Characterization of Unregulated Disinfection By-Products (DBPs) in Chloraminated Water and Estimation of Associated Health Risks

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Abstract

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Water disinfectants are added to inactivate microorganisms during the drinking water treatment process. But they also have the potential to react with natural organic matter (NOM) and form disinfection by-products (DBPs) that could be both cytotoxic and genotoxic. Four species of Trihalomethanes (THM_4) and five species of haloacetic acids (HAA_5) are the only two halogenated organic DBP classes regulated by US Environmental Protection Agency (USEPA). Chloramine is used more and more widely in drinking water utilities as a secondary disinfectant since it can significantly reduce the formation of regulated DBPs. However, chloramination could produce more unregulated DBPs formation than chlorination according to some previous studies. To make it worse, many unregulated DBPs, which generally occur at very low concentration levels in drinking water, are proven to be more toxic than regulated DBPs by many orders of magnitude. Therefore, the investigation of unregulated DBP formation in chloraminated water samples and the associated health risks are warranted. In this study, a liquid-liquid-extraction/gas chromatography-electron capture detector (LLE/GC-ECD) method for haloacetamides (HAMs) was revised and validated. The method showed good accuracy and

precision for 7 HAM species, but not for monochloroacetamide and monobromoacetamide. Together with haloacetamides, a total of 49 DBPs were measured in three batches of chloraminated reverse osmosis concentrates with increasing chlorine contact times. Results showed that samples treated with preformed monochloramine were associated with the least DBP formation for the majority of DBPs measured. Samples with a longer free chlorine contact time had increasing DBP formation. A reproductive and developmental health risk analysis was also performed in the study using the USEPA relative potency factor (RPF) approach. The illustrative health risk analysis was conducted on a subset of DBPs, the 17 DBPs with validated no-observed-adverse-effects-levels (NOAELs) obtained from animal studies. The estimated health risks associated with these 17 DBPs were highest in the chloraminated samples with longest chlorine contact time. The results of the study could provide helpful information for water utilities about DBPs formation in chloraminated water samples and the choice of chloramination options.

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Any opinions, findings, and conclusions expressed in this thesis are those of the author and do not necessarily reflect the views of the U.S. Environmental Protection Agency.

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Lists of Abbreviations

BCAA	Bromochloroacetic acid
BCAM	Bromochloroacetamide
BDCAA	Bromodichloroacetic acid
BDCAM	Bromodichloroacetamide
BDCM	Bromodichloromethane
BIAA	Bromoiodoacetic acid
BMD	Benchmark dose
CIAA	Chloroacetic acid
DBP	Disinfection by-product
DBAA	Dibromoacetic acid
DBAM	Dibromoacetamide
DBCAA	Dibromochloroacetic acid
DBCAM	Dibromochloroacetamide
DCAA	Dichloroacetic acid
DCAM	Dichloroacetamide
DIAA	Diiodoacetic acid
DBCM	Dibromochloromethane
ECD	Electron capture detector
GC	Gas Chromatography
HA	Haloacetaldehyde
HAAs	Haloacetic acids
HAMs	Haloacetamides

HKs	Haloketones
HNM	Halonitromethane
ICED	Index chemical equivalent dose
LLE	Liquid to liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MBAA	Monobromoacetic acid
MCAA	Monochloroacetic acid
MBAM	Monobromoacetamide
MCAM	Monochloroacetamide
MDL	Method detection limit
MTBE	Methyl tert-butyl ether
MS	Mass spectrometry
NOAEL	No observed adverse effect level
PQL	Practical quantification limit
RO	Reverse Osmosis
RPF	Relative potency factor
SPE	Solid phase extraction
TBAA	Tribromoacetic acid
TBAM	Tribromoacetamide
TCAA	Trichloroacetic acid
TCAM	Trichloroacetamide
TBM	Bromoform

TCM	Chloroform
THMs	Trihalomethanes

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1. Introduction

1.1 Disinfection by-products and DBP regulation

Disinfectants are added to water to reduce or prevent illnesses due to consumption of drinking water. There are two types of disinfection. Primary disinfection inactivates harmful microorganism in drinking water while secondary disinfection aims at long-lasting treatment while the water is transported to consumers via water distribution systems [1]. Choice of primary disinfectant varies from chlorine to chlorine dioxide, ozone, and UV. The most common secondary disinfectant in the USA is chlorine (Cl_2) while chloramine (NH_2Cl) is also widely used in water utilities [2]. During water disinfection, natural organic matter (NOM) in drinking water can react with chlorine/chloramine and form halogenated disinfection by-products (DBPs) that are potentially harmful to human health.

In 1976, Rook reported that trihalomethanes (THMs) could be produced from reactions of chlorine with NOM during disinfection of water [3]. There are four species of THMs, including chloroform (trichloromethane, TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform (tribromomethane, TBM). Rook's discovery of THMs in drinking water inspired research on other chemicals produced when disinfectant is added to water. The health effects of these chemical by-products were also investigated. Studies have found that people with high exposure to DBPs may have a higher risk of cancer [4]. Currently, the World Health Organization International Agency for Research on Cancer (IARC) lists chloroform and bromodichloromethane as possible human carcinogens [5]. Consumption of DBPs may also result in reproductive and developmental effects, such as low birth weight [6].

Four species of THMs (THM4) and five species of HAAs (HAA5) are regulated in the current Stage 2 Disinfectants/Disinfection by-products rule (D/DBPR) by USEPA [7].

Concentrations of THM4 and HAA5 cannot exceed 80 µg/L and 60 µg/L, respectively. Typically, THMs and HAAs are present at higher concentration levels than unregulated DBPs [8]. Many unregulated DBPs are present at low levels in drinking water and are more difficult to quantify. Therefore THM4 and HAA5 are used as indicators of total DBP occurrence.

1.2 Chloramination and unregulated DBPs

Since 1976, more than 600 DBPs have been reported, but only a few classes of them have been quantitatively assessed for their occurrence and health effects [9]. A few of these emerging DBPs include haloacetamides (HAMs), haloketones (HKs), halonitromethanes (HNMs), haloaldehydes (HAs), and nitrosodimethylamines (NDMAs) [10, 11]. Since there are so many DBPs species present in drinking water, and they have various toxicological pathways, it is even harder for researchers to assess the health risks for DBPs mixtures as a whole.

Many utilities are switching from chlorine to chloramine since scientific studies show that chloramination forms less regulated DBPs, including THMs and HAAs, compared to chlorination [2]. However, it is also proven that chloramination can increase formation of nitrogen-containing DBPs and iodo-DBPs [2, 8, 12, 13]. The use of chloramine may also be associated with lead contamination of drinking water, such as in Washington DC starting from 2001 [14]. Edwards and Dudi (2004) point out that chloramine-treated water can pick up lead from pipes and solders and carry it along the way in distribution systems, which could result in higher human exposure at the tap [15].

In the real-life application of chloramine disinfection, either preformed chloramine could be added to water directly or ammonia could be added following the addition of chlorine in the water treatment train. When ammonia is added to water after chlorine, the length of free chlorine contact time with drinking water could make a difference in DBP formation and speciation.

1.3 EPA 4Lab study for DBP mixtures

The integrated disinfection byproducts mixtures research project (known as the 4Lab study) was initiated by USEPA [16]. The aim of this study is to evaluate the chemistry and toxicology of a mixture of DBPs, one that represents the compound distribution in typical disinfected drinking waters. The challenge was to simulate drinking water DBP levels and speciation relevant to human exposure, and to scale them for application in an animal exposure study. Reverse osmosis (RO) membrane filtration is used to concentrate NOM from a source water because, compared to XAD resin extraction, RO can concentrate large quantities of water more quickly and efficiently (higher recovery of NOM) [17]. The 4Lab study initially tested the use of RO membranes for concentrating chlorinated water, but found that volatile DBPs were lost during the membrane-concentration procedure [18]. The best approach to produce drinking water concentrate was then identified as concentrating NOM with RO membranes first, and then chlorinating the RO concentrate [17]. Additional bromide and iodide were spiked after RO concentration in order to produce brominated and iodinated species of DBPs. In this way, RO concentrates have much higher NOM concentration than typical raw water and have the potential for much higher formation of unregulated DBPs following disinfection. These conditions make the detection and quantification of unregulated DBPs much more feasible. In the 4Lab animal study, disinfected RO concentrates were placed in rat cages and made available for them to drink for 10 days. DBP concentrations of disinfected RO concentrates were sampled multiple times during the time course of the study to evaluate the stability of DBPs and to accurately assess exposure [19].

1.4 Haloacetamides and the current detection methods

Haloacetamides (HAMs), a group of nitrogen-containing DBPs, were first observed and quantified in drinking water in a 2000-2002 nationwide occurrence study in USA [10]. Among the five species being reported (MCAM, DCAM, TCAM, MBAM, and DBAM), DCAM was observed at higher concentration levels compared to other species, with a maximum concentration of 5.6 µg/L in a finished water plant effluent sample. Six additional species of HAMs (BCAM, BDCAM, DBCAM, TBAM, CIAM, and BIAM) were first reported in a 2012 drinking water study in China [20]. Haloacetamides were also observed in European countries[21]. In a 2015 Greek study, DCAM was reported in a range of 0.28-2.8 µg /L observed in Athens drinking water. That study also reported MCAM was detected at a maximum value of 6.1 µg /L while the average level was below the detection limit.

Haloacetamides were first regarded as hydrolysis products of haloacetonitriles [22]. Amino acids and humic acids were found to be important precursors of haloacetonitriles and haloacetamides [23, 24]. Evidence in a 2012 study suggests that there are alternative formation pathways of haloacetamides without haloacetonitriles in chloraminated water [24].

Plewa and his colleagues tested 13 species of chlorinated, brominated and iodinated haloacetamides in Chinese hamster ovary cells to evaluate *in vitro* toxicity. All 13 species were found to be cytotoxic, and all species but DCAM were found to be genotoxic [25]. As a class, the 13 haloacetamides were 99 times more cytotoxic and 19 times more genotoxic than 13 HAAs. The haloacetamides were proven to be slightly more cytotoxic and genotoxic than HNMs.

Haloacetamides can hydrolyze to form the corresponding HAAs [22]. DCAM and TCAM were found to degrade rapidly in alkaline conditions [26]. DCAM can also hydrolyze under strong acidic conditions (pH=4) [26]. Liew and colleagues investigated DCAM, TCAM, and

DBAM's stability during the 14-day test in 2012 [27]. No significant difference was observed for pH 4 to 8. All three HAM species also showed comparable stability under three preservatives scenarios (no preservatives, ascorbic acid, and ammonium chloride).

EPA has published standard methods for measurement of THMs and HAAs [28, 29], while currently there is no agreement on the method to measure HAMs. In the past 10 years, a number of detection methods for haloacetamides have been published (Table 1.1). Liquid-liquid extraction (LLE) was used more commonly than solid phase extraction (SPE) to isolate the compounds from the sample matrix, but SPE can achieve lower method detection limits due to the much higher concentration factor. Most researchers utilized gas chromatography (GC) to separate haloacetamides chromatographically, while high performance liquid chromatography (LC) was also used for better separation. The electron capture detector for GC is simple and inexpensive to operate, while the use of more expensive mass spectrometry (MS) can significantly improve the method detection limits.

Table 1.1 Summary of analytical methods for haloacetamides

Author, Year	HAM species evaluated	Analysis Method	Method Detection Limits
Weinberg *et al.* 2002 [10]	5 species, including BAM, DBAM, CAM, DCAM, and TCAM	LLE/GC-ECD	PQL: 0.1 µg /L for all 5 species
Plewa *et al.* 2007 [30]	13 species, including all chlorinated, brominated and iodinated	LLE/GC-MS (EI)	N.A.
Chu *et al.* 2009 [26]	DCAM and TCAM	LLE/GC-MS (EI)	N.A.
Liew *et al.* 2012 [27]	5 species, including MBAM, DBAM, MCAM, DCAM, and TCAM	LLE/GC-MS (EI)	MDL: 1 µg /L for MBAM 2 µg /L for MCAM 0.08-0.1 µg /L for DBAM, DCAM, TCAM
Chu *et al.* 2012 [20]	A total of 13 species, including chlorinated, brominated and iodinated	SPE-LC/tqMS	LOD: 0.02-0.05 µg /L
Huang *et al.* 2012 [24]	DCAM	LLE/GC-MS (CI)	N.A.
Samios *et al.* 2015 [21]	DCAM and MCAM	LLE/GC-ECD	LOD: 0.1 µg /L for DCAM 4 µg /L for MCAM. LOQ: 0.3 µg /L for DCAM 12 µg /L for MCAM

CI: chemical ionization. EI: electron ionization.
MDL: method detection limit. LOD: limit of detection. PQL: practical quantification limit. LOQ: limit of quantification.

1.5 Health risk assessment

People have become more aware of the presence of harmful chemicals in the environment, and more apt to question their health impact. What types of health problems could be caused by chemical exposures? Is there a level below which some chemicals will not cause negative health effects? What chemical exposures present the greatest risks? Health risk assessment is a multidisciplinary scientific approach to provide answers to these questions [31]. It can help people to understand how concerned they should be with regard to potential hazards. It can guide policymakers to determine which hazards are the most significant, and to establish the appropriate and feasible regulation limits [32]. Health risk assessment usually involves four steps: hazard identification, exposure assessment, dose-response assessment, and risk characterization [33].

