first, do the following;
    - Rinse the 4 mL quartz cuvette with lab water, fill the cuvette with lab water, wipe the cuvette clean with kimwipes, insert into the chamber, and close the lid completely.

Always insert the cuvette in the same position (with the small Q facing to the towards you)

- Click the 'Experiment Menu' in the toolbar and select 3D
- In the experiment file box, click load and in the scans folder select "DOMCCD_bin1_BDR.xml"
- Data identifier should be C
- Make sure chamber is completely closed. Click "run" to begin the scan
- When the scan is complete, open the data folder and open the S1c/R1c file of the cuvette check scan. Copy the data from the S1c/R1c file (not the S1c file) and paste it into the "BlankNoiseCheck_S1cR1c" worksheet of "Fluorolog_Sample_Log.xlsx".

<u>EEMs – How to run your samples</u>

- Open the Fluorolog_Sample_Log.xlsx on the desktop. This is where you will record all the relevant information about each sample.
- Click the 'Experiment Menu' then click "3D"
- Click "load", go up one folder level, select "scans" and then select your scan
- Start with the scan "DOMCCD_bin1_BDR.xml" – if you need to modify it in any major ways resave it with a new name that is logical.
- Check the scan to make sure the settings are correct!
    - Excitation = 240-450 nm by increments of 5 nm
    - center wavelength = 550 nm
    - Dark offset checked, corrections unchecked
    - Bin=1

    - Gain=Best Dynamic Range

- NOTE: dilute your sample if it is too absorbent for these guidelines. Inner filter correction will not work if your sample is too optically opaque.
- Use the signal output to check if the detector is maxing out. Your signal will have a flat top or butte. If this happens you need to decrease the integration time. If you think you are getting close to maxing out the detector but can't tell by looking at the Fluoressence graph, copy the data into excel and look at the slices individually.
- You must run a blank that corresponds exactly to each variation of the scan you run. In other words, every time you change anything about the scan settings including integration time you must run a new blank with the exact same settings and make sure that you meticulously keep track of which blank corresponds to each sample in the sample log.

<u>Hewlett-Packard UV-Vis Instrument Standard Operating Procedures (SOP)</u>

Start up

1. Turn on the UV-Vis on the back left of the instrument and turn on the attached computer.
2. A "busy" light should come on at the top of the instrument.
3. Let the UV-Vis warm up for 20 minutes.
4. Open up global works (button on desktop).
5. Click on the tab that says "Data Collection".
6. An icon for Diode Array appears; open this.
7. After repeated clicks, and the "busy" light turning on and off, the instrument is ready.

8. You will need 2 cuvettes for your experiment; a blank cuvette and a sample cuvette (if using a 5 or 10 cm pathlength cuvette you will only use one cuvette and will rinse it very very well when collecting the blank and then empty it and use it for the sample).

Collecting absorbance spectra

9. The spec must be blanked every 10 minutes. It is okay to collect multiple absorbance spectra using the same blank as long as it has been less than 10 minutes since the last time it was blanked.
10. Place your blank cuvette against the left opening of the instrument, resting upright on the tray.
11. Click on the tab "Collect Reference".
12. Select "OK."
13. The UV-Vis is now collecting the absorbance of the blank.
14. Click on the tab "Collect Data" with the blank cuvette still in the cuvette holder.

    A clean blank will have even noise around zero.
15. The computer will ask if you want it to transfer the data to Global Works. If the blank was not clean, select "no." If you know you want to keep the blank or if you are not sure, select "yes."
16. Rename the experiment by clicking on the "Experiment 1" in the right hand pane of the software window. Name the experiment by date so that you can search for it if necessary (e.g., 20121003_exp1 for 3 October 2012). Press "enter" after naming the experiment; if you don't the name won't get saved and will revert back to "Experiment 1".
17. If you have obtained a good blank with clean data, replace the blank cuvette with the sample cuvette.

18. Click on the icon that looks like a rectangle with gray and red zig zags at the top of the window.
19. Collect Data repeat as above.
20. When you have completed a scan, name each one with your sample name and press enter; if you do not press enter your name will not be saved. You do not need to name your blank.
21. Click on the graph, then right click; save data as an text file; browse to the folder you wish to save your data in: C:\DATA\Chemistry\Absorbance\HP_UV_VIS\2020_data

Shut down

22. To Shut down, close out the program.
23. You will be prompted to save files and project
24. Select yes.
25. Naming projects – just click yes, it will save the files as the names you have given them.
26. Turn off UV/Vis power.

APPENDIX F: STANDARD OPERATING PROCEDURE FOR CHLORINE DOSING SOLUTION PREPARATION AND RESIDUAL MEASUREMENT

Prepared by Katja Kritsch, updated by Kirsten Studer

1. Reagents and Materials

1.1. Laboratory grade water (LGW), purified using a secondary water purification system (Pure Water Solutions, Hillsborough, NC). Water pretreated with an general in-house purification system was prefiltered (1 µm filter), treated to remove chlorine or chloramine residuals, passed through an activated carbon resin to reduce the total organic carbon content to less than 0.2 µg/L and passed through mixed-bed ion exchange resins to reduce the ion content to less than 18 MΩ cm.

1.2. Sodium hypochlorite (Fisher Scientific, Pittsburgh, PA, USA), 5 - 6%, concentration determined according to procedure 4500 Cl B. Iodometric Method I in Standard Methods for the Examination of Water and Wastewater, 20th edition.

1.3. Acetic acid (Fisher Scientific, Pittsburgh, PA, USA), glacial, trace metal grade

1.4. Potassium iodide (Fisher Scientific, Pittsburgh, PA, USA), reagent ACS, ≥99%

1.5. Sodium thiosulfate pentahydrate (Fisher Scientific, Pittsburgh, PA, USA), reagent ACS, crystalline, ≥ 99.5%

1.6. Ammonium sulfate (Fisher Scientific, Pittsburgh, PA, USA), granular, certified ACS

1.7. Sodium hydroxide (Fisher Scientific, Pittsburgh, PA, USA), 50% w/w/certified

1.8. Hexagonal polystyrene weighing dishes, Fisherbrand

1.9. Intermediate range pH test strips, EMD colorpHast (Fisher Scientific, Pittsburgh, PA, USA), pH 5 – 10 and pH 0 – 6

1.10. Monochlor-F reagent powder pillows (Hach, Loveland, CO, USA)

2. Glassware

All glassware must be washed in detergent (Alconox), rinsed with tap water and LGW, soaked in a 10% Nitric acid solution overnight, rinsed three times with LGW and dried in an 80 °C oven designated for glassware drying. Volumetric glassware must be rinsed three times with methanol and dried in the fume hood and cannot be place in the oven. Caps and Teflon cannot be acid washed. They are washed in a soap bath separate from glassware and each other, rinsed three times with tap water, three times with LGW and three times with methanol. To dry, caps and liners are placed on a clean Kimwipe, covered with another Kimwipe and dried in the fume hood.

2.1. 125 mL amber Boston round bottles with open-top caps and Teflon-lined septa for (Laboratory Supply Distributors, Mt. Laurel, NJ, USA)

2.2. 2 mL clear glass volumetric flasks with open-top caps and Teflon-lined septa for dosing solution (Pyrex, Corning Inc., Corning, NY, USA)

2.3. 10 mL and 100 mL clear glass volumetric flasks with ground glass stoppers for stock solution preparation and UV measurement (Pyrex, Corning Inc., Corning, NY, USA)

2.4. Clear Erlenmeyer flask with ground glass stopper to prepare dosing solution (Pyrex, Corning Inc., Corning, NY, USA); (size depends on amount of dosing solution needed)

2.5. 500 mL clear Erlenmeyer flask, open top, for the standardization of the free chlorine stock solution (Pyrex, Corning Inc., Corning, NY, USA)

2.6. 1 and 10 mL glass pipettes (Pyrex, Corning Inc., Corning, NY, USA) with rubber bulb

2.7. Pasteur pipettes and rubber bulbs for sample transfer

2.8. 2000 mL glass beaker (Pyrex, Corning Inc., Corning, NY, USA), used as ice-bath container

2.9. Glass solids funnel (Pyrex, Corning Inc., Corning, NY, USA), for solids transfer (needs to fit the 100 mL volumetric flasks)

3. Instruments and Apparatus

3.1. Analytical Balance

3.2. UV vis spectrometer

3.3. Hach DR/890 Colorimeter

3.4. Adjustable micro-pipettor with appropriate glass tips

3.5. Stir plate and Teflon-coated stir bar for titration

4. Solution Preparation:

4.1. Hypochlorite Stock Solution (Free Chlorine Stock Solution)

The hypochlorite stock solution comes ready to use and does not need to be diluted. However, the actual free chlorine concentration in mg/L as Cl_2 is usually not known.

4.1.1. Standardization of the Free Chlorine Stock Solution

The entire description of the method is beyond this scope. Please refer to original method for the preparation of the solutions needed for the standardization of the hypochlorite stock solution. The concentration of the hypochlorite stock solution is generally in the range of 40 to 60 mg/mL.

4.2. Place the small funnel on top of the burette and fill burette to the zero mark with 0.01 N sodium thiosulfate titrant prepared and standardized according to procedure 4500 Cl B. Iodometric Method I.