Hazard identification is the process by which scientists determine the specific health problems caused by a chemical. It is based on the review of human or laboratory animal exposure studies conducted with the chemical. Because the health effects of many chemicals have not been studied in humans, it is much more common for scientists to rely on animal studies to judge a chemical's potential human health effect [32].

In the step of exposure assessment, scientists need to determine the exposure route (oral, inhalation and dermal) and duration (short-term and long-term) first, and then estimate the amount of the chemical to which humans or animals are exposed [33]. Breathing rates, water consumption, and daily food intake are examples of important factors that could be used to estimate the exposure [32].

The next step is dose-response assessment. Scientists will further review the information gathered from the hazard identification step to estimate how health effects could be impacted by

different levels of exposure to a chemical. Generally, the dose-relationship for cancer effects is different from non-cancer effects[32]. It is a common assumption that cancer risks are linear in the low-dose range, and the risks can arise from any level of exposure. That is to say, no exposure to a cancer-causing chemical is considered as "zero risk". However, health risks associated with non-cancer causing chemicals could be minimal or negligible if the exposure is below a certain threshold limit [34]. No-observed-adverse-effect-level (NOAEL) or lowest-observed-adverse-effect-level (LOAEL) is used to estimate the safe limit, and can be evaluated based on animal studies of chemicals causing non-cancer health effects [31]. The determination of NOAELS and LOAELs depends highly on the study design and the experimental dose spacing.

Risk characterization is the last step, which takes all information obtained from previous steps to estimate the risk level of health effects in an exposed human or animal population. The risk of cancer, which is caused by long-term exposure, is usually expressed as the number of new cases of cancer that could occur in a million people due to exposure to the cancer-causing chemical over a 70-year lifetime [32, 33]. Non-cancer health risk, on the other hand, can be determined by comparing the actual level of exposure to the reference level obtained during the step of dose-response assessment [32]. If the human exposure exceeds the reference level, it is considered to be a human health risk related to the chemical, and a further investigation or actions should be recommended. It should be pointed out that many reference levels are determined based on the NOAEL from laboratory animal studies. When scientists are using NOAELs derived from animal studies to assess human health risks, a safety factor (usually 100 to 10,000 times) should be used to generate a much lower health reference level [32]. The safety

factor is used to adjust possible differences in health effects between laboratory animals and humans, and to ensure that real health risks are not underestimated.

In addition to the reference level approach, recently the benchmark dose (BMD) approach has become the preferred method within USEPA. BMD, calculated from USEPA Benchmark Dose Software, is defined as the central estimate of the dose that leads to a predetermined change in response rates of an adverse health effect [35]. BMD is supposed to be less affected by experimental dose spacing and can appropriately account for uncertainty and variability in the experimental results.

1.6 DBP health risk assessment and USEPA Relative Potency Factor (RPF) method

Health risk assessment of DBPs can provide evidence for the establishment of USEPA DBP regulation rules. There are many challenges remaining for health risk assessment of DBPs. First, for quantitative risk assessment, a dose response curve developed from *in vivo* (animal) studies is necessary. However, most toxicological studies of DBPs are *in vitro* (cell or test tube) studies, while fewer *in vivo* (animal) studies are available. This significantly limits the amount of data available for health risk assessment. In addition, it is even harder for researchers to assess the health risks for DBP mixtures as a whole since there are so many DBP species in drinking water, and they have various toxicological pathways.

Rice et al. (2008) at USEPA used the EPA Relative Potency Factor (RPF) approach to estimate reproductive and developmental risks posed by DBP mixtures for the 4Lab study [16]. The DBP mixture that they tested was produced by concentrating finished water samples by RO membranes and spiking back some volatile DBPs after concentrating.

$$RPF_i = \frac{Toxicity\ potency(index\ chemical)}{Toxicity\ potency(the\ ith\ chemical)} \qquad \text{Equation 1.1}$$

As mentioned in Equation 1.1, RPF is defined to be the ratio of toxicity potency of the index chemical to that of the ith chemical in the mixture (i=1,…,n). The no-observed-adverse-effect level (NOAEL) is commonly used to indict chemicals' toxicity potency.

$$P_{Effect} = f_1 \sum_{i=1}^{n} (RPF_i \times D_i) \qquad \text{Equation 1.2}$$

P_{Effect} , the possibility of effect presented by the chemical mixture, could be determined as illustrated in Equation 2. f_1 stands for the dose-response function of the index chemical 1. D_i is the dose of the ith mixture component (i=1,…,n).

Rice and his colleagues chose bromodichloromethane (BDCM) as the index chemical. The dose-response curve of BDCM was developed using the USEPA benchmark dose software [35]. With The RPFs of all other DBPs are calculated based on ratios of the no-observed-adverse-effect level (NOAEL) for BDCM to the NOAELs for other DBPs. Index chemical equivalent dose is calculated as the sum of products of each DBP concentration multiplied exposure and the corresponding RPF. Finally, the probability of effect is determined based on BDCM's dose response curve and BDCM equivalent dose. In this way, they estimated that the health risks of rats posed by chlorinated RO concentrate (7×10^{-5}) was about two fold higher than the risks posed by RO concentrates with ozonation/post-chlorination (3×10^{-5}). The result suggested that pre-ozonation might reduce health effects resulting from drinking water disinfection with chlorine.

This RPF approach is based on two assumptions. First, the toxicity mode of action (MOA) of the index chemical is similar to the MOA for all other DBPs. Second, the dose-response function for the index chemical is similar to those of all other DBPs. Clearly, these two assumptions are uncertain. Therefore, the results from RPF approach cannot be considered as a definitive risk assessment, while it can still provide some insights.

1.7 Specific aims

The first aim of the study is to revise and validate a LLE/GC-ECD method for measurement of haloacetamides (HAMs) evaluation in drinking water. The hypothesis is that we can meet the required accuracy and precision for determination of all haloacetamides.

The second aim of the study is to measure targeted unregulated DBPs concentrations in reverse osmosis (RO) concentrates for three chloramination treatment options.

The last aim of the study is to estimate developmental and reproductive health risks associated with 17 DBPs formed in the treated RO concentrates by using the US EPA RPF approach.

2. Methods and Materials

2.1 Liquid-liquid extraction/gas chromatography-electron captor detector (LLE/GC-ECD) method for haloacetamides

2.1.1 Reagents and Chemicals

Pure compounds of nine haloacetamides as listed in Table 2.1 were obtained from commercial companies. Monochloroacetamide, and monobromoacetamide were purchased from Sigma-Aldrich (St. Louis, MO). Dichloroacetamide and trichloroacetamide were obtained from Alfa Aesar (Ward Hill, MA) and Fisher Scientific (Fair Lawn, NJ), respectively. The other five species, including dibromoacetamide, bromochloroacetamide, bromodichloroacetamide, dibromochloroacetamide and tribromoacetamide, were all purchased from Orchid Cellmark (synthesized by Bruce McKague, Toronto, Ontario, Canada). High purity Omnisolv ethyl acetate for stock solutions and extractions was purchased from EMD Millipore (Billerica, MA). Anhydrous sodium sulfate used to facilitate the extraction was purchased from EMD Chemicals (Gibbstown, New Jersey). 1,2-dibromopropane used as the internal standard was obtained from Sigma-Aldrich (St. Louis, MO). The pH of water samples was adjusted with ACS Reagent grade acetic acid (Avantor, Center Valley, PA) and sodium hydroxide (Fisher Scientific, Fair Lawn, NJ). MilliQ water (MQ) was used for all blanks, extractions, and dilutions via a Barnstead Easypure system (Thermo scientific, Dubuque, IA).

Table 2.1 Haloacetamides to be analyzed by the LLE/GC-ECD method

Compounds	Acronyms
9 Haloacetamides	HAM_9
Choroacetamide	MCAM
Bromoacetamide	MBAM
Dichloroacetamide	DCAM
Dibromoacetamide	DBAM
Bromochloroacetamide	BCAM
Dibromochloroacetamide	DBCAM
Bromodichloroacetamide	BDCAM
Trichloroacetamide	TCAM
Tribromoacetamide	TBAM

2.1.2 Safety

All extraction experiments were carried out in a properly functioning fume hood to prevent chemical inhalation exposure. Personal protective equipment (PPE), including lab coat, gloves, and safety glasses, was worn at all times to prevent skin exposure.

Volumetric glassware was rinsed at least three times with ethyl acetate to remove residual haloacetamides before being used for preparation of haloacetamides standard solutions preparation. All glassware was cleaned in soap bath, rinsed with tap and MQ water, soaked overnight in 10% nitric acid bath, rinsed with MQ water and then rinsed with methanol before use. Pure compounds, stock and standard solutions were stored in freezer at -20°C. New stock and standard solutions were made every six months to prevent potential degradation.

2.1.3 Standard preparation

Individual primary stock solutions of all haloacetamides were prepared at a concentration range of 5-10 mg/mL in ethyl acetate. Then they were diluted with ethyl acetate to generate a mixed secondary stock solution with a concentration of 20 mg/L for all haloacetamides.

Method calibration standards were prepared fresh with the water sample extraction by diluting the secondary stock solution with pH-adjusted MQ water (10% acetic acid for pH 5) in volumetric glassware. A total of five calibration standards were made in duplicate, with a concentration range of 1-100 µg/L HAMs (1, 5, 20, 50, 100 µg/L).

Matrix spike standards were also prepared by diluting individual primary stock solutions into ethyl acetate. Due to high detection limit and poor recovery of MCAM and MBAM at low concentrations, these two species were prepared at twice the concentration of other haloacetamides species. As a result, matrix spike standards were prepared at a concentration of 40mg/L for MCAM and MBAM, while the concentration of other HAM species in matrix spike standards was 20 mg/L. For a matrix spike sample, 20 mL of sample was spiked with 15 µL of matrix spike standards to yield 30 µg/L for MCAM and MBAM, and 15 µg/L for all other HAMs. The pH-adjusted MQ water was used as lab blank while a 200 µg/L 1, 2-dibromopropane solution in ethyl acetate was used as the extraction solvent for HAMs.

2.1.4 Sample extraction

Before extraction, pH-indicator strips (McolorpHast, EMD Millipore Corporation, Billerica, MA) were used to check pH of water samples. If the original pH was not within the range of 4.5-5.5, a 10% (volume percentage) acetic acid solution was added dropwise to adjust pH. This should be completed during sample collection and prior to extraction. Aliquots of 20mL water samples were then poured gently into clean 40mL glass sample vials. Then, 4mL of prepared ethyl acetate extraction solution (with 200 µg/L 1, 2-dibromopropane) and 10g of sodium sulfate were added to vials. Capped with PTFE-faced septas, vials of samples were vortexed for 1 min. After 5 minutes to allow for solvent layer separation, about 2 mL of the top organic layer was placed into a GC auto-sampler vial using a disposable glass Pasteur pipette.

The GC auto-sampler vials were kept in the freezer at -20°C overnight to separate any residual water in the extract. Then, a portion of the organic extract was transferred to a new GC auto-sampler vial with insert for analysis on GC/ECD.

2.1.4 Gas chromatography/ electron capture detector (GC/ECD)

The GC was an Agilent 6890N (Santa Clara, CA) connected with a micro-ECD. It was also equipped with an Agilent 7683 injector and 7683 autosampler. The chromatography column was a HP-5MS UI, 30m, 0.25 mm inner diameter column with a 0.25 um film thickness (Agilent Technologies, Santa Clara, CA). The temperature of the injector was kept at 180 °C. It was set at the splitless mode, purging 40 mL/min of Helium, the carrier gas, to split vent at 0.5 minute. Helium flowed through the column at a constant rate of 1.6 mL/min with a pressure of 16.3 psi. The make up gas was nitrogen with a constant flow of 60 mL/min. The detector temperature was maintained at 300°C during the analysis. The injection volume was 2 µL. The oven was held at 37 °C for 1 minute initially. Then the temperature went up at 5°C/minute until it reached at 280 °C. The total time for one run was 49.6 minutes.

ECD responses of chromatograms were collected and analyzed using the Agilent GC ChemStation software (Version: B.04.03).

2.2 Analytical methods for additional DBPs

2.2.1 HAA analysis

A total of 12 species of HAAs were evaluated in this study in Table 2.2. The HAA extraction and analysis was revised based on EPA method 552.3 and Weinberg et al (WRF report, 2011) [29, 36]. In order to protonate all of the HAAs including iodoacetic acids, 2 mL concentrated nitric acid was added to a 30 mL aliquot of water sample in a 60mL glass vial to enable a pH value < 0.5. For extraction, 3 mL methyl tertiary butyl ether (MTBE) spiked with

200 µg/L 1,2-dibromopropane and 12 g of sodium sulfate were added to the sample vial. After vortexing for 1 minute, a 1 mL aliquot of the MTBE extract was mixed with 1mL of 10% (v:v) sulfuric acid-methanol in a 10mL test tube. The test tube was kept at 50 °C in a heating block for 2 hours of derivatization. After derivatization, 1 mL of pure MTBE and 4 mL of 10% sodium sulfate solution (150g/L) were added to the mixed solution [37]. After 1 minute of vortexing, the organic extract was transferred to a GC autosampler vial for analysis. The extracted HAA samples were analyzed by the same GC-ECD as the HAM, but with a HP-1MS UI column (30m, 0.25 mm inner diameter, 1 µm film thickness, also from Agilent Technologies). The GC temperature program starts at 37°C for 21 min, increases to 136°C at a rate of 5°C/min and held for 3 min, increases to 250°C at a rate of 20°C/min and held for 3 min, for a total run time of 53 min. The injector is set at 180°C and detector at 300°C. The injection volume is 1µL. Detailed GC/ECD settings are described in Appendix III.

Table 2.2 12 targeted HAAs to be analyzed by LLE/GC-ECD method

Compounds	**Acronym**
12 Haloacetic acids	HAA_{12}
Monochloroacetic acid	MCAA
Monobromoacetic acid	MBAA
Dichloroacetic acid	DCAA
Dibromoacetic acid	DBAA
Bromochloroacetic acid	BCAA
Dibromochloroacetic acid	DBCAA
Bromodichloroacetic acid	BDCAA
Trichloroacetic acid	TCAA
Tribromoacetic acid	TBAA
Chloroiodoacetic acid	CIAA
Bromoiodoacetic acid	BIAA
Diiodoacetic acid	DIAA

2.2.2 SPE/GC-MS method for 28 DBPs

The SPE/GC-MS method to measure 28 DBPs listed in Table 2.3 was based on Chinn et al. (2007) [38] and Weinberg et al. (2002) [10] and is described in detail in Appendix IV. The solid phase extraction was performed with a 12-port Visiprep vacuum manifold, using 6mL Bond Elut-PPL cartridges (Agilent, Lake Forest, CA). The cartridges were first conditioned with 8 mL of methanol at a flow rate of 3 mL/min. Water samples were pre-acidified with nitric acid to bring pH below 3.5 prior to extraction. To provide a sample reservoir, the barrel portion of a 30 mL polypropylene syringe (BD syringe, Franklin Lakes, New Jersey) was attached to each cartridge via Teflon adapter prior to applying 36mL of water samples. Water samples were loaded under vacuum at a flow rate no greater than 3 mL/min. Once the sample loading had finished, the valves of the manifold were turned off, and the elution solvent was added to each cartridge (1.6 mL of MtBE with 200 µg/L 1,2-dibromopropane). Each cartridge was soaked in the elution solvent for 2 minutes before turning the valves on again. Then the eluent was allowed to pass through a disposable flow control valve liner (Supelco, Bellefonte, PA) at a flow rate of 1 mL/min into a 2mL GC-autosampler vial. The extracts were stored in the capped GC-autosampler vial overnight at -20 °C to remove excess water. An aliquot of each sample was transferred to a 500µL autosampler insert in a 2 mL autosampler vial for GC/MS analysis. The GC was set up with an Agilent HP-1MS UI column (30-m, 0.25-mm inner diameter, 1-µm film thickness). The column was kept at 35 °C for 1 min at first, then increased at 4 °C /min until it reached 103 °C. Without any hold time at 103 °C, the column was further heated to 292 °C at 27 °C /min with 2-min hold time. The GC run time for each run is 57 min, with 18 min equilibration time between two runs. The injector temperature was kept at 90 °C to prevent analyte

degradation, and increased to 200 °C after 0.5 min. Detailed GC/ECD settings are described in Appendix IV.

Table 2.3 DBPs to be analyzed by the SPE/GC-MS method

10 Trihalomethanes (THM_{10})	**12 Haloketones (HK_{12})**	**4 Haloacetonitriles (HAN_4)**
Chloroform (TCM)	Chloropropanone	Dichloroacetonitrile (DCAN)
Bromodichloromethane (BDCM)	1,1-dichloropropanone	Bromochloroacetonitrile (BCAN)
Dibromochloromethane (DBCM)	1,3-dichloropropanone	Dibromoacetonitrile (DBAN)
Bromoform (TBM)	1,3-dibromopropanone	Trichloroacetonitrile (TCAN)
Dichloroiodomethane (DCIM)	1,1,1-trichloropropanone	
Bromochloroiodomethane (BCIM)	1,1,3-trichloropropanone	**1 Halonitromethane (HNM_1)**
Dibromoiodomethane (DBIM)	1-bromo-1,1,dichloropropanone	Chloropicrin
Chlorodiiodomethane (CDIM)	1,1,1-tribromopropanone	
Bromodiiodomethane (BDIM)	1,1,3-tribromopropanone	**1 Haloaldehyde (HA_1)**
Iodoform (TIM)	1,1,1,3-tetrachloropropanone	Chloral hydrate
	1,1,3,3-tetrachloropropanone	
	1,1,3,3-tetrabromopropanone	

2.3 Water sample characteristics

2.3.1 Water samples and RO concentrates

We collaborated with USEPA labs in Cincinnati, OH for this study. The USEPA Cincinnati lab collected clarified/filtered water samples (1X) and then concentrated water samples via reverse osmosis (RO) membrane filtration (See Appendix I for more information). The RO concentration method is described in a previous study, Pressman et al. [17]. In this study, the 142X RO concentrate was generated by concentrating the 1X sample by a factor of 142 with respect to its dissolved organic carbon concentration. Bromide and iodide were spiked to meet 1X conditions of 115 µg/L for bromide and 11.5 µg/L for iodide. The conditions of sample collection, concentration and disinfection treatment are detailed in a forthcoming publication by Kennicutt et al. [39].

Treated drinking water samples and RO concentrates in Table 2.4 were packed in ice and sent to UW via overnight shipping after quenching disinfectant with ascorbic acid and necessary pH adjustment. Samples were kept at 4 °C before extraction and the extraction was completed within 2 weeks upon arrival.

2.3.2 Treatment Options

Three different chloramination approaches were performed in the study as described in Table 2.4 and Kennicutt et al. [39] (See Appendix I for details).

Table 2.4 Description of treatment methods among 3 batches of samples [39]

Sample	Chloramination Treatment (1X)	Chloramination Treatment (142X)
Batch A	Preformed monochloramine is added to water	Preformed monochloramine is added to water
Batch B	Short free chlorine contact time (3 min) before addition of ammonia to convert to chloramines	Short free chlorine contact time (2 min) before addition of monochloramine
Batch C	Long free chlorine contact time (20 min) before addition of ammonia to convert to chloramines	Long free chlorine contact time (8 min) before addition of monochloramine

Experiments for 1X drinking water samples were performed prior to RO concentrates. In Batch A, 1X drinking water samples were treated with preformed chloramines. In Batch B, 1X samples were treated with free chlorine first, and then dosed with ammonia at about 3 minutes. In Batch C, 1X samples were treated with free chlorine as Batch B samples. But Batch C has a much longer contact time (about 20 minutes) with free chlorine before the addition of ammonia. Results of the initial experiments for 1X samples were applied to kinetic models to obtain the comparable free chlorine contact time and dosage of chemical for RO concentrates. The major difference between treatment methods of 1X samples and 142X RO concentrates was the

addition of monochloramine to batches B and C for 142X RO concentrates. The free chlorine contact time in 142X RO concentrates was also adjusted to scale up the reaction appropriately.

2.4 USEPA RPF method for health risk assessment

For this study, we will follow the EPA RPF method to estimate health risks posted by chloraminated water samples and RO concentrates. A systematical toxicological review is performed for DBPs to derive non-observed adverse effect levels (NOAELs) from published animal studies. The accuracy of NOAELs is strongly dependent on the quality of the scientific studies. Therefore animal studies must be validated first before incorporating into risk assessment. It is a daunting task to validate animal studies for all 49 DBPs included in the chemical analysis and beyond the scope of this work. Therefore, we will use the NOAELs data previously utilized by Rice et al. (2008) since the data has already been reviewed and validated. Since Rice et al. (2008) aimed at the endpoint of reproductive/developmental effects, we will also focus our study on this endpoint. We also investigated the chemical's Kow information in toxicological review since it could affect the actual exposure dose of human to these chemicals.

BDCM is chosen as the index chemical for the study. BDCM is also used as the index chemical in Rice et al. (2008) since it has adequate animal dose-response data for developmental effects [16]. Based on the available dose-response data, the dose-response curve was developed using EPA Benchmark Dose Software [35].

3. Results and Discussion

3.1 HAMs Method Revision and Validation

3.1.1 LLE/GC-ECD method revision

The method is developed based on the original LLE/GC-ECD method used for the EPA nationwide DBP occurrence study [8]. Weinberg and colleagues investigated silylation, acid-catalyzed hydrolysis and a LLE/GC-ECD method, and the LLE/GC-ECD method was determined to be the best since the other two methods had relatively higher detection limits or could result in formation of additional DBPs during extraction [10].

According to the original method, 4 mL ethyl acetate with 100 µg/L of 2,3-dibromopropane as internal standard is added to 20mL sample water. The organic layer is extracted after 1 min vortexing and 5 min solvent layer separation time.

A significant problem of emulsion formation was observed when extracting 142X RO concentrates. The emulsion also led to lower recovery ratios (less than 50%) when concentrations in water samples were compared with concentration of direct HAM standard in ethyl acetate. Note that these values are not matrix-spike recoveries, but rather ratios of analyte area in extracted samples to those in pure standards. The addition of anhydrous sodium sulfate was added to reduce emulsion, and improve recoveries by LLE as shown in Table 3.1.

Table 3.1 Comparison of area-based recovery ratios with and without salt addition

	MCAM	DCAM	TCAM	MBAM	DBAM	TBAM	BCAM	BDCAM	DBCAM
No salt	BDL	16%	48%	BDL	37%	37%	28%	40%	37%
With salt	4%	61%	150%	11 %	66%	50%	65%	78%	34%

Table 3.2 Haloacetamide Percent Recoveries from Matrix-Spiked Dilutions

Compounds	142X 1:100	142X 1:10
MCAM	36%	174%
MBAM	107%	120%
DCAM	118%	94%
BCAM	108%	108%
TCAM	108%	109%
DBAM	102%	96%
BDCAM	94%	95%
DBCAM	93%	94%
TBAM	81%	84%

Note: For 142X 1:2 dilution, percent recovery was not determined due to emulsion

We analyzed 142X RO concentrates in three different dilutions: 1:2, 1:10 and 1:100. Percent recoveries of haloacetamides in 1:100 and 1:10 dilutions were determined during the method validation stage, not during the full study. The recoveries of these two dilutions were very similar, as shown in Table 3.2. Percent recoveries of haloacetamides cannot be determined in 1:2 dilutions due to serious emulsion. In the full study sample analysis, haloacetamide concentrations were measured in 1:10 dilutions easily within the range of the calibration curve.

We also improved the original method by specifying the sample collection protocol, choosing the optimal pH condition for extraction and the quenching agent needed for analyte stability. According to Chu and colleagues, brominated and iodinated HAMs may degrade in the presence of excess amount of ascorbic acid [26]. In this study, the amount of ascorbic acid to be added was determined to be 105% of the molar amount of residual total chlorine (chlorine + chloramines). After quenching with ascorbic acid, the pH of the samples was adjusted by 10% acetic acid solution to 5 ± 0.5 since fast hydrolysis of HAMs could be prevented at pH 5 [26].

The GC/ECD set-up was the same as Weinberg et al. [10]. A HP-5MS UI, 30m, 0.25 mm inner diameter column with a 0.25 um film thickness was used for the analysis. The oven was

held at 37 °C for 1 minute at first. Then the temperature went up at 5°C/minute until it reached at 280 °C. The temperature of the injector was kept at 180 °C, and the injection volume was 2 µL. The injector was operated at the splitless mode. The detector temperature was maintained at 300°C during the analysis.

3.1.2 Method Validation

3.1.2.1 Retention times of HAMs

The retention times of each HAM species in 100ppb calibrant are listed in Table 3.3. Figure 3.1 shows separation of HAMs peaks in the chromatogram. Long tails of HAMs are observed from the chromatogram, and it could result from the structure of HAMs. HAMs are more polar than other compounds (esters, for example), and therefore they can easily stick to the HP5 column and cause peak tailing. The peak of MBAM has a long tail, and therefore coelutes with DCAM (See Figure 3.2).