4.3. Pipette 5 mL acetic acid in the open top Erlenmeyer flask.

4.4. Weigh 1 g potassium iodide into a weighing dish and transfer the solids to the Erlenmeyer flask.

4.5. Pipette 100 µL of the free chlorine stock solution into the flask using a micropipettor. Be careful not to disturb any solids in or on the hypochlorite stock bottle.

4.6. Mix the sample mixture with a glass rod. A yellowish color will develop.

4.7. Place the stir plate under the burette and adjust height for Erlenmeyer flask to fit. Place the flask on the stir plate and add the stir bar. Turn on the stir plate.

4.8. Carefully titrate (stopping often and waiting for reaction) until the yellow color is almost gone.

4.9. Add 1 mL starch solution. A blue color should develop.

4.10. Titrate drop-wise until the solution turns colorless, note the volume of titrant used.

4.11. Calculate the concentration of the free chlorine standard according to the following equation:

$$mg\ Cl\ as \frac{Cl_2}{mL} = \frac{A \times N \times 35.45}{V}$$

A = mL volume of titrant used to titrate to endpoint

N = 0.01, normality of sodium thiosulfate titrant

V = 0.1 mL, sample volume in mL

5. Hypochlorite Working Solution

5.1. The concentration of the hypochlorite working solution to prepare depends on the desired chlorine-to-ammonia ratio.

5.2. Pipette the appropriate volume of free chlorine stock solution into a 100 mL glass volumetric flask and fill to the mark with LGW.

5.3. Stopper the flask and invert three times to mix.

6. UV-Vis and Hach Kit Monochloramine Analysis Procedure

6.1.UV-Vis Analysis

6.1.1.1. In order to create a calibration curve for use on the UV-Vis spectrometer in combination with the indophenol method, prepare at least three to four calibration points bracketing the target residual selected for your experiment.

6.1.1.2. Pipet a 10 mL sample of each calibration standard and the sample into separate 20 mL vials.

6.1.1.3. Record the absorbance of each calibration solution at 255 nm using the indophenol method and create a calibration curve.

6.1.1.4. Measure the absorbance of your sample at 255 nm using the same procedure and use the calibration curve to determine the monochloramine residual in the sample.

6.2.Hach Colorimeter Chlorine Analysis Procedure

6.2.1.1. Chlorine residual to be measured using HACH Chlorine Pocket Colorimeter with Method 8021 DPD free chlorine reagent powder pillows and Method 8167 DPD total chlorine reagent powder pillows

7. Chlorine Demand

The chlorine demand of the sample determines the amount of dosing solution needed to achieve a desired target residual, which can either be an immediate target or a residual after a specified contact time.

7.1. Prepare a sample representative of the experimental batch containing the same concentration of any precursors, dissolved organic carbon content, pH (buffers) and any other additives.

7.2. Split this sample in at least 5 equal aliquots and dose these aliquots with chloramine doses at and above the selected target concentration.

7.3. The pH of the sample has a major influence on the chloramine residual. A low pH will favor the formation of dichloramine and deplete the monochloramine residual.

APPENDIX G: STANDARD OPERATING PROCEDURE (SOP) FOR IODOACETIC ACIDS

Prepared by Katja Kritsch, modified by Kirsten Studer

1. Materials:

1.1. Commercially Available Iodoacetic Acids (Sigma–Aldrich, St Louis, MO, USA)

1.1.1 Iodoacetic acid, CAS # 64-69-7, puriss. p. a. ≥ 99.5 %

1.2. Synthesized Iodoacetic Acids (CanSyn, Toronto, Ontario, Canada)

1.2.1. Bromoiodoacetic acid, CAS # 71815-43-5, 85-90%

1.2.2. Chloroiodoacetic acid, CAS # 53715-09-6

1.2.3. Diiodoacetic acid, CAS # 598-89-0

1.3. Commercially Available Haloacetic Acids, (Sigma–Aldrich, St Louis, MO, USA)

1.3.1. EPA 552.2 Acids calibration mix, 200 - 2000 µg/mL in MtBE

chloroacetic acid (ClAA)	614.0 µg/mL
dichloroacetic acid (Cl_2AA)	596.6 µg/mL
trichloroacetic acid (Cl_3AA)	199.3 µg/mL
bromoacetic acid (BrAA)	407.9 µg/mL
dibromoacetic acid (Br_2AA)	200.6 µg/mL
tribromoacetic acid (Br_3AA)	1962 µg/mL
bromochloroacetic acid (BrClAA)	400.0 µg/mL
bromodichloracetic acid ($BrCl_2AA$)	375.3 µg/mL
chlorodibromoacetic acid ($ClBr_2AA$)	1009 µg/mL

1.4. Commercially Available Reagents & Materials

1.4.1. Laboratory grade water (LGW), purified using a secondary water purification system (Pure Water Solutions, Hillsborough, NC). Water pretreated with an

general in-house purification system was prefiltered (1 µm filter), treated to remove chlorine or chloramine residuals, passed through an activated carbon resin to reduce the total organic carbon content to less than 0.2 µg/L and passed through mixed-bed ion exchange resins to reduce the ion content to less than 18 MΩ-cm.

1.4.2. 2,3-dibromopropionic acid, internal standard, (Sigma–Aldrich, St Louis, MO, USA), Supelco Standard, 1000 µg/mL in MtBE

1.4.3. 2-bromobutyric acid, surrogate standard, (Sigma–Aldrich, St Louis, MO, USA), Supelco Standard, 1000 µg/mL in MtBE

1.4.4. Ascorbic acid, (Sigma–Aldrich, St Louis, MO, USA), SigmaUltra, ≥99%

1.4.5. Di (ethylene glycol) ethyl ether, (Sigma–Aldrich, St Louis, MO, USA), ≥99%

1.4.6. Diazald (Sigma–Aldrich, St Louis, MO, USA), 99%

1.4.7. Magnesium sulfate (Fisher Scientific, Pittsburgh, PA, USA), anhydrous, powder, ≥98%

1.4.8. Methanol (Sigma–Aldrich, St Louis, MO, USA), Chromasolv®, 99.9%

1.4.9. Methyl tert-butyl ether (Sigma–Aldrich, St Louis, MO, USA), Chromasolv®, 99.9%

1.4.10. Potassium hydroxide (Fisher Scientific, Pittsburgh, PA, USA), 45% w/w

1.4.11. Silicic acid (Sigma–Aldrich, St Louis, MO, USA), purum p.a. ≥99%

1.4.12. Sodium hydroxide (Fisher Scientific, Pittsburgh, PA, USA), pellets, certified ACS, ≥97%

1.4.13. Sodium sulfate (Fisher Scientific, Pittsburgh, PA, USA), anhydrous, granular, 99.3%

1.4.14. sulfuric acid (Fisher Scientific, Pittsburgh, PA, USA), certified ACS plus, 95-98%

1.4.15. Strata Florisil cartridges (Phenomenex, Torrance, CA, USA), 200 mg/ 3 mL

1.4.16. Narrow range pH paper, pH 0 to 2

1.5.Glassware

All glassware must be washed in detergent (Alconox), rinsed with tap water and LGW, soaked in a 10% Nitric acid solution overnight, rinsed three times with LGW and dried in an 80 °C oven designated for glassware drying. Volumetric glassware must be rinsed three times with methanol and dried in the fume hood and cannot be place in the oven. Caps and Teflon cannot be acid washed. They are washed in a soap bath separate from glassware and each other, rinsed three times with tap water, three times with LGW and three times with methanol. To dry, caps and liners are placed on a clean Kimwipe, covered with another Kimwipe and dried in the fume hood.

1.5.1. 1000 mL and 250 mL amber Boston round bottles with open-top caps and Teflon-lined septa for sample collection and the liquid-liquid extraction procedure (Laboratory Supply Distributors, Mt. Laurel, NJ, USA)

1.5.2. 1000 mL clear separatory funnels with ground glass stoppers (Pyrex, Corning Inc., Corning, NY, USA)

1.5.3. 2 mL clear glass volumetric flasks with open-top caps and Teflon-lined septa for primary standard preparation (Pyrex, Corning Inc., Corning, NY, USA)

1.5.4. 100 mL and 1000 mL clear glass volumetric flasks with ground glass stoppers for sample preparation prior to liquid-liquid extraction and solution preparation (Pyrex, Corning Inc., Corning, NY, USA)

1.5.5. 50 mL, 100 mL and 1000 mL clear measuring cylinder to measure sulfuric acid and water sample volume during the liquid-liquid extraction procedure (Pyrex, Corning Inc., Corning, NY, USA)

1.5.6. 15 mL graduated centrifuge tubes to collect eluent from solid-phase extraction and further use the same tubes for part of the backextraction procedure (Scientific, Pittsburgh, PA, USA)

1.5.7. 4 mL clear straight-walled sample vials with open-top caps and Teflon-lined septa for the backextraction procedure and derivatization (Laboratory Supply Distributors, Mt. Laurel, NJ, USA)

1.5.8. 1 mL and 10 mL glass pipette (Pyrex, Corning Inc., Corning, NY, USA) with rubber bulb

1.5.9. Pasteur pipettes and rubber bulbs for sample transfer

1.5.10. 1.8 mL amber glass autosampler vials with 300 µL flat-bottom glass inserts Teflon-lined silicon crimp tops (Laboratory Supply Distributors, Mt. Laurel, NJ, USA)

1.5.11. 250 mL glass beaker (Pyrex, Corning Inc., Corning, NY, USA), premarked at approximately 100 g sodium sulfate for salt additions during liquid-liquid extractions

1.5.12. Small glass funnel (needs to fit the 4 mL vials)

1.6. Instruments and Apparatus

1.6.1. Gas Chromatography column: Zebron ZB-1701, 30 m length, 0.25 mm inner diameter, 1µm film thickness, (Phenomenex, Torrance, CA, USA)

1.6.2. HP 6890 series gas chromatographic system equipped with an HP 7683 series injector with autosampler tray and G2397A μ-ECD detector (Agilent Technologies, Santa Clara, CA, USA)

1.6.3. Manually operated 24 port Visiprep™ SPE manifold (Supelco) with appropriate connectors, Teflon transfer lines and trap

1.6.4. Vortexer

1.6.5. Adjustable micropipettors with appropriate glass tips

2. Solution Preparation:

2.1. Standard Acid Solutions

2.1.1. Surrogate standard 2-bromobutyric acid

2.1.1.1. Primary Solution, 10 mg/L: transfer 100 μL of 2-bromobutyric acid (1000 μg/mL) and dilute to volume with MtBE.

2.1.2. Iodoacetic acid primary standards, contain only one neat iodoacetic acid species

2.1.2.1. Primary Standards, 2 mg/mL (BrIAA, CLIAA, I_2AA, and IAA): One standard at a time, accurately weigh approximately 4 mg of the neat iodoacetic acid species directly into a 2 mL volumetric flask and dilute to volume with MtBE. Make sure the solution is clear. Transfer to a 4 mL sample vial, cap, seal and wrap in aluminum foil. Do not expose solution to light longer than necessary to prepare one standard.

2.1.3. Iodoacetic acid standard mix contains bromoiodoacetic acid, chloroiodoacetic acid, diiodoacetic acid and monoiodoacetic acid

2.1.1.2. Spiking Solution Mix (IAs), 1 mg/L of four iodoacetic acid species: transfer 5 µL of each primary iodoacetic acid standard to the same 10 mL volumetric flask and dilute to volume with MtBE.

2.2. Internal Standard Solution (MtBE+IS)

2.2.1. Primary solution, 5mg/mL: fill a 2 mL volumetric flask with approximately 1 mL MtBE using a Pasteur pipette without touching the glass walls of the volumetric flask and place the flask on a scale. Using sandwich technique, pull up 5 – 10 µL of the liquid 1,2-dibromopropane and transfer to the volumetric without touching the solvent surface. Quickly read scale, place in hood and fill to volume with MtBE.

2.3. Sodium Bicarbonate Solution

2.3.1. Combine 50 g of sodium bicarbonate with 500 mL LGW in a 1000 mL amber Boston round bottle.

2.3.2. Drop Teflon-coated stir bar into the solution and stir, uncapped, on a stir plate for 30 minutes until dissolved.

2.4. Acidic Methanol

2.4.1. Fill a 100 mL clear volumetric flask with approximately 50 mL methanol and place on an ice bath.

2.4.2. Measure 10 mL concentrated sulfuric acid using a glass pipette and slowly add the acid to the volumetric flask.

2.4.3. Swirl and fill to volume with methanol.

2.5. MtBE containing 10% methanol

2.5.1. Measure 10 mL methanol using a glass pipette and transfer to a 100 mL clear volumetric flask.

2.5.2. Fill to volume with MtBE.

2.6. Ascorbic acid solution, 100 g/L

2.6.1. Accurately weigh 10 g of ascorbic acid, transfer to a 100 mL volumetric flask.

2.6.2. Fill flask with approximately 50 mL LGW, swirl until dissolved, fill to volume with LGW.

3. Sample Handling & Preservation:

3.1. Quenching Agents

3.1.1. Ascorbic acid

3.1.1.1. To a 1000 mL bottle, transfer 100 µL of the 100 g/L ascorbic acid solution.

4. Sample Preparation & Analysis:

4.1. Sample Preparation (Addition of Surrogate Standard)

4.1.1. Label the sample bottle as “bottle 1”.

4.1.2. Transfer pre-cooled sample (4 hours at 4°C) to a 1000 mL volumetric flask; do not exceed the 1000 mL mark.

4.1.3. Transfer 100 µL of the surrogate standard primary solution.

4.1.4. Cap flask and invert three times.

4.2. Sample Fortification (Standard Addition Calibration)

4.2.1. Sample fortification is only completed for the calibration curve, skip to 4.3. if this is a sample that does not belong to the calibration curve.

4.2.2. Add appropriate amounts of the Spiking Solution Mix, 1 mg/L and EPA 552.2 Acids calibration mix, 200 - 2000 µg/mL. Select three concentration levels appropriate for the expected iodoacetic acid concentrations. Spike with iodoacetic acids and three concentration levels of haloacetic acids. Cap and invert three times.

4.3. Extraction Procedures

4.3.1. Liquid-Liquid Extraction (LLE)

4.3.1.1. Label sample bottle as “bottle 1”.

4.3.1.2. Using a 1000 mL measuring cylinder, measure 500 mL of the sample and transfer to a second 1000 mL amber bottle. Label this bottle “bottle 2”. Return remaining sample into original 1000 ml amber bottle (bottle 1).

4.3.1.3. Using a 50 mL clear measuring cylinder, carefully measure 30 mL concentrated sulfuric acid.

4.3.1.4. Slowly add the acid to the water sample in bottle 1, holding the bottle at an angle and letting the acid hit the glass surface first. Only add a little at a time, turn the bottle a ¼ turn before adding more. Repeat until the acid is added to the sample. Swirl to mix.

4.3.1.5. Confirm pH of 0.5 or lower using a narrow range pH paper. Do not immerse. Use a Pasteur pipette to transfer a small amount of sample onto the paper.

4.3.1.6. Using the pre-marked beaker, transfer 100 g baked sodium sulfate to bottle 1; do not let the bottles sit to prevent compaction of the salt.

4.3.1.7. Measure 100 mL of MtBE (plain, without internal standard) and transfer to bottle 1.

4.3.1.8. Measure 100 mL of MtBE (plain, without internal standard) and transfer to bottle 1.

4.3.1.9. Manually agitate the bottle for 2 minutes.

4.3.1.10. Pour sample from bottle 1 into 1000 mL clear separatory funnel, place empty sample bottle under the funnel, cap funnel, wrap with aluminum foil and wait 5 minutes to allow for separation of the phases.

4.3.1.11. Carefully drain aqueous phase into the sample bottle, stopping just before the last drop of aqueous phase drains. Place a 250 mL amber glass bottle containing 5 g baked anhydrous magnesium sulfate under the funnel and drain the organic phase.

4.3.1.12. Place this extract in the refrigerator until the second part of the extraction procedure is completed.

4.3.1.13. Repeat steps 4.3.1.6. to 4.3.1.12. with the bottle 2.

4.3.1.14. Remove the extract from step 4.1.15. from the refrigerator and place it under the funnel. Drain the organic phase.

4.3.1.15. Cap the bottle and swirl. Observe the magnesium sulfate. If enough drying agent was added, the magnesium sulfate will swirl around

similar to a “snowglobe”. If the magnesium sulfate sticks to the bottom or sides of the bottle, add an additional small scoop of baked anhydrous magnesium sulfate. Check effectiveness of drying again and repeat until the “snowglobe” effect appears.

4.3.1.16. Place the bottle containing the combined sample extract in the freezer until further processing.

4.3.2. Solid-phase Extraction (SPE)

4.3.2.1. Place manifold in fume hood and attach appropriate liquids trap between manifold and vacuum outlet. Do not turn on vacuum.

4.3.2.2. Place Strata Florisil cartridges on manifold.

4.3.2.3. Condition with 6 mL methanol using a graduated 10 mL glass pipette to measure volume.

4.3.2.4. Condition with 6 mL MtBE using a graduated 10 mL glass pipette to measure volume.

4.3.2.5. Stop flow from cartridges by turning cartridges clockwise. Refill each cartridge with MtBE one more time.

4.3.2.6. Prepare an ice-bath big enough to fit on a shelf above the manifold and to hold all sample bottles being processed in this step.

4.3.2.7. Remove 250 mL bottles containing MtBE extracts (step 4.3.1.16.) from freezer and immediately decant each sample into a clean 250 mL amber glass bottle.

4.3.2.8. Secure Teflon-lines to each bottle using laboratory tape and connect to the cartridges.

4.3.2.9. Turn on vacuum.

4.3.2.10. One cartridge at a time start flow to start liquid transfer from the bottle, quickly lift the connector, close the valve again and let the cartridge fill with the extract. Be careful to put the connector back on in time before the cartridge overfills! Turn cartridge anti-clockwise to restart flow and adjust to 30 drops per minute (approximately 1.5 mL/min).

4.3.2.11. Repeat step 4.3.2.10. with remaining cartridges. Place some aluminum foil around cartridges to protect from light.

4.3.2.12. After the samples have passed through the cartridges, remove the tubing, connectors and bottles. Turn off vacuum.