Table 3.3 Retention times of HAMs in the 100ppb calibrant

Chemicals	Retention Time (min)
MCAM	7.808
MBAM	10.889
DCAM	11.412
BCAM	13.882
TCAM	15.038
DBAM	16.330
BDCAM	17.613
CDBAM	20.113
TBAM	22.533

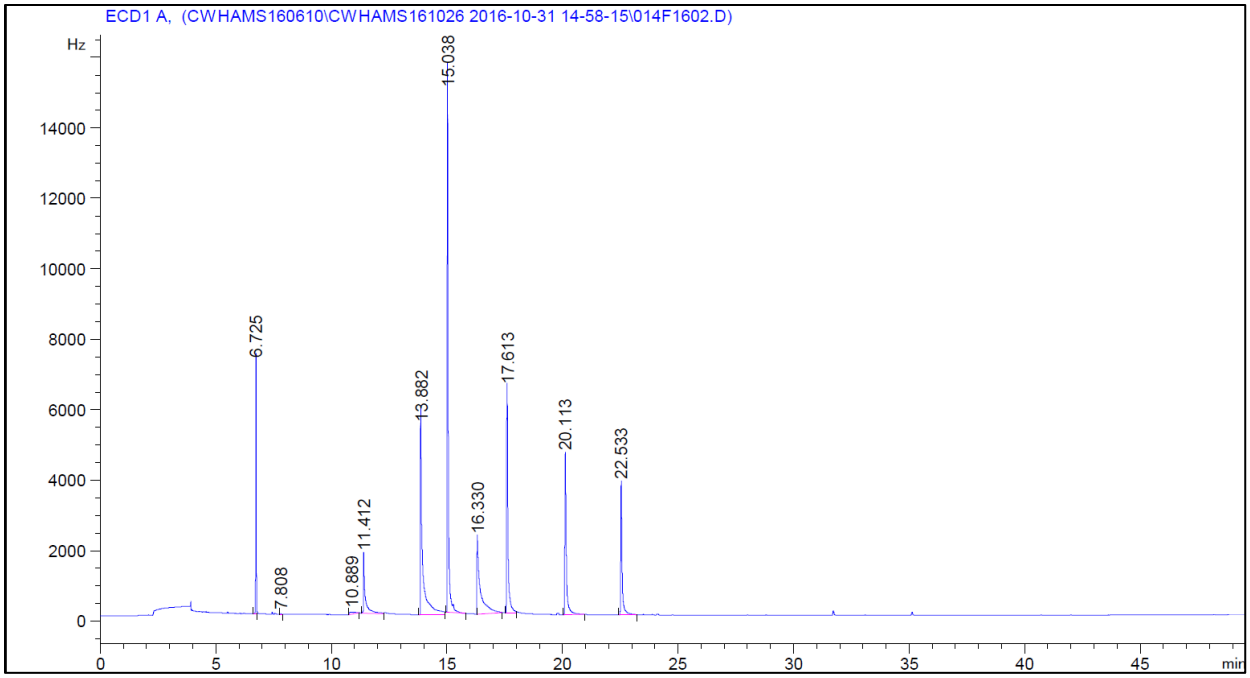

Figure 3.1 Chromatographic separation of 9 HAMs and internal standard in the 100ppb method calibrant

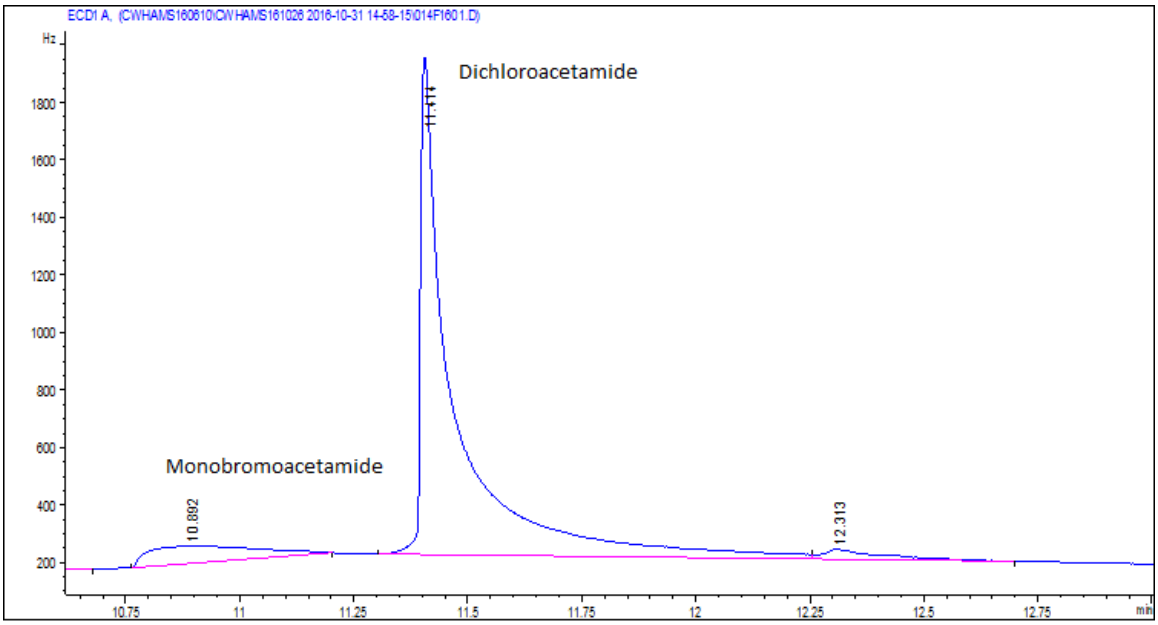

Figure 3.2 Coelution of MBAM and DCAM in the 100ppb method calibrant

3.1.2.2 Linearity, Accuracy, and precision

In this study, linear calibration curves were observed for all chlorinated species. However, brominated species (DBAM, BCAM, DBCAM, BDCAM and TBAM) matched better with quadratic models instead of linear. Figure 3.3 shows TBAM's calibration curve as an example of all brominated species. It was clear that the quadratic model better matched method calibrants, especially in higher concentration ranges. Therefore, polynomial model was used for all brominated species calibration curves in this study.

Table 3.4 Method calibration curves for HAM quantification in 142X samples

Compound	Batch A		Batch B and C	
	Line Equation †	R^2	Line Equation †	R^2
MCAM	$y = 1E\text{-}04x - 0.0002$	0.9865	$y = 0.0004x - 0.0123$	0.9672
MBAM	$y = -1190.1x^2 + 661.28x + 12.784$	0.9951	$y = -510.15x^2 + 488.44x + 16.932$	0.9985
DCAM	$y = 0.0095x - 0.001$	0.9991	$y = 0.0099x - 0.0057$	0.9972
BCAM	$y = -1.9541x^3 + 5.5754x^2 + 35.577x + 1.6015$	0.9993	$y = 5.6714x^3 - 27.808x^2 + 64.457x + 1.8548$	0.9994
TCAM	$y = 0.0356x - 0.0412$	0.9976	$y = 0.044x - 0.0836$	0.9991
DBAM	$y = -8.8755x^3 + 18.241x^2 + 50.437x + 1.7361$	0.9991	$y = 38.846x^3 - 116.63x^2 + 132.46x + 1.284$	0.9987
BDCAM	$y = -0.2604x^3 - 10.019x^2 + 80.239x + 0.9979$	0.9979	$y = 23.184x^3 - 70.536x^2 + 107.33x + 1.2673$	0.9988
DBCAM	$y = 8.5969x^3 - 36.96x^2 + 115.79x + 1.3916$	0.9954	$y = 24.394x^3 - 69.504x^2 + 124.84x + 2.0737$	0.9924
TBAM	$y = 4.4776x^3 - 54.377x^2 + 158.09x + 2.0645$	0.9912	$y = 55.743x^3 - 145.33x^2 + 195.2x + 3.9591$	0.9795

† For linear equation, y=relative area, x=concentration (µg/L), for quadratic equations, y= concentration (µg/L), x= relative area

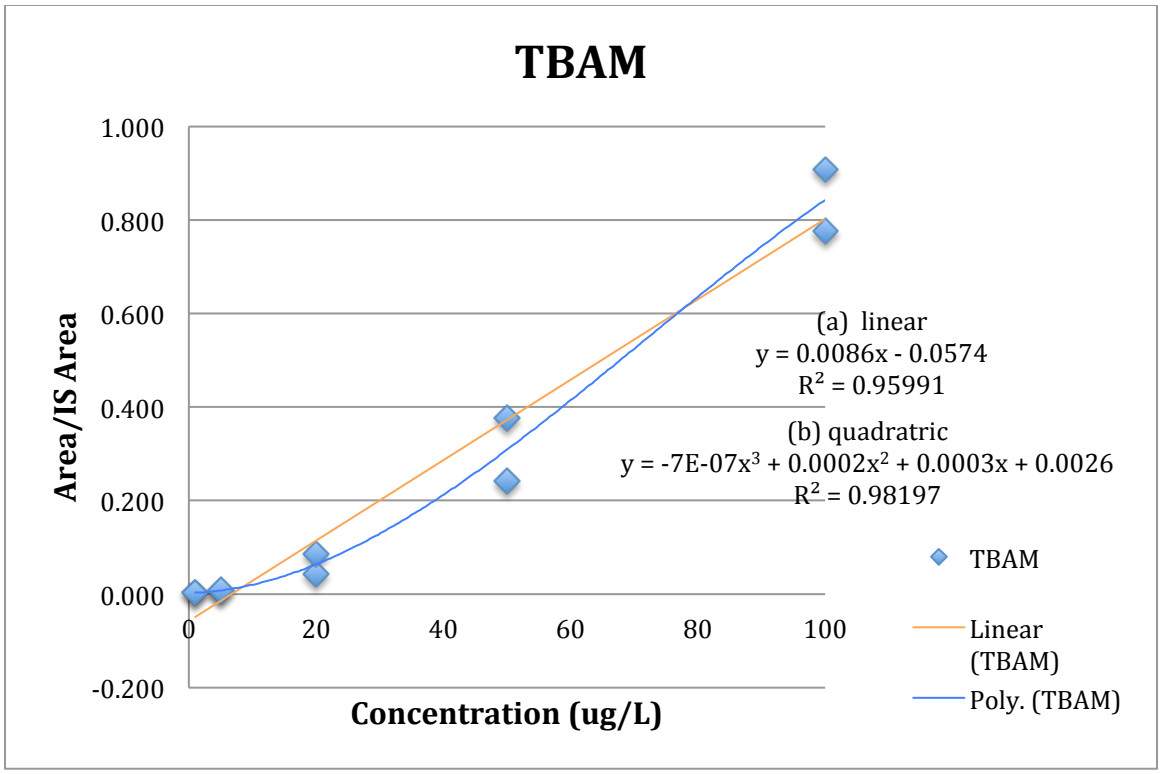

Figure 3.3 TBAM's calibration curve: (a) linear (b) quadratic

Accuracy of the method was verified by matrix-spike recovery ratios. If a chemical's matrix-spike recovery ratio is in the range of 75% to 125%, it is confirmed that the chemical could be measured accurately by the method. Good method and instrument precision were determined by relative standard deviations (RSD) <25% for replicate extractions and injections, respectively.

In this study, MCAM was determined to be "unquantifiable" based on low matrix recovery as listed in Table 3.5. MBAM had a better matrix-spike recovery than MCAM, but still did not meet the criteria. In addition, MBAM was observed to coelute with DCAM. The coelution made it very difficult to accurately integrate MBAM's peak in the chromatogram. Therefore, the determination of MHAMs in this study was considered semi-quantitative.

Table 3.5 Matrix-spike recovery and %RSD of HAMs for 1:10 142X concentrates

Chemicals	Matrix-spike Recovery (n=3)	Batch A %RSD		Batch B and C % RSD	
		Method Precision (n=9)	Instrument Precision (n=10)	Method Precision (n=9)	Instrument Precision (n=10)
MCAM	41%	16%	6%	2%	1%
MBAM	69%	13%	1%	2%	3%
DCAM	108%	17%	3%	3%	1%
BCAM	88%	16%	2%	4%	3%
TCAM	87%	14%	4%	1%	1%
DBAM	98%	16%	4%	8%	11%
BDCAM	122%	16%	4%	4%	2%
DBCAM	117%	16%	5%	6%	2%
TBAM	112%	16%	4%	9%	2%

3.1.2.3 Detection limits

The method detection limit (MDL) for each analyte was determined first by measuring the standard deviation (s) of the relative area of 7 replicate injections. Different calibrant levels were chosen for different analytes to make sure that the calibrant level is within 1-5 times of the calculated detection limit[40].

Table 3.6 Method detection limits of HAMs [39]

Chemicals	MDL (µg/L)
MCAM	~50
MBAM	~20
DCAM	0.3
BCAM	2
TCAM	2
DBAM	2
BDCAM	1
DBCAM	1
TBAM	3

3.2 DBP formation in 142X RO concentrates

The 142X RO concentrates were diluted at ratios of 1:2, 1:10, and 1:100 before measurement. The concentrations in 142X RO concentrates were then determined by multiplying the DBP concentration in the chosen dilutions by the dilution factor. The final concentrations were further adjusted by subtracting DBP concentrations detected in untreated samples, only necessary for HAAs and HAMs. All samples were analyzed in triplicate, and the average concentration and standard deviation were calculated and shown in following figures.

3.2.1 DBP compound classes detected in 142X RO concentrates

Figure 3.4 shows 9 DBP classes detected in 142X RO concentrates. Among them, the EPA-regulated THM_4 had substantially higher concentrations than unregulated DBP classes. HAA_9, including the five HAA species regulated by EPA, had lower concentration levels than but similar in scale to THM4. HAMs, which followed THM4 and HAA9, were the third highest DBP class and the highest unregulated DBP class in this study. The relative high occurrence of HAMs observed in this study is consistent with previous studies where chloramination favors HAMs formation [24]. Iodinated THMs, which were mostly reported as "not detected " in the 2002 national occurrence study and EPA 4Lab study chlorinated RO concentrates [8, 10, 17], were observed as the second highest unregulated DBP class in the study. Chloropicrin and iodinated HAAs appeared to be the two least formed DBP classes in the study, with concentration levels even lower than HKs, HAN_4 and chloral hydrate.

THM_4 concentration was highest in Batch C (5.4 ppm or 5400 ppb), which was 11 and 0.4 times higher than concentration in Batches A and B. Similarly, HAA_9 concentration was also highest in Batch C (3.1 ppm), which was almost as high as 3.5 times and twice as concentrations in Batch A and B, respectively.