4.3.2.13. Wash cartridges with 6 mL MtBE containing 10% methanol.

4.3.2.14. Push three syringes air through each cartridge to remove wash solution prior to elution.

4.3.2.15. Place 15 mL graduated centrifuge tubes in the vacuum manifold.

4.3.2.16. Elute cartridges with 3 mL acidic methanol.

4.3.2.17. Push two syringes of air through each cartridge to maximize solvent recovery.

4.3.2.18. Store acidic methanol extracts in freezer until further processing.

4.3.3. Backextraction into MtBE+IS

4.3.3.1. Remove extracts from 4.3.2.18. from the freezer and place in the refrigerator to equilibrate to 4 °C for 30 minutes.

4.3.3.2. Remove one vial from the refrigerator at a time for the backextraction procedure.

4.3.3.3. Add 1 mL MtBE+IS using glass pipette.

4.3.3.4. Add 7 mL sodium sulfate solution.

4.3.3.5. Vortex 2 minutes, then let phases separate for 5 minutes.

4.3.3.6. Using a Pasteur pipette, quantitatively transfer the yellowish MtBE layer into a clear 4 mL vial. Make sure to transfer entire amount of MtBE. This will also transfer a small amount of the aqueous layer which is removed in the next step.

4.3.3.7. Tip the 4 mL vial slightly, put pressure on pipet bulb and insert the previously used Pasteur pipette to the bottom edge of the vial, remove most of the aqueous layer without removing MtBE.

4.3.3.8. Add 1 mL sodium bicarbonate solution.

4.3.3.9. Vortex five times in five-second spurt, carefully loosening the cap in between spurts to release evolving CO_2.

4.3.3.10. Allow for the layers to separate within 10 minutes. The MtBE layer is now clear and colorless.

4.3.3.11. Using an adjustable micropipettor, transfer 500 µL of the MtBE layer to a clear 4 mL vial.

4.3.3.12. Add a small scoop (approximately 5 – 10 mg) of baked magnesium sulfate using the small glass funnel. Swirl. Check to confirm the drying agent swirls freely and does not stick to the bottom or sides. Otherwise add another small scoop and recheck.

4.3.3.13. Keep vial cool and do not expose to light more than necessary for handling.

4.3.3.14. Place vial in fridge until further processing.

4.3.4. Derivatization (Diazomethane)

4.3.4.1. Be sure enough diazomethane solution is available to derivatize all samples in a set with the same batch. Otherwise prepare a fresh batch of diazomethane following the diazomethane generation procedure.

4.3.4.2. Prepare an ice bath big enough to hold all processed vials.

4.3.4.3. Derivatization blank: add one vial containing MtBE to the group of extracts.

4.3.4.4. Derivatization standard: add the amounts for the highest calibration standards to an empty 4 mL vial (see Table 1) and add appropriate amount of MtBE to achieve 500 µL total volume.

4.3.4.5. Using an adjustable micropipettor, add 250 µL cold diazomethane solution to each extract and place vial in the ice bath.

4.3.4.6. Let react for 1 hour, remove and check for yellow color indicating a sufficient amount of derivatizing agent was present for the reaction.

4.3.4.7. Equilibrate to room temperature for 15 minutes.

4.3.4.8. To quench residual diazomethane, add a small scoop (approximately 10 - 20 mg) of silicic acid. Do not use too much, this will reduce the amount of extract that can be recovered. The extract will turn colorless.

4.3.4.9. Allow silicic acid to settle for 5 minutes.

4.3.4.10. Transfer to two autosampler vials with glass insert (analysis and backup sample). Using a crimper, crimp top and double-check cap tightness by turning top slightly. Re-tighten if necessary.

4.3.4.11. Store extracts in freezer until analysis.

APPENDIX H: STANDARD OPERATING PROCEDURE FOR HALOGENATED VOLATILE ANALYSIS

Prepared by: Bonnie Lyon, Updated by Kirsten Studer

Halogenated Volatiles

Abbrev.	Compound	CAS #	mol. wt. (g/mol)
TCM	chloroform	67-66-3	119.38
TCAN	trichloroacetonitrile	545-06-2	144.39
DCAN	dichloroacetonitrile	3018-12-0	109.94
BDCM	bromodichloromethane	75-27-4	163.83
TCA	chloral hydrate	302-17-0	165.4
11DCP	1,1-dichloropropanone	513-88-2	126.97
TCNM	trichloronitromethane (chloropicrin)	76-06-2	164.38
DBCM	dibromochloromethane	124-48-1	208.28
BCAN	bromochloroacetonitrile	83463-62-1	154.39
111TCP	1,1,1-trichloropropanone	918-00-3	161.42
TBM	bromoform	75-25-2	252.73
DBAN	dibromoacetonitrile	3252-43-5	198.85
DCIM	dichloroiodomethane	594-04-7	210.83
BCIM	bromochloroiodomethane	34970-00-8	255.28
CDIM	chlorodiiodomethane	638-73-3	302.28
DBIM	dibromoiodomethane	593-94-2	299.73
BDIM	bromodiiodomethane	557-95-9	346.73
TIM	triiodomethane	75-47-8	393.73

Equipment

• Clear 60-mL, clean, prewashed glass screw cap sample vials with teflon-lined silicone septa.

• Gas tight syringes: 25 µL, 50 µL, 100 µL, 250 µL

• 50-250 µL micropipetter fitted with clean glass capillary tips

• Eight 100-mL glass volumetric flasks with glass stoppers

• 1-L amber bottle mounted with 10-mL pump pipetting dispenser

• 23-cm disposable glass Pasteur pipettes with rubber Pasteur pipette bulb

• pH indicator strips pH 0-6

• GC vials - Amber glass vials, Laboratory Supply Distributors, (catalog #20211ASRS-1232)

• GC Caps - with silicone septa, 40 Mils thick, Supelco (catalog #27360-U)

• GC vial inserts – flat bottom, Laboratory Supply Distributors, (catalog #20870-530)

• Hand crimper for sealing gas chromatography autosampler vials

• Vortexer

• Teflon tape

• Stainless steel scoopula

Instrumentation

• Hewlett-Packard GC5890 Series II with autosampler/autoinjector tower

• Capillary Column: ZB-1, 30 m length x 0.25 mm inner diameter, 1.0-µm film thickness

• Electron Capture Detector (ECD): Hewlett-Packard Model ECD

• Data System: Hewlett-Packard ChemStation

• Carrier Gas-Ultra High Purity helium (He)

• Makeup Gas-Ultra High Purity nitrogen (N_2)

Reagents

• Laboratory Grade Water (LGW)

• Extraction solvent: OmniSolv Methyl-t-Butyl Ether, (Fisher Scientific – catalog # MMX08266)

• Sodium sulfate (Na_2SO_4), Mallinckrodt, granular, ACS grade (catalog #8024). Bake at 400ºC for 24 hours and stored in glass-stoppered bottle in desiccator.

• Methyl-t-Butyl Ether (OmniSolv, Fisher Scientific – catalog # MMX08266)

• L-Ascorbic Acid, Certified ACS grade (Fisher Scientific –catalog #A61-25)

• Sulfuric Acid, Certified ACS Plus (Fisher Scientific – catalog #A300-212)

Standards

• THM Calibration Mix, 2000 µg/mL each in methanol (Supelco)

• EPA 551B Halogenated Volatiles Mix, 2000 µg/mL each in methanol (Supelco)

• Internal Standard (IS): 1,2-dibromopropane neat standard, 99+% (Sigma)

• Dichloroiodomethane, CanSyn Chemical Corporation

• Bromochloroiodomethane, CanSyn Chemical Corporation

• Chlorodiiodomethane, CanSyn Chemical Corporation

• Dibromoiodomethane, CanSyn Chemical Corporation

• Bromodiiodomethane, CanSyn Chemical Corporation

• Triiodomethane, CanSyn Chemical Corporation

Sample Handling

Samples should be collected headspace-free in pre-cleaned 60 mL glass vials with screw caps and PTFE-lined silicone septa containing 1.4 mg ascorbic acid. Samples should be filled headspace free and holding vial at an angle so halogenated volatiles do not escape through volatilization. Store samples in fridge at 4ºC. Samples should be extracted within 24 hours of quenching.

Procedure

1. Internal Standard: Stock solution of internal standard (IS) at ~2000 µg/mL in MtBE - prepared by injecting 10 µL of the neat standard and injecting into a 5 mL volumetric flask containing MtBE, fill to line with MtBE. Primary dilution at 100µg/mL: prepared by injecting

250 µL of IS stock solution using a micropipette into a 5 mL volumetric flask containing MtBE, fill to line with MtBE. Extracting solution at 50 µg/L or 100 µg/L (depending on what expected concentration of analytes in samples): calculate how much extracting solvent will be needed for all of your samples and calibrations (3 mL for each sample and calibration).

2. Halogenated Volatiles Calibration Standards: These are prepared as a mix of THM4, 551B Halogenated Volatiles and chloral hydrate. Calibration Standard #1: 100 µg/mL, 100 µL of each THM4 and EPA 551B stock calibration mix and 200 µL of chloral hydrate to 2 mL volumetric flask containing MtBE, fill to line with MtBE. Calibration Standard #2: 1 µg/mL, 20 µL of Calibration Standard #1 into 2 mL volumetric flask containing MtBE, fill to line with MtBE.

3. Transfer standards to a 2-mL amber glass vial and store in laboratory standards freezer at -15ºC.

4. Check calibration standards a few days before extraction. Make up two dilutions (50 µg/L and 1 µg/L) in MtBE containing internal standard. Standards should be monitored for degradation and contamination by comparing standard chromatographic peak area values obtained on the performance evaluated designated GC to those obtained during initial calibration of standard. The responses obtained on the same instrument are normalized relative to the freshly prepared internal standard to account for instrument detector drift. New standards should be made from the stock solution if check exceeds 20% drift. If the drift persists, purchase new stock solutions from two suppliers and compare the responses making a note of the stock batch number.

5. Prepare a laboratory reagent blank (the level 1 calibration standard - see step 6) and the laboratory fortified blank (level 3 calibration standard – see step 6) at the beginning of each day

and analyze on the GC before extracting samples. If quality control criteria fail, troubleshoot and correct the problem, reanalyzing these check standards before proceeding to the next step.

6. Prepare calibration standards in 100 mL LGW according to the range of concentrations expected in the samples. Example concentrations: 0, 1, 5, 10, 20, 50, 100, 200 µg/L.

7. Prepare matrix spike and matrix spike duplicate in 25mL samples should be ~2-3 times halogenated volatile levels in samples.

8. Measure 30 mL from all calibration standards using a 50 mL measuring cylinder starting from lowest to highest concentration and then follow with the samples all in duplicate and transfer into 60 mL vials. Rinse cylinder 3 times with LGW and once with sample to be measured next between each. Pour at an angle so halogenated volatiles are not lost through volatilization.

9. Adjust all samples and calibrations to approximately pH 3.5 with 0.2 N H_2SO_4. (Amount required for pH adjustment will likely be different for calibrations compared to samples. Use remaining 30 mL aliquot from 60 mL vial to determine how much H_2SO_4 will be needed.)

10. Add 3 mL extracting solvent from a solvent dispenser bottle to each 30 mL aliquot. Make sure there are no bubbles in the dispenser addition line.

11. Add ~6 g pre-baked sodium sulfate to each 30 mL sample/calibration standard. Vortex samples for 1 minute immediately after adding sodium sulfate to avoid clumping. Let samples settle for 5 minutes.

12. Using a disposable 23-cm glass Pasteur transfer ~1.5 mL from the middle of the MtBE layer (top layer) to a GC autosampler vial. Do not transfer any sodium sulfate crystals as they will clog the GC. Cap and crimp vial. Fill two GC vials for each sample (one for halogenated volatile analysis and one backup), and two GC vials with each calibration. Use GC vial inserts.

Store in the laboratory freezer at -15ºC in a tray covered in aluminum foil if not analyzed immediately. Also fill two autosampler vials with MtBE and 2 vials of extracting solvent containing MtBE + IS. Analyze within 4 weeks.

13. Analyze according to specified GC method (see GC temperature programs below) on the designated GC. Instructions for GC use for this method are provided by the instrument that is available at the time.

Quality Control

Precision is measured as the average and relative percent difference of the duplicate analyses of each sample. The relative percent difference should be less than 10% otherwise sample has to be flagged as suspect. The coefficient of variation of all the internal standard responses for the complete set of samples must be less than 15%. Individual samples responsible for elevating this value above the threshold should be flagged and considered suspect.

A calibration check standard is prepared in the mid-range of the standard calibration curve and is injected every 10 samples. If the detector response for this sample varies more than 10% from the previous injection, all samples analyzed between the two injections are flagged for investigation.

Each sample bottle set is accompanied by replicate field and travel blanks.

Instrumental Method

GC-ECD analysis on Hewlett-Packard GC5890 Series II:

Injector: Syringe size = 10µL; Injection volume = 2µL

Wash solvent = MtBE; Pre-injection washes = 3; Post-injection washes = 3; Pumps = 3

Injector Temperature = 200°C; Injection splitless (split after 0.5 min)

Oven/Column: Oven equilibration time = 3 min; Oven max °C = 300°C

Initial: temperature of 35°C with a ramp of 1°C/min for 22 min; Level 1: a ramp of 10°C/min over 2 min until 145°C; Level 2: a ramp of 20°C/min over 10 min until 225°C; and Level 3:a ramp of 20°C/min over 5 min until 260°C (Total time = 55.75 min)

Gas = helium; Column flow = 1 mL/min

Column type = ZB-1, 30.0 m length, 0.25 mm inner diameter, 1 μm film thickness

Split flow = 1 mL/min; Split ratio = 1:1

Electron Capture Detector (ECD): Detector temperature = 290°C, Injector temp: 117^{o}C

APPENDIX I: STANDARD OPERATING PROCEDURES FOR DISINFECTION BYPRODUCT ANALYSIS BY GAS CHROMATOGRAPHY/ION TRAP MASS SPECTROMETRY-ELECTRON IONIZATION

Prepared by: Kirsten Studer

HCB Preparation

1. Before using the instrument on any given day, the operator first needs to prepare a dilution of hexachlorobenzene (HCB) in hexane as an instrument performance standard. Stock solutions of HCB should be prepared around 1 mg/mL. Working solutions of HCB are stored in freezer at a concentration of 10 mg/L. HCB is relatively stable, so the working solution usually lasts 4 to 6 months. Create the dilution of HCB in hexane as follows:

   Dilution: 100 µL of the 10 mg/L working solution in 10 mL Hexane = 100 µg/L
2. First evaluate the hexane that will be used to prepare the HCB stock and dilutions.
3. Unless there is a documented instrument issue that has been reported to HSW and accepted, compare your HCB absolute response (ion counts) at 100 µg/L to the previous time you analyzed. If it differs by more than 20%, remake the working solution. If that doesn't resolve the issue, remake the stock. If there is still a problem, the instrument needs intervention.

Instrument Evaluation

1. Perform System Daily Checks according to "Saturn 2000 GCMS Procedures EI" document prepared by Stanford (04/06/2006). Record relevant information in the system checks log books.

2. Manually inject 1μL of hexane solvent to observe the background chromatogram. After the solvent chromatogram has been confirmed as clean, inject 1 μL of 100 μg/L HCB in hexane using the method "Sens check for EI-Cold for 1701.mth"
3. To analyze the samples, go to MS Data Review and go to Chromatogram > Select Chromatogram Data To Plot… Data files are stored under the users name with the date, time, and sample name included.
    a. Select the 100 μg/L HCB files from your analysis date. Then under "Data" select "Ion(s)" and type in 284. Do this for both HCB samples.
    b. The retention time of HCB is around 10.9 minutes. Record the concentration of HCB in the lab notebook that you are able to see and work with.
    c. Next, right click on the HCB peak and select "Calculate signal/noise". Record this S/N as S/N@284 in the log book and repeat for the m/z ion at 249.
4. Repeat at least once per sample batch at a dilution of 10 μg/L HCB.

Sample Injection and Analysis

1. Hexane or relevant solvent blanks should be placed every 5 to 10 samples and at the beginning and end of each sample list segment.
2. After sample injections, record the remaining information about the solvent you used, your derivatizing agent (if any), your target analytes, the number of injections from your sample list and the sum of all injections since the last septum change (just add your values to the previous number).

5. To analyze the samples, go to MS Data Review and go to Chromatogram > Select Chromatogram Data To Plot… Data files are stored under the users name with the date, time, and sample name included (i.e., C:\VarianWS\data\Kirsten\07-13-15 3-20-08 PM 100HCB).
3. Conclude the sample run with the HCB sensitivity check and a GC bakeout method.
4. Determine if the sensitivity has improved or worsened by re-injecting the HCB standard. If the sensitivity is worse, inform the lab manager.

GC-MS Method for Haloacetic Acids including Iodoacids

Internal Standard: 1,2-Dibromopropane; Formula: $C_3H_6Br_2$; Molar mass: 201.9 g/mol

Surrogate Standard: 2,3-dibromopropionic acid; Formula: $C_3H_4Br_2O_2$; Molar mass: 231.9 g/mol

2-bromobutyric acid; Formula: $C_4H_7BrO_2$; Molar mass: 167.0 g/mol

Injector temperature program: 90°C, hold for 0.10 minutes;

90 to 180°C at 100°C/minute, hold for 51 minutes

Oven temperature program: 37°C, hold for 21 minutes;

37 - 136°C at 5°C/minute, hold for 3 minutes;

136 - 250°C at 20°C/minute, hold 3 minutes

Total run time: 52.0 minutes.

Detector temperature: 300°C

APPENDIX J: PROCEDURE FOR TOTAL/DISSOLVED ORGANIC CARBON AND TOTAL NITROGEN ANALYSIS IN WATER SAMPLES

Standards Preparation

Dissolved Organic Carbon (DOC) Stock Standard (1,000 mg/L as C)

- Dissolve 2.125 g potassium hydrogen phthalate in 1-L lab grade water (LGW);
- Store in fridge in amber bottle with teflon-lined septa/cap. Good for 2 months

Total Nitrogen (TN) Stock Standard (1000 mg/L as N)

- Dissolve 7.219 g Potassium nitrate in 1-L LGW; mix with a magnetic stir bar
- Store in fridge in amber bottle with teflon-lined septa/cap. Good for 2 months

HCl solution (2 N)

- Carefully add 41 mL concentrated HCl (12.1 N) to a 250 mL volumetric flask.
- Fill to line with LGW. Store in amber bottle with teflon-lined septa/cap.