As seen with THM_4 and HAA_9, Batches B and C have much higher formation of unregulated DBPs than in Batch A. In general, unregulated DBPs concentrations in Batch C were significantly higher than concentrations in Batch B, such as HAMs, HAN_4, chloropicrin and chloral hydrate. HK concentrations in Batch B and C were at a similar range. But iodinated THMs and HAAs had higher formation in Batch B, revealing iodide oxidation and iodine incorporation during Batch B's chloramination.

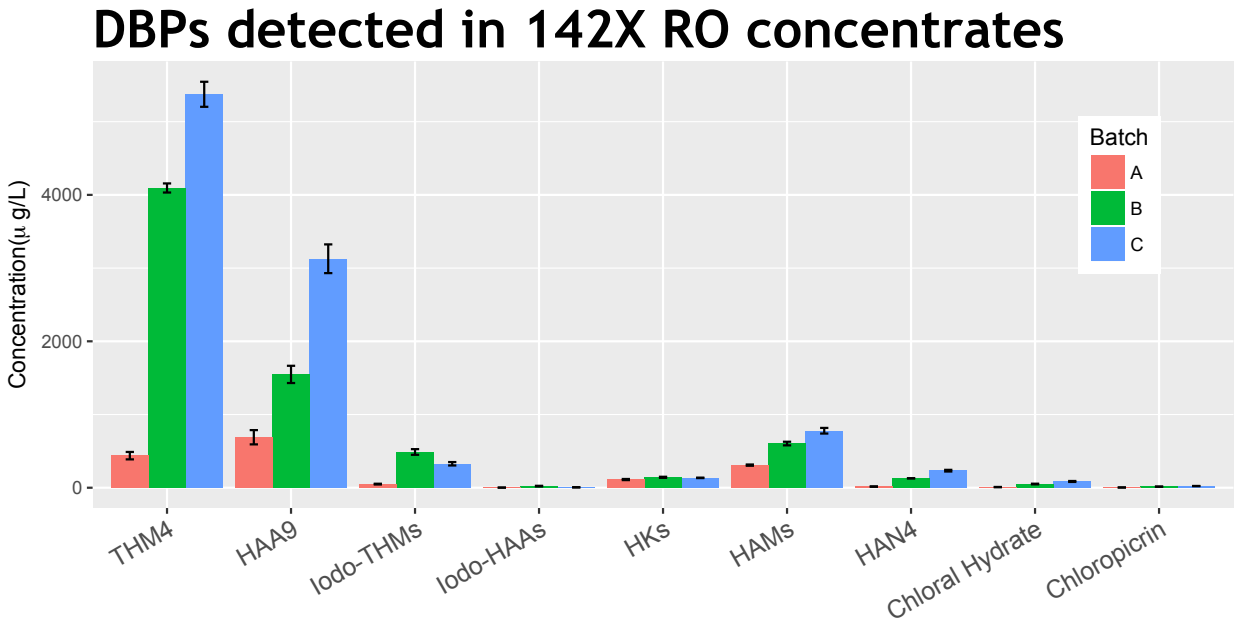

Figure 3.4 DBP compound classes detected in 142X RO concentrates (N=3) [39]

3.2.2 DBPs speciation in 142X RO concentrates by DBP class

3.2.2.1 THM_4 and HAA_9

1:10 dilutions were used to determine concentrations of TCM and BDCM in Batch A, while 1:2 dilutions were used for DBCM and TBM in the same batch. Concentrations of TCM and BDCM in Batch B were determined based on a combination of 1:100 and 1:10 dilutions.

Concentrations of DBCM and TBM in Batch B were determined based on 1:10 dilutions. 1:100 dilutions were chosen for all four THM species in Batch C.

Figure 3.5 shows the speciation of THM4 in three batches. Front the left to the right, DBPs are first ranked in the order of the number of halogenated atoms. Then they are ranked in the order of most chlorinated species to most brominated species. As shown in Figure 3.5, concentrations of the four THM species were lowest in Batch A. TCM was formed at a higher concentration in Batch B than C (1990 and 1820 μg/L). All other three species were detected at highest levels in Batch C. The greater bromine incorporation makes sense and it is consistent with reaction scheme 1 as shown in Figure 3.6 since free chlorine can oxidize bromide and produce oxidized NOM as precursors of brominated DBPs. Concentrations of BDCM, DBCM, and TBM in Batch C were 35%, 112%, and 256% higher than that in Batch B, respectively. Therefore, more bromine was incorporated in Batch C than B. In general, chloroform (TCM) was the dominant THM species among all Batches, except for Batch C where BDCM was slightly higher than chloroform in the same batch (1860 and 1820 μg/L, respectively). TBM was formed at the lowest levels among the four THM species, and it was not detected in Batch A.

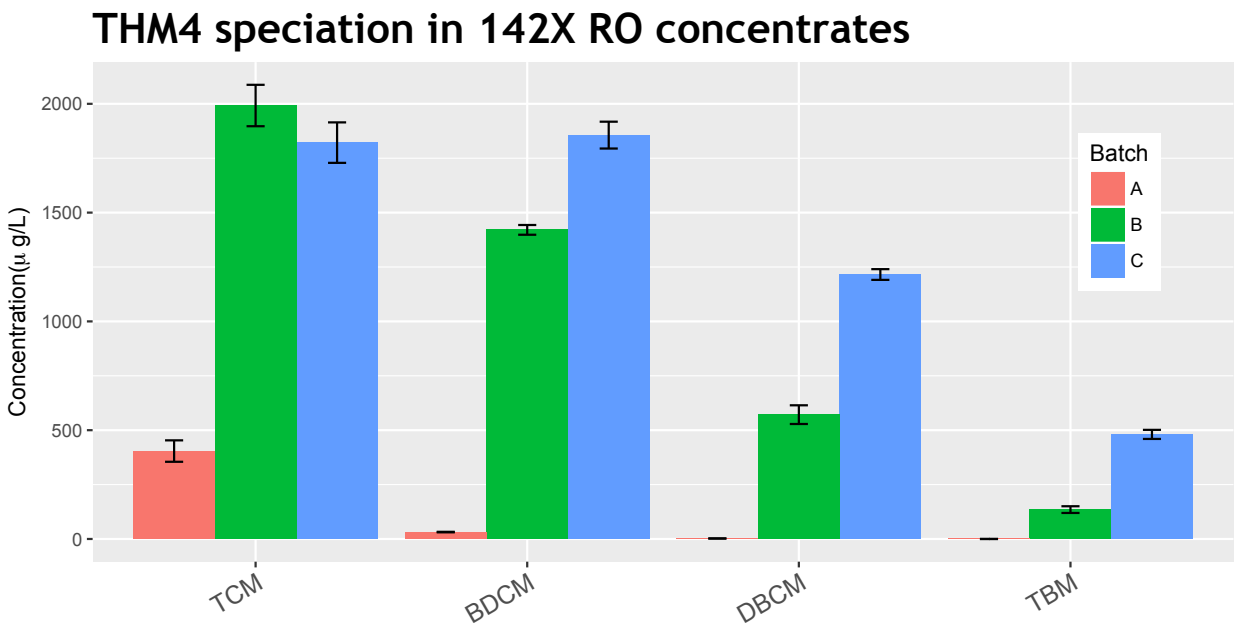

Figure 3.5 THM_4 speciation in 142X RO concentrates (N=3) [17, 39]

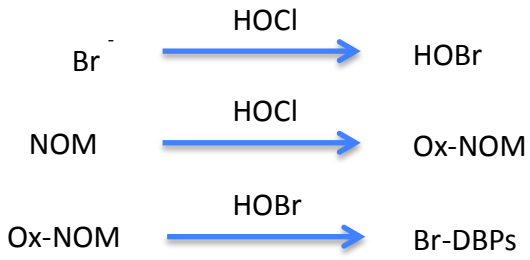

Figure 3.6 Reaction scheme 1: Oxidation of bromide and NOM by chlorine.

Note: Ox-NOM = oxidized NOM

All HAA concentrations reported here were determined based on 1:10 dilutions. Similar to THM_4, all HAA species were formed at the lowest level in Batch A except for DCAA, which were formed at equal levels in Batch A and Batch B (590 µg/L for both). All HAA species were detected at highest levels in Batch C. Concentrations of TCAA and TBAA in Batch C were 72% and 341% higher than that in Batch B, respectively. This shows that the longer chlorine contact

time has a significant impact on bromine incorporation as illustrated in Figure 3.6. DCAA was the dominant HAA species for all batches. Concentrations of MCAA and MBAA in Batch A and B were below method detection limits and therefore were not shown in Figure 3.7. BDCAA was also not detected in Batch A. The regulated HAA_5 formation contributed to 88%, 61% and 57% of the total HAA_9 formation for Batch A, B and C, respectively.

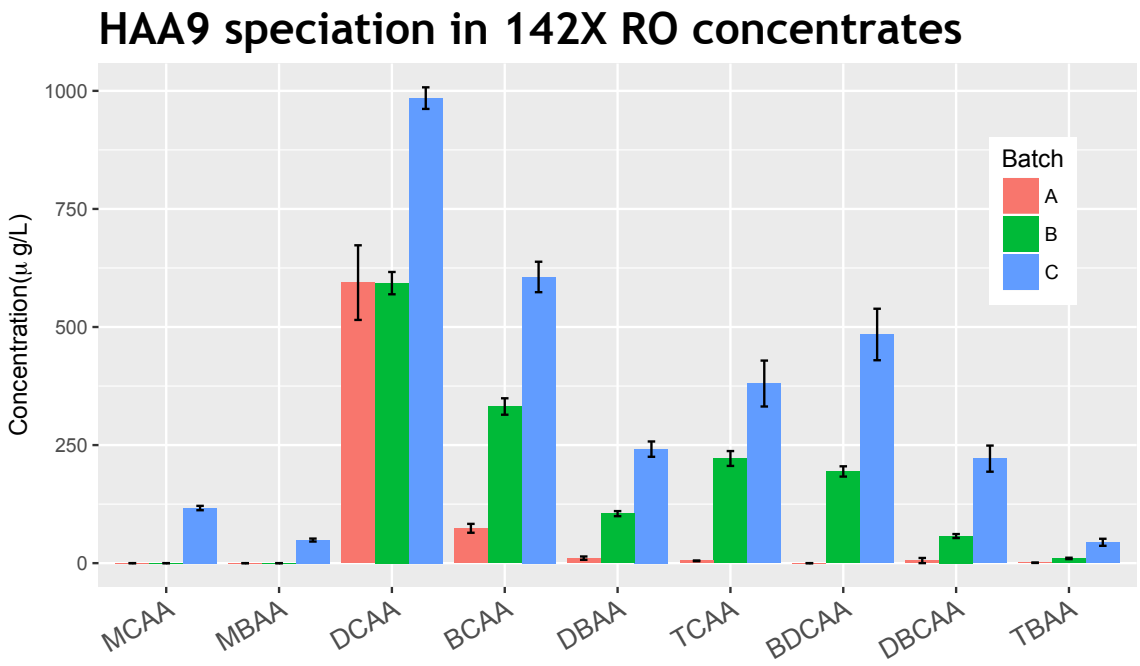

Figure 3.7 HAA_9 speciation in 142X RO concentrates (N=3) [39]

3.2.2.2 HAMs

Concentrations of All HAMs reported here were quantified using 1:10 dilutions.

Only 7 species of HAMs were shown in Figure 3.8, since MCAM and MBAM could not be accurately quantified due to method limitation. This study is the first to quantitatively measure BCAM, BDCAM and DBCAM in RO concentrates, since these three species were only qualitatively identified in EPA 4Lab study [17]. Most HAM species were detected at highest

levels in Batch C, while DCAM and BDCAM were the two exceptions. As shown in Figure 3.5, DCAM was formed at equal levels in all three batches, while Batch B had a slightly higher BDCAM concentration than Batch C (33 and 27 µg/L, respectively). Except for DCAM, all other HAM species had their lowest formation in Batch A. Concentrations of TCAM, BDCAM, DBCAM and TBAM were even below detection limits in Batch A. DCAM was formed at highest level among all HAM species, which was consistent with data from the EPA 4Lab study chlorinated RO concentrates [17].