DOC Working Solution (100 mg/L as C)

- Pipette 10-mL of DOC Stock Standard into a 100 mL volumetric flask; fill to line with LGW; invert stoppered flask three times
- Store in fridge in amber bottle with PTFE-lined septa/cap. Good for 1 week

DOC/TN Working Solution (100 mg/L as C, 100 mg/L as N, 0.05 M HCl)

- Pipette 10-mL of DOC Stock Standard, 10 mL of TN Stock Standard, and 2.5 mL of 2 M HCl into a 100 mL volumetric flask; fill to line with LGW; invert stoppered flask three times
- Store in fridge in amber bottle with PTFE-lined septa/cap. Good for 1 week

Calibration Points should be made fresh for every run

- To make 0.5 mg-C/L Calibration Point, pipette 0.5 mL of DOC Working Solution into a 100-mL volumetric flask; fill to line with LGW; invert stoppered flask three times
- Additional Calibration Points are made in an analogous fashion

Procedure

*The concentrations of the samples need to be less than 10 mg/L as C or N – you should first test a highly diluted sample to make sure you will be in the correct range.

*If you do not plan to analyze your water samples soon after you collect them, adjust to pH 4.5 and store them in the fridge.

*Before you start running samples, you need to talk to person in charge of the TOC/TN about what type of samples you will be running – to make sure they will not compromise the instrument

*Salts in excess of 50 ppm will crystallize out causing the catalyst to be impaired much quicker than normal. If the 50ppm contains a high proportion of chloride, Cl radicals can impact the accuracy of Carbon measurement. Always run a dilute sample with salt below 50 first to see if C is detectable.

1. Prepare calibrations (for example: 0, 0.5, 5, 10 mg C and N/L) and samples (dilute if necessary – concentration needs to be less than 10 mg/L as C or N).

2. Pour your samples and calibrations into acid-washed TOC vials.

3. Acidify all samples and calibrations to pH 2-2.5 using 2 N HCl. A typical surface water requires about 2-4 drops of 2 N HCl if using 24 mL sample vials, but you need to test your actual

sample matrix using a pH meter to be sure you adjust the pH to this value. Cover each vial with aluminum foil.

4. Check the system. Before using the instrument, check a day or two in advance that the pressure in the air tank is above 500psi by opening the regulator attached to the air tank and reading the pressure. Use only ultra-high purity air ("air grade zero").

5. Start the system. On the day of use turn on computer (login Weinberg Lab, password chocolate), turn on TOC analyzer, and open the air tank at the regulator.

Open Software (TOC ControlV)

Sample table

File → New → sample run → TOC-TN 24 mL system (default)

Instrument → connect →Use settings on computer

Check the following on the instrument:

(a) Carrier gas flow = 150 (TOC analyzer); Pressure = 200 (TOC analyzer)

(b) Continuous bubbles in the plastic bottle (TOC analyzer)

(c) N flow ~ 0.5 (Nitrogen unit)

(d) Fill the humidifier tank with laboratory grade water (LGW) of TOC < 0.5ppm water if it is empty or almost empty.

(e) Check the water level in the waste bottle underneath the bench. If nearly full, empty it in an appropriate drain.

Instrument → Background monitor → run and wait for all points to be checked and green (about 20 mins)

6. Create your calibration curve

File → New → Calibration curve → 24mL system (default) → Non Purgeable Organic Carbon Standard, TOC, Linear Regression (uncheck the 'zero shift'), Check 'multiple injections'

Put the number of standards and the range of the concentrations

Adjust the concentrations of each standard and save

Change the injection volume to 100 µL AFTER entering all calibration points

7. Create your sequence

(a) First excel cell → insert autogenerate→ choose your method → put 3-4 blank LGW vials to rinse the system; (b) Run a 5 mg/L as C and N standard after LGWs. (c) Click on next excel cell → insert calib curve TOC → enter the vial #s in the ASI vial view; (d) Next excel cell → insert calib curve TN → enter the vial #s; (e) Next excel cell → insert auto generate → choose your method → enter the number of samples and start vial # (only after the standards) → Enter your sample name in the excel cells→ Save as your sequence

Samples and standards should be arranged as follows:

- LGW blank x3
- 5 mg/L as C and N standards
- LGW blank
- Calibration curve
- LGW blank
- Samples, arranged from lowest to highest expected concentration
- Insert 5 mg/L as C and N standards after every 10-15 samples
- LGW blank
- 5 mg/L as C and N standards

- LGW blank x3

8. Check the system: Recheck the previous signals, if all lights are green,

Maintenance → replace flow line content (cleans the syringe)

Maintenance → Mechanical Check → Vertical Arm Reset

9. Run the sequence

Instrument → Start → Shut down → make sure external acid addition is checked → run

10. The instrument will shut down once sample run is finished, but you need to come in and manually turn off the gas tank at the regulator when run is done.

11. In the notebook by the instrument, record the method and calibration you used next to your name and the date. When your samples have finished running, record the calibration curve information: slope, y-intercept, R^2, and the area counts for the first non-zero calibration point area. Also record the area counts for the 5 mg/L standards at the start and end of your run.

12. After running your samples, remove vials from instrument immediately and clean them. Any vial containing environmental samples (tap water or dirtier) needs to be rinsed and put in the 10% nitric acid bath overnight. Then rinse at least 3x with LGW and dry in 180ºC oven overnight. Any vial containing LGW or standards made up in LGW can be rinsed 3x with LGW and dried in 180ºC oven overnight.

13. Maintenance – All users are expected to contribute their time in maintaining the instrument, troubleshooting problems, and providing resources to replace consumables.

APPENDIX K: PROCEDURE FOR INDUCTIVELY COUPLED PLASMA MASS SPECTROMETER FOR IODINE ANALYSIS IN WATER SAMPLES

Instrument: Agilent Inductively-Coupled Plasma – Mass Spectrometer (ICP-MS) 7500 Series

Start-up

1. Turn on water cooler and exhaust fan.
2. Close out of all software windows.
3. Select the Configuration shortcut. Correct the autosampler set up for the desire rack size. (i.e. correct to Rack 1 60 vials for 15-mL volume samples).
4. Connect the lines on the peristaltic pump, where the waste line is in the inner position, the IS line is in the middle position, and the sample line is in the outer position. Check the direction of flow for each line.
5. Place the internal standard line into the 2% HNO_3 vial near the pump.
6. Take off caps for Bottle 1 (2% HNO_3) and Bottle 5 (5% NH_4OH).
7. Take off caps in Rack 1 positions 02 (tuning solution), 05 (10% NH_4OH), and 07 (Pulse/Analog Factor tuning).
8. Move Automatic Liquid Sampler to position 1. (Automatic Liquid Sampler menu > GoTo > 1)
9. Open the Online Instrument window and turn plasma ON. Wait 15 minutes for instrument to warm up and for the plasma to stabilize. (Make the Sample Sequence now.)
10. Go to the Tune Panel on the instrument window and send the Automatic Liquid Sampler to Rack 1 Position 02 for the tuning solution.
11. On the Tune window, hit the Start button and click the Auto Vert Range box. Observe the signal increasing and stabilizing. Select the Stop button.

12. Go to the Tune menu > AutoTune > Select He mode, Resolution/Axis, Torch Position, Tuning Report > Start.
13. Check the printed tune against passed ion intensities, double-charged percentage, and oxide percentage.
14. Send Automatic Liquid Sampler to Rinse for 30 sec.
15. Send Automatic Liquid Sampler to Rack 1 Position 07 for the Pulse/Analog Factor tune.
16. On the Tune window, press Start to observe the signals to ensure the lines have are flushed. The signals should be higher in the Pulse/Analog tuning solution.
17. For the Pulse/Analog factor tuning, detune the instrument by changing Extract 2 to 90 and the Carrier gas to 0.82. The Pulse/Analog factor helps maintain the linear response between digital and analog signals.
18. Go to the Tune menu > Pulse/Analog factor > Run. When the run is finished, select accept.
19. Restore the Extract 2. Go to the Tune menu > Pulse/Analog factor > Select Merge with results > Run. When the run is finished, select accept.
20. Restore the Carrier gas. Go to the Tune menu > Pulse/Analog > Select Merge with results > Run. When the run is finished, select accept. Check that all ions have a response value.
21. Generate and print the Pulse/Analog Factor report. Be sure to restore all parameters on the tune window.
22. Send the Automatic Liquid Sampler to Rinse for 30 sec then send Automatic Liquid Sampler to Bottle 1.
23. Switch the IS line to the 100ppb Te solution vial, but first wipe off the line from the 2% HNO_3.

24. Load the he.u tune file to run in helium mode.

Sequence

25. Set up the Sequence method, by loading your last sequence.
26. Go to the Sequence menu > Edit Sample Log Table to create the injection sequence.
    a. Note: Each injection is ~2mL depending on number of runs/signal time/modes.
27. Load method “iodide.m”.
28. Save the Sequence as KSYYMDD#.S. For example, KS16D141.S was the first (#) sequence created on April (month D) 14th, 2016.
29. Do not change the Source Batch Directory from <u>your</u> old sequence that ran the same method. This directory retrieves information from the previous sequence.

Shutdown

30. Send the Automatic Liquid Sampler to Rinse for 30 sec.
31. Send the Automatic Liquid Sampler to Bottle 1 for 5 min to rinse the system.
32. Move the internal standard line to 2% HNO_3 to rinse the line.
33. Turn off the plasma.
34. When the cooling water flow is at zero, turn off the chiller and the exhaust fan.

APPENDIX L: DIONEX ION CHROMATOGRAPH STANDARD OPERATING PROCEDURES FOR ANION ANALYSIS

Prepared by Ryan Kingsbury, updated by Kirsten Studer

Initial Startup:

1. Fill all eluent bottles and the regenerant bottle with the appropriate solutions.
2. Turn on pressure at the helium tank
3. Turn the eluent degas module ON. Set all bottles to sparge and turn each individual bottle to ON. Loosen the cap on each bottle.
4. Verify that gas is flowing out of each sparge line that is turned on. Connect the sparge lines to the bottles. Allow the eluents to sparge for 20 minutes
5. While the eluents are sparging, take out the suppressor and remove the caps on all four ports
6. Hydrate the suppressor membranes by using a syringe and the luer-lok adapter to push ~5 mL LGW through the REGEN IN port and ~3 mL through the ELUENT OUT port. Be careful not to push through the ELUENT OUT port too fast or you may damage the membrane
7. Uncap the ends of the regenerant and eluent lines in the sink
8. Install the suppressor in the cabinet and connect the REGEN OUT port of the suppressor to the appropriate line
9. Connect the column and guard column to the injection assembly
10. When sparging is complete, remove the sparge lines. Tighten the caps on the bottles and switch them to PRESSURIZE. Adjust the regulator on the degas module to 7 +/- 2 psi
11. Turn the pump ON

12. Prime the pump. For each eluent bottle, set the flow to 100% and 1.0 mL/min. Turn the silver bar on the pump perpendicular to the pump face and attach a 3 mL syringe. Press START and draw about 3 mL from the port into the syringe. Discard. Repeat two more times or until no air bubbles are seen. On the third time, loosen the black knob and push the syringe contents back into the pump while tapping on the clear tube to remove any air bubbles. Re-tighten the knob.
13. Begin pumping eluent through the system at 1.0 mL/min. As soon as you see eluent dripping out of the column line, connect it to the ELUENT IN port of the suppressor.
14. When you see eluent emerge from the ELUENT OUT port of the suppressor, connect it to the detector.
15. Tighten the cap and the gas line connection on the regenerant bottle. Carefully turn on pressure to the regenerant bottle at the regulator, watching to see when regenerant begins to flow in the line. When regenerant begins to flow, connect the line to the REGEN IN port of the suppressor.
16. Adjust the pressure until the desired regenerant flow rate is achieved (measure flow out of the REGEN OUT line in the sink with a graduated cylinder and a watch). Consult the suppressor manual for optimal regenerant flow rates for each eluent strength.
17. Turn the ACI and Detector ON. Turn the cell OFF.
18. Allow the system to equilibrate for 30 minutes
19. Turn the cell ON. Record the baseline conductivity and the pump back-pressure in the log book.
20. Run samples.

Short Term (Daily operation) Shutdown:

1. Flush the system with LGW at 1.0 mL/min for 10 minutes.
2. Turn the cell OFF. Turn the detector OFF.
3. STOP and turn off the pump.
4. Turn off pressure to the regenerant bottle at the regulator. Loosen the regenerant bottle cap to relieve the pressure. Re-tighten the cap.
5. Cap the ends of the eluent and regenerant lines in the sink to keep them from drying out.
6. Leave the Eluent Degas Module ON with pressure to the eluent and LGW bottles.

Short Term (Daily operation) Startup:

1. Uncap the ends of the regenerant and eluent lines in the sink.
2. Turn on pressure to the regenerant bottle at the regulator. Adjust until the desired regenerant flow rate is achieved (measure flow with a graduated cylinder in the sink).
3. Turn the pump ON.
4. Prime the pump. For each eluent bottle, set the flow to 100% and 1.0 mL/min. Turn the silver bar on the pump perpendicular to the pump face and attach a 3 mL syringe. Press START and draw about 3 mL from the port into the syringe. Discard. Repeat two more times or until no air bubbles are seen. Re-tighten the knob.
5. Begin pumping eluent through the system at 1.0 mL/min.
6. Turn the ACI and Detector ON. Turn the cell OFF.
7. Allow the system to equilibrate for 30 minutes. Turn the cell ON. Record the baseline conductivity and the pump back-pressure in the log book.
8. Run samples.

Long Term (> 1 week) Shutdown:

1. Flush the system with LGW at 1.0 mL/min for 10 minutes
2. Turn the cell OFF. Turn the detector OFF. STOP the pump.
3. Turn off pressure to the regenerant bottle at the regulator. Loosen the regenerant bottle cap to relieve the pressure.
4. Remove the suppressor. Cap both ends of the regenerant out line. Cap the ELUENT IN and ELUENT OUT ports with the original plugs.
5. Using a disposable syringe and the luer-lock adapter in the drawer, push 5-6 mL of LGW through the REGEN IN port on the suppressor.
6. Cap the REGEN IN and REGEN OUT ports with the original plugs.
7. Connect the column directly to the detector. Flush the system with operating eluent for 10 minutes.
8. Remove the column and guard column. Cap the ends with the original caps and place in their respective boxes. Be careful not to tap, drop, or otherwise shock the columns as this will disturb the packing.
9. Connect the detector directly to the injection assembly. Flush the system with LGW at 9.9 mL/min for 10 minutes.
10. STOP and turn OFF the pump.
11. On the eluent degas module, switch all bottles to SPARGE. Loosen the caps to relieve the pressure. Switch the entire module OFF. Switch each bottle OFF. Switch all bottles to PRESSURIZE. Turn off the gas supply.

APPENDIX M: ANALYSIS OF TOTAL ORGANIC HALOGEN (TOX) IN DRINKING WATER

Instrumentation

1) Adsorption Module (Tekmar-Dohrmann)

   Model: AD-2000 Adsorption Module, Model n^{o}: 890-161, Serial n^{o}: 99292009

   For 100mL sample size: Range: 4-1000 μg AOX/L; Precision: ±2 μg/L or ±2%

2) Organic Halide Analyzer

   Model: DX-2000 Organic Halide Analyzer, Model n^{o}: 890-162, Serial n^{o}: 99292009

   For 100 mL sample size: Range: 4-1000 μg TOX/L; Precision: ±2 μg/L or ±2%

   For 10 mL sample size: Range: 40-10,000 μg TOX/L; Precision: ±20 μg/L or ±2%

3) Software

   AOX/TOX by column – Copyright 1993-1996 Rosemount Dohrmann Div. – Version 2.10

The process of the system operates in such a way that it meets international methods including:

- EPA Methods 9020A, 9076, 450.1, and 1650, ASTM-D-4744, Standard Methods 5320B, DIN 38409-H14, ISO Method 9562, SCAN-W Method 9:89, and NEN Method 6402

Reagents

- Lab grade water (LGW, deionized water)
- 70% by wt. Acetic Acid (Glacial, Fisher Scientific) in LGW
- 80% H_2SO_4 (ACS Plus Grade, Fisher Scientific) in LGW
- Concentrated H_2SO_4 (ACS Plus Grade, Fisher Scientific)
- Silver acetate (≥99% purity, Sigma-Aldrich)

- 40 mg/mL Na_2SO_3 (anhydrous, ACS Grade, Fisher Scientific) in LGW
- 5,000 mg NO_3^-/L of KNO_3 (ACS Grade, Fisher Scientific) in LGW
- 200 ng Cl/μL of NaCl (ACS Grade, Fisher Scientific) in LGW
- 500 ng Cl/μL 2,4,6-Trichlorophenol (98%, Aldrich) in high purity methanol
- Sodium bicarbonate (Industrial grade, Fisher Scientific)
- Methanol (halogen free, highest purity, LCMS Grade, Fisher Scientific)
- Dry GAC (100-200 mesh GAC, <1.0 μg/40 mg, Rosemount Dohrmann)
- Glass-packed Carbon Columns 2mm ID (CPI International)
- Ultra high purity Helium, 220ft^3
- Oxygen, 99% purity, 220ft^3

Sample Collection & Dechlorination

Samples for TOX analysis should be collected in 12-mL or-250 mL amber bottles with open-top PTFE-lined septa. To quench about 3 mg/L of free chlorine, 40 μL of a 40 mg/mL solution of sodium sulfite (Na_2SO_3) should be added to the sample bottles prior to collecting the samples. Before collecting samples, the sample tap should be opened and allowed to run to waste for 2-3 minutes. The flow should then be reduced, the bottle placed at a slant and the water allowed to run down the side. When the bottle is almost full, cap the bottle with the Teflon side of the liner facing inwards. Invert the bottle to mix and then open the cap and completely fill so that no air bubbles remain. Invert to confirm absence of air. After collection, samples should be kept in a refrigerator at 4^oC until analysis, which should take place within 14 days of collection.

Sample Pre-Treatment

Before the analysis, allow sample bottles to achieve room temperature. Then, add 25 drops of concentrated sulfuric acid (A.C.S. Plus) to the 125-mL samples, or 50 drops to 250-mL bottles, with a glass Pasteur pipette.

Sample Preparation – Adsorption

1. The sample volume, adsorption rate of sample to the carbon columns, channel fill rate, and use of sample prime can be adjusted in the control panel using the arrow keys after selecting the channel in use and pressing the keys "SAMPLE" and "MENU."