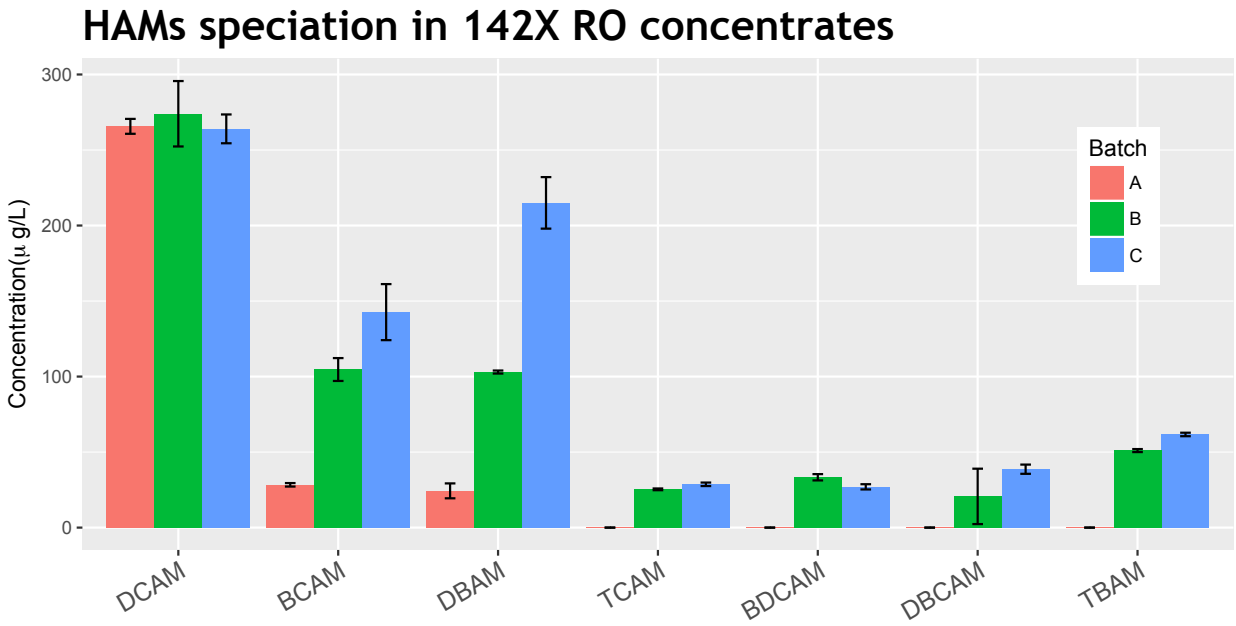

Figure 3.8 HAMs speciation in 142X RO concentrates (N=3) [39]

3.2.2.3 Iodinated THMs and HAAs

Most iodinated THMs were quantified using 1:2 dilutions. But DCIM in all three batches, and BCIM in Batch B and C, were quantified using 1:10 dilutions. All iodinated HAAs were quantified based on 1:10 dilutions.

Different from all other DBP classes discussed before, most iodinated THMs had higher formation in Batch B instead of Batch C as shown in Figure 3.9. The exception was that Batch C had slightly higher concentrations of BCIM and DBIM (112 and 39 µg/L, respectively) than Batch B (106 and 38 µg/L, respectively). Batch A was associated with least formation of iodinated THMs for all species. Among all species, DCIM was the one with highest concentration level. It was consistent with the 2002 national occurrence study [8, 10, 17]. Both DBIM and BDIM were not detected in Batch A. TIM (iodoform) was only detected in Batch B.

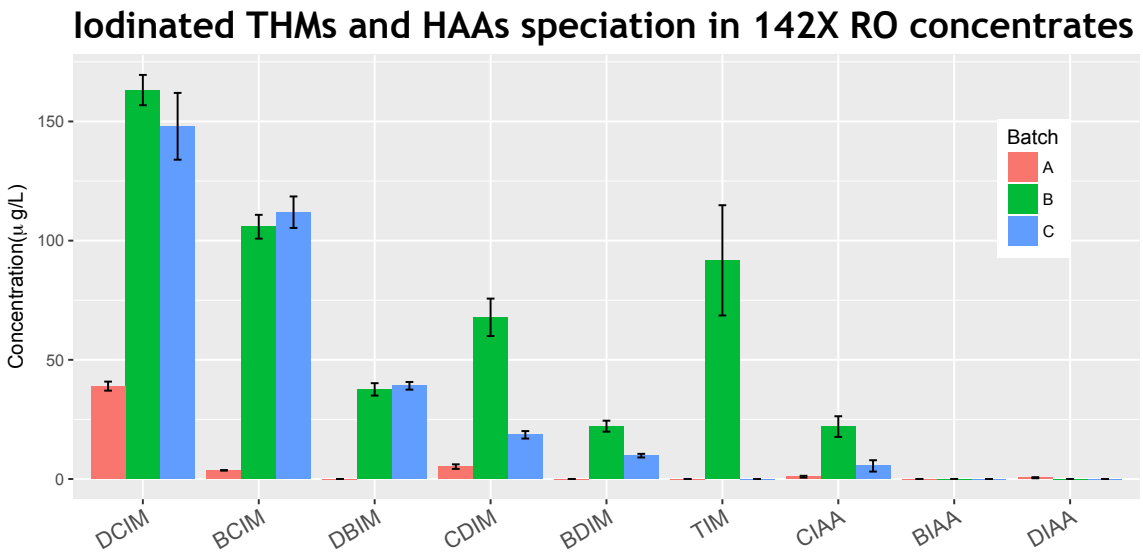

Figure 3.9 Iodinated THMs and HAAs speciation in 142X RO concentrates (N=3) [39]

Three iodinated HAAs, which were not investigated in both the 2002 national occurrence study and EPA 4Lab study, were first reported in this study. BIAA and DIAA were not detected in all three batches. CIAA was observed in Batch B and C, with a highest concentration at 22 µg/L in Batch B.

Highest iodinated DBPs formation observed in Batch B could be explained as the reaction scheme shown in Figure 3.10. Free chlorine can oxidize iodide into hypoiodous acid

(HOI), and convert NOM into precursors of iodinated DBPs. But overdose of free chlorine can further oxidize hypoiodous acid into iodate. That explains why iodinated DBP formation in Batch B was higher than in Batch C. On the other hand, chloramine is a weak oxidizer, which can oxidize iodide into HOI but not further into iodate, and it is not very effective at converting NOM into DBP precursors. Therefore chloramine addition alone produces low levels of iodinated DBPs, as observed in Batch A.

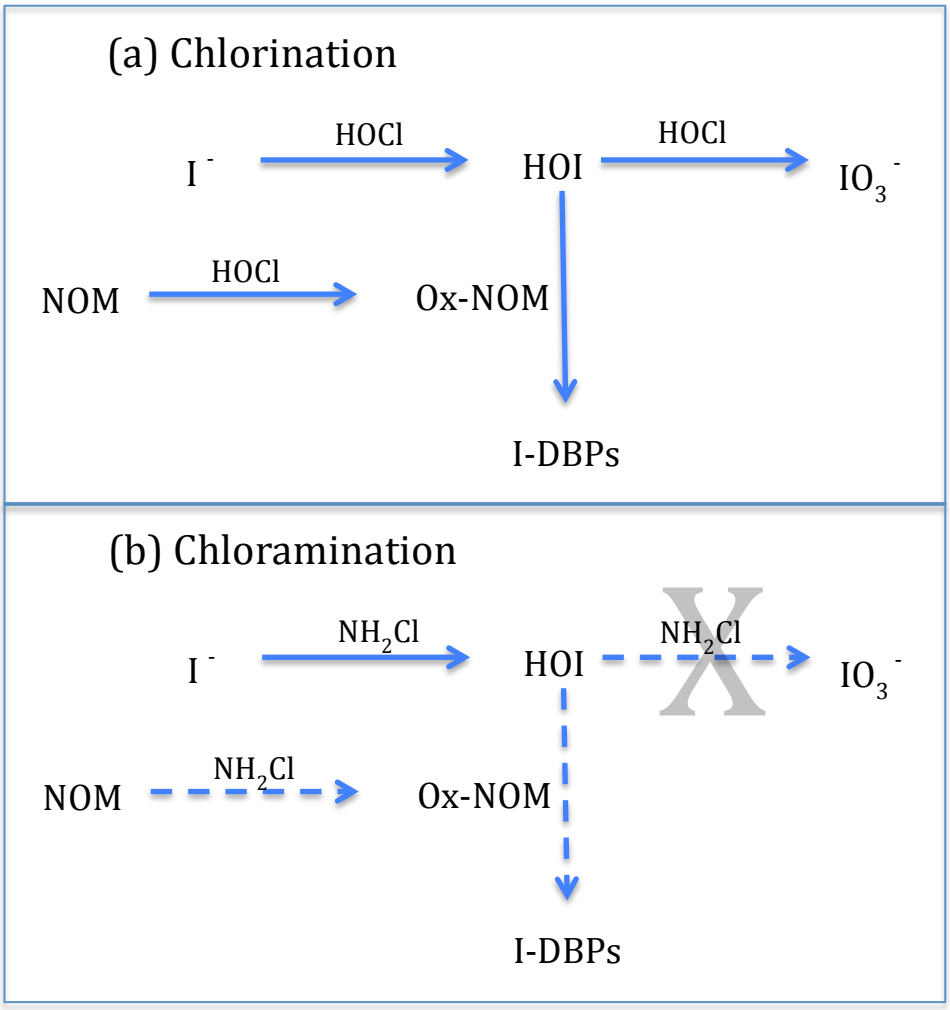

Figure 3.10 Reaction scheme 2: Iodinated DBP formation from (a) chlorination and (b) chloramination

Note: Ox-NOM = oxidized NOM

3.2.2.4 HKs

Concentrations of most HKs were determined based on 1:2 dilutions. Only chloropropanone and 1,1-dichloropropanone in Batch A were determined based on 1:10 dilutions. Figure 3.11 shows detection of 9 out of 12 HKs measured in this study. 1,1,3-tribromopropanone, 1,1,1,3- tetrachloropropanone, and 1,1,3,3-tetrabromopropanone were not included in the figure since they were not detected in any of the batches. Chlorinated species had much higher formation than brominated species. 1,1,1-trichloropropanone was the most dominant HK species in chlorinated RO concentrates that EPA investigated before [17], but that was not the case in this study. Instead, chloropropanone and 1,1-dichloropropanone were formed at significantly higher concentration levels than all other species, and at equal levels to each other. 1,1-dibromopropanone and 1,1,1-trichloropropanone were not detected in Batch A, while 1-bromo-1,1-dichloropropanone and 1,1,1-tribromopropanone were only detected in Batch C.

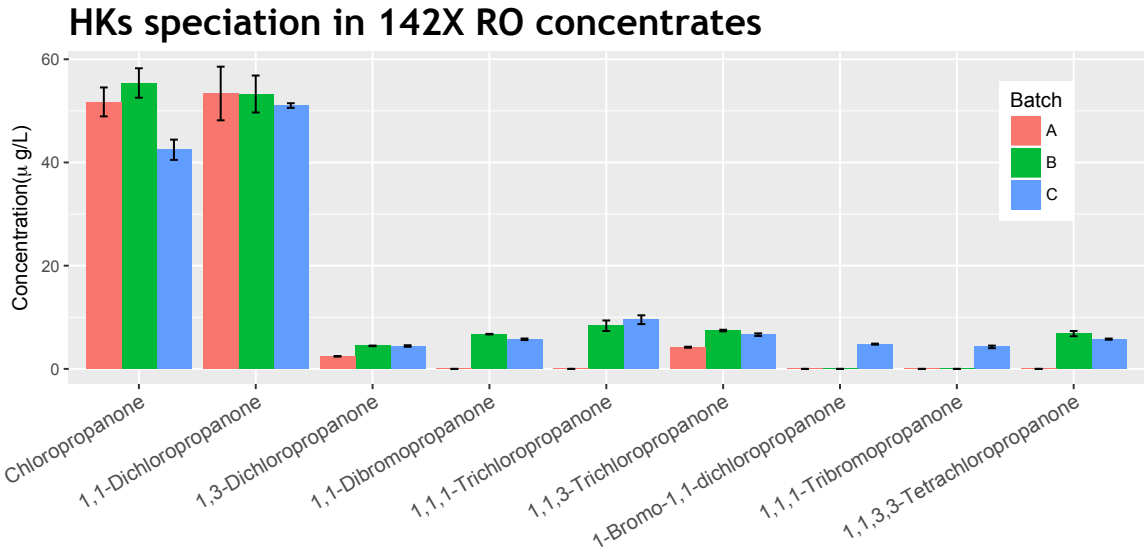

Figure 3.11 HKs speciation in 142X RO concentrates (N=3) [39]

3.2.2.5 HAN_4, chloropicrin and chloral hydrate

1:2 dilutions of 142X RO concentrates were chosen for quantification of HANs, chloropicrin and chloral hydrate.

In this study, TCAN was not detected in all batches. The other three HAN species were shown in Figure 3.12. All three species were detected at highest levels in Batch C. Concentrations of DCAN, BCAN, and DBAN in Batch C were 38%, 108%, and 147% higher than that in Batch B, respectively. This trend was similar to THM_4 and HAA_9 in that there was a more significant difference in concentrations between Batch B and C and brominated species were higher in Batch C compared to Batch B. In the chlorinated RO concentrates from the 4Lab study, DCAN was formed at concentration levels significantly higher than all other HAN species [17]. But in this study, BCAN was formed at similar concentration levels to DCAN in all three chloraminated RO concentrates. Batch A was associated with least formation for all HAN species, and DBAN was not detected in Batch A.

Formation of chloral hydrate and chloropicrin in all three batches followed the same trend: Batch C>Batch B>Batch A.