2. Before sample adsorption, make sure that the previous user rinsed the sample channels with LGW. Program used for sample channels (1-4): Sample volume: 100 mL (can also use 50, 25 or 10-mL volume, depending on range of TOX expected); Adsorption rate: 2 mL/min; Fill rate: Slow (33 mL/min); Sample prime: NO; Priming volume: 0 mL

3. To load the samples in the channels, choose one of the channels (1-4) keys, press the "START/STOP" key, connect the sample to the channel using one of the fill tubes (Figure 1a) and press "OK." After the desired volume of sample is in the channel, the screen will display the message "Connect columns (then press OK)."

4. Disconnect the fill tube, carefully pierce the endcaps of two glass carbon columns (if holes are too big, carbon will come out during adsorption), connect the columns in series using a connector, and press the "OK" key. The aqueous sample is then passed through the two carbon columns, a top column and a bottom column for breakthrough, connected in series at a flow rate that permits complete adsorption of the organic halogens. The sample will drip out at the end of

the bottom carbon column. Collect this waste in a beaker. Water samples may be discarded in the drain.

5. To remove inorganic chloride ions, the samples must be rinsed with a nitrate wash. Connect the top column to the nitrate wash channel and wash the column with 2 mL of the nitrate wash solution (5,000 mg NO_3^-/L KNO_3) at a rate of 1 mL/min. Do the same for the bottom column. If the sample columns are not ready to be analyzed on the DX-2000 Organic Halide Analyzer, cover the ends of the columns with aluminum foil again and store in a beaker covered with foil. For sample columns that are ready to be analyzed, transfer them to the DX-2000 Organic Halide Analyzer.

DX-2000 Organic Halide Analyzer Instrument Preparation

1. Before using the DX-2000 Organic Halide Analyzer module for sample analysis, make sure that the gas supplies, oxygen for combustion and helium as carrier gas, are above 500 psi. Change gas tanks when pressure of the gas tanks reaches 500 psi.

2. When the instrument is not in use, the scrubber should always be disconnected from the combustion tube. To change the acid in the scrubber vial, pour the old acid into an acid waste container. Add fresh 80% H_2SO_4 to the marked line on the scrubber vial. This acid should be changed every day of TOX use and may need to be changed again during the day if the TOX is used for more than 6-7 hours at a time.

3. Change the sodium bicarbonate in the spill tray after making sure that the acid is neutralized. If acid is still present in the tray, use more sodium bicarbonate to neutralize it, and then empty the tray into aluminum foil and empty the contents in the garbage. Before throwing it

in the garbage, be sure that no acetic acid smell can be detected. Keep it in the hood until this condition is met.

4. After rinsing the tray with tap water and drying it, fill it to about 1/3 with sodium bicarbonate to neutralize cell electrolyte. Empty the acid fume trap contents in the sink, rinse it with LGW and add sodium bicarbonate to about 1/4 full and LGW to about 1/2 full.

5. Change acid in the cell by removing the cell fill plugs, opening the stopcock valve to drain the acid from the cell to the spill tray, closing the valve, and filling the cell to the cell neck with fresh 70% acetic acid. Make sure that the reference electrode has no bubbles in it. Do not remove the metal reference electrode from the reference electrode assembly. Problems in obtaining a stable baseline are likely to occur if the metal reference electrode is moved as it connects to the silver acetate reagent.

6. To replace silver acetate solution in the top of the reference electrode, first prepare a slurry of solid silver acetate in 70% acetic acid. Use glass stir rod to stir slurry. Empty out old silver acetate/acetic acid mixture from reference electrode using a Pasteur pipet (can use a kimwipe to break off tip of pipet for easier use) and rinse bottle of acetic acid. Be very careful not to get any silver acetate into bottom compartment of reference electrode. This will require rinsing and refilling the entire electrode. Hold a kimwipe around top of electrode so nothing drips down. Use another broken-tip Pasteur pipet to add slurry to now-empty top compartment of reference electrode. Allow to settle for a few minutes and fill to top with 70% acetic acid, making sure there are no bubbles. The silver acetate should be about 2/3 of the top compartment, with 70% acetic acid filling the rest. Cover with aluminum foil while slurry is settling. Reconnect reference electrode to cell.

DX-2000 Organic Halide Analyzer Settings

To begin sample analysis in the computer program, choose the icon "AOX/TOX by Column" and click "OK."

1. Gas & Temperature Settings

In the "System Setup" menu bar click "Open" and turn the system to "Standby." Slowly increase temperature in 50^{o}C increments to 550^{o}C. Make sure to press tab or click in another box each time you increase the temperature. Once system reaches 550^{o}C, make sure temperature in "Ready" box is set at 550^{o}C. Open both gas tank main valves (keep fly valves shut for now). Turn system to "Ready." Slowly open the fly valves of both gas tanks. You should see vigorous bubbling in the scrubber vial and cell. Make sure the injection port hatch door is completely closed. On the front panel of the TOX, check that the gas gauge for oxygen is around 50 and helium is around 25. Adjust the flow up and down by turning the knobs on gauges, if necessary.

Increase the furnace temperature to 850^{o}C from its standby temperature of 550^{o}C in increments of 50^{o}C. When the temperature reaches the desired ready mode temperature, an orange light in the temperature control panel indicator will appear in the "Ready" light.

2. Baseline Monitor

Select in the "System Checks" menu the "baseline monitor" option and wait for about 15 minutes until the baseline is stable. The voltage reading should be higher around 250 V. If the voltage reading is lower than 250 V, flush the cell with fresh 70% acetic acid, or inject 5 µL of the 200 ng Cl/µL NaCl solution until the desired voltage value is obtained.

In the “System Setup” menu bar click “Open” and turn the cell to the “ON” position. In the “System Checks” menu, choose again the “Baseline Monitor” option and wait until the baseline is stable. If you wish to see the current instead of the voltage reading, select in the “Options” menu the “Graph Mode” option of your choice. Both current and voltage will always be displayed in the bottom of the computer screen.

3. Cell Check

To verify that the cell is working properly, inject 5 µL of a 200ng Cl/µL NaCl solution and check the recovery obtained. In the “System Checks” menu choose the “Cell Check” option and fill the “Run Info” menu with the information of the solution injected. Before pressing the “Start” key have the syringe ready for the injection. Press “Start,” wait for a message saying, “Inject to cell then press OK,” remove the white cell fill plug from main cell cap, inject the desired volume into the titration cell and click “OK.” If the resulting percentage recovery is between 90-110%, then the cell is working properly. Perform this check three times for consistent results.

4. Clean Boat

Before analyzing the samples, the boat has to be cleaned. Select the “Clean Boat” option in the “System Checks” menu.

5. Combustion Check

To verify the furnace performance, inject 2 µL of a 500 ng/µL solution of 2, 4, 6-trichlorophenol into the boat on top of a scoop of dry GAC (or contents of a carbon column) and check the recovery obtained. Run a blank carbon to obtain a background level that you will subtract from the trichlorophenol spike value.

Carefully place a scoop of dry GAC in the boat and close the lid tightly. In the "System Checks" menu choose the "Combustion Check" option, fill the "Run Info" menu with the information of the solution injected. Before pressing the "Start" key have the syringe ready for the injection. Press "Start", wait for a message saying, "Inject to boat then press OK", carefully inject the volume through the lid septum and press "OK." Make sure that the furnace is completely pyrolyzing the carbon: fresh carbon is black, while pyrolized carbon is a light orange color. If the carbon is not completely pyrolizing, check for gas leaks. The lid above the boat should be sealed tightly. 90-110% recovery of the trichlorophenol solution indicates good recovery. Perform this check three times for consistent results. Remove the pyrolized carbon from the boat using a vacuum tube attached to a trap. Perform this check during analysis of samples to verify the furnace's performance.

6. Sample Analysis

Remove the plastic endcaps from the sample column. Open the injection port lid and use the T-shaped ejector tool to inject the sample-adsorbed carbon into the glass boat. Be careful to not touch the boat with the tool, as the boat is extremely fragile. Close the lid and make sure that the seal is tight by checking the bubbling in the titration cell and the scrubber vial.

Under the "Run" menu, select "Manual Run." Select the type of sample (blank, sample, standard). Next select common run parameters: print results, enter comments, sample ID name, and enter adsorption volume. Select the column parameters for the type of sample (sample, blank, standard) to be analyzed: top/bottom column, blank value, dilution factor, standard concentration if the sample is a standard, nitrate if the sample is

a nitrate blank. Verify that the information is correct. Click "OK" to save and "Start Run" when ready to run the analysis. A graph of voltage (or amperes) vs. time in seconds will appear on the computer screen during the analysis. When the sample has finished undergoing combustion, the computer will output a raw TOX value in "µg Cl." Vacuum the boat once analysis is over. Repeat the procedure for other samples.

To ensure the validity of the data collected, it is extremely important to perform cell checks and combustion checks before analysis and sporadically during the sample analysis process. If the recoveries obtained range between 90-110% the system is being effective in the determination of the total organic halide content of the samples.

7. Shutdown

First check that there are no runs in progress. Next, clean the boat as necessary. Turn off the cell. Set the system into Standby mode and set temperature to 35°C. Close the fly valves and main valves of the gas tanks. Let system reach 35°C – this will take several hours. Shut down software and turn off TOX power.