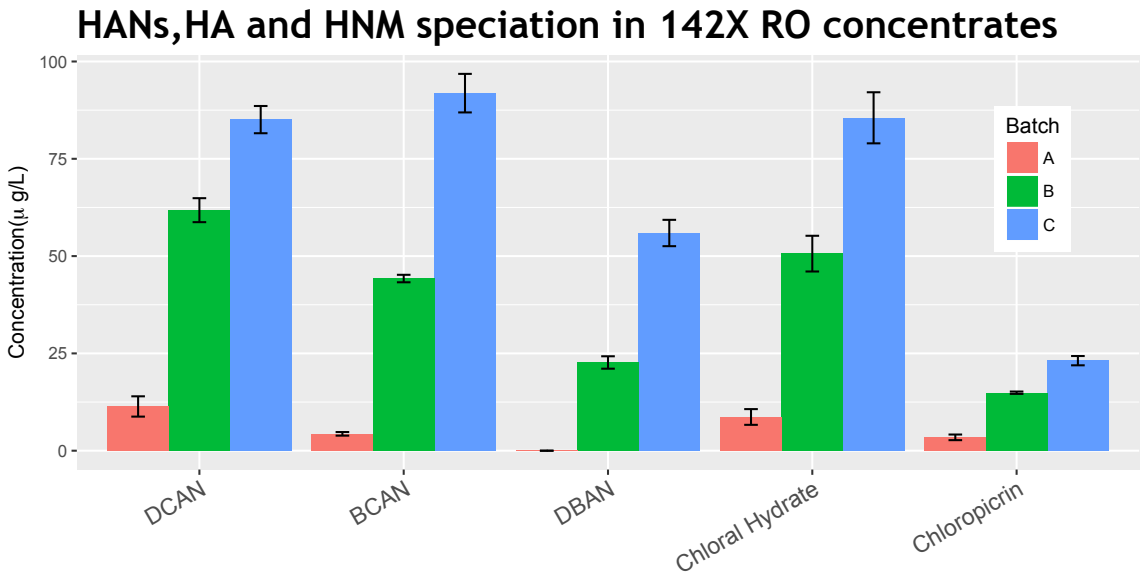

Figure 3.12 HANs, HA and HNM speciation in 142X RO concentrates (N=3) [39]

3.3 Health risk analysis of DBP mixture

3.3.1 RPF analysis

BDCM was chosen as the index chemical for the RPF method. BDCM was identified as a reproductive/developmental toxicant for rats which could induce full-litter resorption in a study in which ingestion was the only exposure route [41]. A dose-response function was developed based on the animal study data obtained from the same study. A log-logistic model (Equation 3.1) was chosen when the function was developed using the USEPA Benchmark Dose Software [35]. It was chosen because that log-logistic model fits best the dose/effect data and it was consistent with Rice et al. (2008) approach [16]. More information about the animal study, and the model fitness is provided in Appendix VI and VII.

$$P(effect) = 1/[1+\exp(11.7 - 2.5 \times \ln(dosage))] \qquad \text{Equation 3.1}$$

Table 3.7 shows the results of RPFs developed for the 17 DBP with NOAELs for ingestion obtained from the literature. RPF values were developed by dividing the NOAELs of the individual DBPs by the NOAEL of BDCM, the index chemical (Equation 2.1). NOAELs for 15 DBPs in the table were taken from Rice et al. (2008), while those for DBCAA and 1,1,3,3-tetrachloropropanone were obtained elsewhere [42, 43].

Table 3.7 Comparison of individual DBP NOAELs and their corresponding RPF values

Chemical	NOAEL (mg/kg bodyweight/d)	RPF
Chloroform	50	0.5
Bromoform	100	0.3
Bromodichloromethane	25	1.0
Dibromochloromethane	40	0.6
Monochloracetic acid	70	0.4
Dichloroacetic acid	14	1.8
Trichloroacetic acid	33	0.8
Monobromoacetic acid	50	0.5
Dibromoacetic acid	13	1.9
Bromochloroacetic acid	20	1.3
Tribromoacetic acid	39	0.6
Dibromochloroacetic acid	89[42]	0.3
Dichloroacetonitrile	55	0.5
Bromochloroacetonitrile	5.5	4.5
Dibromoacetonitrile	5	5.0
Trichloroacetonitrile	1	25
1,1,3,3-tetrachloropropanone	4.8[43]	5.2

The individual chemical exposure rates were calculated for the 17 DBPs in the 142X RO concentrates based on the measured individual DBP concentrations and assumed water consumption rates (0.1g water/g bodyweight/day) in Table 3.8. The water consumption rate was the same as used in Rice et al. (2008) [16]. DBP concentrations measured below method

detection limits were considered as zero when taken into calculations. The individual DBP exposure rates were also converted into index chemical equivalent dose (ICED) using previously obtained RPF values.

Table 3.8 Exposure data for individual DBPs and the corresponding ICEDs

Chemical	Individual DBP Exposure data (mg/kg/d)			ICED Exposure Data (mg/kg/d)		
	Batch A	Batch B	Batch C	Batch A	Batch B	Batch C
Chloroform	4.04E-02	1.99E-01	1.82E-01	2.02E-02	9.96E-02	9.11E-02
Bromoform	0.00E+00	1.35E-02	4.81E-02	0.00E+00	4.05E-03	1.44E-02
Bromodichloromethane	3.17E-03	1.42E-01	1.86E-01	3.17E-03	1.42E-01	1.86E-01
Dibromochloromethane	2.20E-04	5.71E-02	1.22E-01	1.32E-04	3.43E-02	7.29E-02
Monochloracetic acid	0.00E+00	9.34E-03	1.17E-02	0.00E+00	3.74E-03	4.67E-03
Dichloroacetic acid	5.94E-02	5.93E-02	9.85E-02	1.07E-01	1.07E-01	1.77E-01
Trichloroacetic acid	3.63E-03	1.18E-03	2.52E-02	2.90E-03	9.44E-04	2.02E-02
Monobromoacetic acid	0.00E+00	1.22E-03	4.89E-03	0.00E+00	6.10E-04	2.45E-03
Dibromoacetic acid	1.03E-03	2.27E-02	3.86E-02	1.96E-03	4.31E-02	7.33E-02
Bromochloroacetic acid	7.38E-03	3.32E-02	6.06E-02	9.59E-03	4.31E-02	7.88E-02
Tribromoacetic acid	1.33E-03	1.18E-03	5.70E-04	7.98E-04	7.08E-04	3.42E-04
Dibromochloroacetic acid	1.93E-03	2.85E-03	6.26E-03	5.79E-04	8.55E-04	1.88E-03
Dichloroacetonitrile	1.14E-03	6.18E-03	8.51E-03	5.70E-04	3.09E-03	4.26E-03
Bromochloroacetonitrile	4.30E-04	4.42E-03	9.19E-03	1.94E-03	1.99E-02	4.14E-02
Dibromoacetonitrile	0.00E+00	2.27E-03	5.60E-03	0.00E+00	1.14E-02	2.80E-02
Trichloroacetonitrile	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1,1,3,3-tetrachloropropanone	0.00E+00	6.80E-04	5.80E-04	0.00E+00	3.54E-03	3.02E-03

The ICED were summed for each RO concentrate and the risks were estimated according to Equation 3.1 as listed in Table 3.9. The detailed calculation is available in Appendix VIII.

Table 3.9 RPF-based risk estimates associated with 17 DBPs in 142X RO Concentrates

RO Concentrates	ICED Total Exposure (mg/kg/d)	P(effect)
Batch A	1.45×10^{-1}	7.33×10^{-8}
Batch B	5.08×10^{-1}	1.58×10^{-6}
Batch C	7.89×10^{-1}	4.65×10^{-6}

3.3.2 Discussion of RPF-based estimation results

The chemical property K_{ow} could affect the actual dose since it predicts the amount of ingested DBPs to be absorbed by body tissues and organs. K_{ow} information was investigated in toxicological review as well as NOAELs since it could also affect the actual dose (See Appendix VI). The results showed only 9 out of 17 DBPs had K_{ow} values determined by experiments. Estimated K_{ow} values of the other 8 DBPs could vary a lot depending on the different estimation methods used. Therefore no further evaluation of K_{ow} was performed in this study.

Based on the RPF analysis, the risk of a reproductive or developmental effect associated with the mixture of 17 DBPs in Batch A, B, and C is 7.33×10^{-8}, 1.58×10^{-6}, and 4.65×10^{-6}, respectively. The substantially low level of risk associated with these 17 DBPs in the 142X RO concentrates was mainly because the calculated individual DBP exposure dose values (Table 3.6) were significantly lower than their corresponding NOAELs (Table 3.5).

The RPF-based estimate for Batch A was lowest among three. It suggested that chloramination with addition of pre-formed chloramines may reduce the reproductive and developmental risks associated with the target 17 DBPs. Chloraminated RO concentrates with long free chlorine contact time could produce significant health risks compared to ones with short contact time. Brominated DBPs were associated with higher health risks due to their

relatively low NOAELs, and therefore Batch C with highest bromine incorporation was evaluated with higher risk than Batch B.

In the EPA 4Lab study [17], unregulated DBPs formation was measured in 136X RO concentrates. Chlorinated 136X RO concentrates were obtained by RO concentrating untreated 1X sample water at a factor of 136 in terms of TOC (318 mg/L for 136X RO concentrates, and 2.34 mg/L for 1X), and then treated with free chlorine. EPA has performed a rat multigenerational reproductive toxicity study for the 136X chlorinated RO concentrates [44]. The results were predominantly negative, while slight but significant effects were observed for puberty, sperm production, and thyroid cells. It is valuable to compare RPF-based risk estimates of chloraminated RO concentrates to that of chlorinated RO concentrates, but the DBP input must first be normalized to organic carbon concentration. The DBP results in these chlorinated 136X RO concentrates were available as the equivalent 1X concentration[17]. Since TOC concentration of 142X chloraminated and 136X chlorinated RO concentrates (286 and 318 mg/L, respectively) didn't match, DBPs concentrations were first normalized to TOC=1 mg/L before RPF analysis (See detailed calculation process in Appendix IX). As shown in Table 3.10, it was clear that chlorinated RO concentrates measured in EPA 4Lab study had a much higher ICED exposure rate than three chloraminated RO concentrates measured in this study. As a result, chloraminated RO concentrates appeared to be less likely associated with reproductive and developmental effects compared with chlorinated RO concentrates.

Table 3.10 Comparison of RPF-based risk estimates associated with 17 DBPs based on DBP levels in 136X RO Concentrates measured in EPA 4Lab study [19]

RO Concentrates	DOC-normalized ICED (mg/kg/d)	P (effect)
Batch A	5.08E-04	7.06E-14
Batch B	1.78E-03	1.52E-12
Batch C	2.77E-03	4.48E-12
EPA 4Lab study chlorination [38]	7.60E-03	5.33E-11

3.3.3 Assumptions and limitations of the RPF-based health risk analysis

The RPF-based risk estimates mentioned above, although they could provide helpful information for choice of chloramination options, are uncertain and cannot be viewed as quantitative health risk analysis. There are several factors involved in the restriction.

First, RPF method was based on two primary assumptions: (1) the mode of action (MOA) by which BDCM causes toxicity is similar to the MOA of all other DBPs, and (2) the shape of the dose-response curve for BDCM-induced full-litter resorption is similar to those of other DBPs with potential reproductive and developmental effects[16]. Obviously, these two assumptions are uncertain and cannot be used in definitive risk assessment before evaluation.

Second, the RPF method only provides health risk estimated attributable to additivity which does not take interaction effects into consideration. If the toxicity of the chloraminated RO concentrates were to exceed these estimates in animal studies, it is likely that additivity could not explain a substantial part of toxicity. And in this circumstance, the investigation for interaction effects among these DBPs may be necessary for further study.

The restriction also comes from the limited species of DBPs included in this analysis. In this study, only 17 DBPs were included in the RPF analysis while a total of 49 DBPs were

measured in the chemical analysis. This was mainly because only these 17 DBPs had previously-reported NOAELs for reproductive and developmental effects. It is a pity that although many unregulated DBPs were measured in the chemical analysis, they cannot be included in the RPF-based health risk analysis since they don't have available NOAELs. The risk estimation could be improved if DBP classes, such as halonitromethanes, iodinated DBPs, and haloketones, could be included since these DBPs were proved to be highly cytotoxic and genotoxic in previous studies[45].

Finally, the utility of NOAELs highly depends on the study design and the experimental dose spacing. Therefore NOAELs might not be good indicators of chemicals' toxicity. Instead of NOAELs, benchmark dose (BMD) is supposed to be less affected by experimental dose spacing and can appropriately account for uncertainty and variability in the experimental results. However, in this study, the application of BMDs instead of NOAELs was impossible since not enough dosage/response data was available to determine BMDs of these DBPs included in the RPF-based analysis.

4. Conclusions

In this study, a simple and inexpensive LLE/GC-ECD method for analysis of haloacetamides in drinking water was revised and validated. The method was optimized resulting in excellent method accuracy and precision for 7 HAM species, except MCAM and MBAM. The method was then applied to measure HAM formation in chloraminated RO concentrates.

Including HAMs, a total of 49 DBPs were measured in the 142X chloraminated RO concentrates provided by USEPA. Batch A treated with preformed monochloramine was associated with the least DBP formation. Batch B and C were both treated with free chlorine first and then followed by monochloramine addition. Results showed that Batch B and C had much higher formation of DBPs than Batch A. In fact, many unregulated DBPs that were below detection limits in Batch A could be evaluated in Batches B and C. The trend was more obvious for brominated and iodinated species. In general, unregulated DBPs concentrations in Batch C were significantly higher than concentrations in Batch B, such as THM_4, HAA_9, HAMs, HAN4, chloropicrin and chloral hydrate. This could result from the longer free chlorine contact time for Batch C. HK concentrations in Batch B and C were at a similar range. Iodinated DBPs were minimized in Batch A since little precursors of iodinated DBPs could be generated via chloramine addition. Free chlorine addition in Batch B and C could oxidize NOM into precursors of iodinated DBPs, and generate iodinated DBPs in the presence of HOI. However, in Batch C, the longer chlorine time potentially resulted in more complete oxidation of iodide (I^- → HOI → IO_3^-) before significant iodine incorporation into DBPs could occur [12].

A RPF-based health risk analysis was performed on 17 DBPs for reproductive and developmental effects. The results suggest that the reproductive and developmental health risks associated with these 17 DBPs in Batch C is much higher than in Batch A and B, although the

risks with all three batches are too low to be a health concern with available data and toxicity information. The health risk analysis could provide helpful information in terms of choice of chloramination options, but could not be used as quantitative health risks assessment due to unverified method assumptions.

Future work of the study could be aimed at a better HAM detection method, which would accurately quantify MCAM and MBAM as well. The RPF of DBPs in health risk analysis could be better estimated based on BMDs instead of NOAELs. BMDs of iodinated DBPs, haloketones, and halonitromethanes could be helpful to perform more meaningful risk estimation associated with DBP mixtures since they were proved highly cytotoxic and genotoxic in previous studies. Total organic halide (TOX) concentration could be evaluated to provide more insights about unidentified DBP concentrations.

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Appendix I Sample water and experimental conditions at EPA

All information in Appendix I is described in a forthcoming paper [39].

DOC concentration for 1X samples was 2.01 mg/L. 142X RO concentrates had 142-times the DOC concentration of 1X samples. Bromide and iodide were spiked to meet 1X conditions of 115 ug/L for bromide and 11.5 ug/L for iodide, and the concentrates were spiked 142-times of these values.

For the 1X waters, a 3-minute ("short") and a 20-minute ("long") free chlorine contact time respectively corresponded to 80% and 100% bromide oxidation and 65% and 100% iodide oxidation. Chloramination conditions of 142X RO concentrates were determined based on initial experiment results of 1X samples and kinetic models to scale up reactions.

Batch A: Preformed monochloramine

- 1X waters: dosed with 2.5 mg/L monochloramine
- 142X waters: dosed with 50 mg/L monochloramine

Batch B: "Short" free chlorine contact time

- 1X waters: dosed with 2.5 mg/L of free chlorine; at 3 minutes dosed with ammonia (4.75:1 chlorine to ammonia-nitrogen ratio)
- 142X waters: dosed with 72 mg/L free chlorine, at 2 minutes dosed with 20 mg/L preformed monochloramine
    - Additional dosing of 3.27 mg/L bromide and 0.57 mg/L iodide at 2.25 minutes

Batch C: "Long" free chlorine contact time

- 1X waters: dosed with 2.5 mg/L of free chlorine; at 20 minutes dosed with ammonia (4.75:1 chlorine to ammonia-nitrogen ratio)
- 142X waters: dosed with 119 mg/L free chlorine, at 8 minutes dosed with 17 mg/L preformed monochloramine

All 1X samples and 142X RO concentrates in Batch A were quenched after 72 hours of contact time, while 142X RO concentrates in Batch B were quenched after 48 hours.

Appendix II SOP for analysis of HAMs in drinking water

A. PURPOSE AND SCOPE

This Standard Operating Procedure (SOP) describes the extraction and analysis of the nine haloamides chloroacetamide, bromoacetamide, dichloroacetamide, bromochloroacetamide, dibromoacetamide, trichloroacetamide, bromodichloroacetamide, dibromochloroacetamide, tribromoacetamide in finished drinking water by liquid-liquid solvent extraction and gas chromatography with electron capture detection (GC/ECD) analysis. This method is applicable when determining haloamides in concentrations greater than 0.1μg/L

B. SUMMARY

This SOP describes a liquid-liquid solvent extraction and GC/ECD direct injection analysis of nine haloamide disinfection by-products (DBPs) in finished drinking water. Four milliliters of ethyl acetate are used to extract analytes from 20mL samples, and separated by GC on an HP5-MS (or DB-5) column. GC-MS should be used for analyte confirmation.

C. SAFETY AND SAMPLE HANDLING

Exposure to chemicals should be kept to a minimum. All procedures should be carried out under a functional fume hood. Safety Data Sheets should be accessible to all lab workers for proper chemical handling. Personal protective equipment including lab coat, gloves, and goggles must be worn at all times. Standard solutions are stored at -20°C. Samples are stored at 4°C.

D. APPARATUS AND MATERIALS

- 20 mL glass screw cap sample vials with polytetrafluoroethylene (PFTE)-lined screw caps.
- Glass volumetric flasks with glass stoppers
- 20 mL graduated glass pipettes and bulbs
- Vortexer
- Positive displacement pipettes with glass capillary tips (VWR Scientific)
- GC autosampler vials (Agilent Technologies, Cat #5183-4493)
- pH strips
- Disposable glass Pasteur Pipets (VWR Scientific Cat# 14673-043)
- GC-ECD (Shimadzu) with GC Solution Software
- GC Column: 30-m, 0.25 mm ID, 0.25 μm film thickness HP5-MS (Hewlett-Packard/Agilent, Folsom, CA)

E. REAGENTS AND CHEMICALS

- Trichloroacetamide (Fisher Scientific, Fair Lawn, NJ)
- Tribromo acetamide (Orchid Cellmark, Burlington, NC)
- Dibromoacetamide (Orchid Cellmark, Burlington, NC)
- Dichloroacetamide (Alfa Aesar, Ward Hill, MA)
- Monobromoacetamide (Sigma-Aldrich, St. Louis, MO)
- Monochloroacetamide (Sigma-Aldrich, St. Louis, MO)
- Bromochloroacetamide (Orchid Cellmark, Burlington, NC)
- Bromodichloroacetamide (Orchid Cellmark, Burlington, NC)

- Dibromochloracetamide (Orchid Cellmark, Burlington, NC)
- Organic-free deionized water (Barnstead Easypure (Dubuke, IA))
- 1,2-dibromopropane in EtOAc (200 µg/L as internal standard)
- Ethyl acetate (EtOAc) EMD OmniSolv Grade (Fisher)
- L-Ascorbic acid in granular form (Fisher Scientific, Fair Lawn, NJ)
- 10% acetic acid (Avantor, Center Valley, PA)
- Helium gas (Praxair, 99.999% purity)
- Nitrogen gas (Praxair, 99.999% purity)

F. QC SAMPLES

1. ***Primary stocks.***
   Individual primary stock solutions of all haloacetamides were prepared at a concentration range of 5-10 mg/mL in ethyl acetate.

2. ***Secondary Stocks.***
   Primary Stocks are diluted to a concentration of 20 mg/L in EtOAc as a secondary mixed stock solution.

3. ***Calibration standards.***
   Calibration standards are made fresh daily by diluting secondary stocks with pH adjusted DI water (to 5 with acetic acid) in volumetric glassware. A total of five calibration standards are made in duplicate per haloamide. Concentration range for each is 1-100µg/L (1,5,20,50,100 ug/L)

4. ***Internal Standard***
   A 200 µg/L 1, 2-dibromopropane solution is prepared for use as internal standard with ethyl acetate.

5. ***Matrix Spikes*.**
   Matrix spike standards are prepared at a concentration of 40mg/L for MCAM and MBAM, while the concentration of other HAM species in matrix spike standards was 20 mg/L.
   For a matrix spike sample, 20 mL of sample was spiked with 15 µL of matrix spike standards to yield 30 µg/L for MCAM and MBAM, and 15 µg/L for all other HAMs.

6. ***Blank samples.***
   - Lab Blank: pH adjusted DI water to 5 with 10% acetic acid
   - Field Blank: DI water shipped along with samples

H. SAMPLE COLLECTION and PREPARATION

- A solution of 10 % acetic acid is added by drops to adjust pH to 5.
- Samples are poured gently into 20mL glass sample vials . After adding 4mL of EtOAc with internal standard and 10 g sodium sulfate, the vials are capped with PTFE-faced septas.

- Samples are vortexed for 1 min.
- Solvent layer is allowed to separate in 5 min.
- 1 mL of organic layer is placed in GC auto-sampler vials for analysis using a disposable glass Pasteur pipette.
- 1 mL of organic layer is stored for back up at -10°C.

I. GC ANALYSIS

1. Load the samples on the autosampler of the GC/ECD.
2. Load a method into software with the following
   - Column parameters:
     Type: HP5-MS UI column; Length: 30 m; Internal Diameter: 0.25 mm; Thickness: 0.25µm
     Carrier gas: Helium with a 1.6 mL/min flow.
   - Oven Temperature: Initial: 37°C for 1 minute,
     Temperature ramp: 5°C per minute
     Final temperature: 280°C
     Total time for the program: 49.6 minutes.
   - The injector should be in splitess mode and maintained at 180ºC . Helium is used as the carrier gas, and is purged 40 mL/min, to split vent at 0.5 minute. Injection volume is 2uL
   - The detector temperature should be maintained at 300°C with a make up gas (nitrogen) flow of 60 mL/min

J. Detection of Chemicals

Table A2.1 Retention times of HAMs and MDLs

Compounds	Acronyms	Retention Time (min)	MDL (µg/L)	MDLx (µg/L)
9 Haloacetamides	HAM_9			
Choroacetamide	MCAM	7.808	~50	500
Bromoacetamide	MBAM	10.889	~20	200
Dichloroacetamide	DCAM	11.412	0.3	3
Dibromoacetamide	DBAM	13.882	2	20
Bromochloroacetamide	BCAM	15.038	2	18
Dibromochloroacetamide	DBCAM	16.330	2	19
Bromodichloroacetamide	BDCAM	17.613	1	15
Trichloroacetamide	TCAM	20.113	1	14
Tribromoacetamide	TBAM	22.533	3	26

MDL: method detection limits for dilutions
MDLx: method detection limits for 142X RO concentrates corrected for dilution factors. HAMs concentration in 142X RO Concentrates were all quantified based on 1:10 dilutions.

K. REFERENCES

Weinberg, H. S.; Krasner, S. W.; Richardson, S. D.; Thruston Jr., A. D. The occurrence of disinfection by-products (DBPs) of health concern in drinking water: Results of a nationwide DBP occurrence study; EPA/600/R-02/068; U.S. EPA, NERL, Ecosystems Research Division: Athens,GA, September 2002 (www.epa.gov/athens/publications/DBP.html).

Chu, Wenhai, et al. "Trace determination of 13 haloacetamides in drinking water using liquid chromatography triple quadrupole mass spectrometry with atmospheric pressure chemical ionization." *Journal of Chromatography A* 1235 (2012): 178-181.

Appendix III SOP for analysis of HAAs in drinking water

A. PURPOSE AND SCOPE

This Standard Operating Procedure (SOP) describes the extraction and analysis of the nine chlorinated and brominated haloacetic acids (chloroacetic acid, bromoacetic acid, dichloroacetic acid, dibromoacetic acid, bromochloroacetic acid, trichoroacetic acid, tribromoacetic acid, bromodichloroacetic acid, dichlorobromoacetic acid) and three iodinated acetic acids (chloroiodoacetic acid, bromoiodoacetic acid, and diiodoacetic acid) in finished drinking water by liquid-liquid solvent extraction and gas chromatography with electron capture detection (GC/ECD) analysis.

B. SUMMARY

This SOP describes a liquid-liquid solvent extraction and GC/ECD direct injection analysis of nine haloacetic acids disinfection by-products (DBPs) in finished drinking water. Three milliliters of ethyl acetate are used to extract analytes from 30mL samples, and separated by GC on an HP1-MS (or DB-1) column. GC-MS should be used for analyte confirmation.

C. SAFETY AND SAMPLE HANDLING

Exposure to chemicals should be kept to a minimum. All procedures should be carried out under a functional fume hood. Safety Data Sheets should be accessible to all lab workers for proper chemical handling. Personal protective equipment including lab coat, gloves, and goggles must be worn at all times. Standard solutions are stored at -10°C. Samples are stored at 4°C.

D. APPARATUS AND MATERIALS

- 60 mL glass screw cap sample vials with polytetrafluoroethylene (PFTE)-lined screw caps.
- Glass volumetric flasks with glass stoppers
- 20 mL graduated glass pipettes and bulbs
- Vortexer
- 20ml Test tubes and screw caps
- GC autosampler vials (Agilent Technologies, Cat #5183-4493)
- Disposable glass Pasteur Pipets (VWR Scientific Cat# 14673-043)
- GC-ECD (Shimadzu) with GC Solution Software
- GC Column: 30-m, 0.25 mm ID, 0.25 µm film thickness HP1-MS (Hewlett-Packard/Agilent,
  Folsom, CA)

E. REAGENTS AND CHEMICALS

- EPA 552.2 Methyl Ester Calibration Mix w/Surrogate (Sigma-Aldrich, St. Louis, MO)
- Diiodoacetic acid

- Chloroiodoacetic acid
- Bromoiodoacetic acid
- EPA 55.2.2 Haloacetic Acids Mix(Sigma-Aldrich, St. Louis, MO)
- Surrogate,2,3-Dibromopropionic acid solution(Sigma-Aldrich, St. Louis, MO)
- 1,2 dibromopropane in MTBE (100 µg/L as internal standard)
- Methyl Tertiary Butyl Ether(MTBE) EMD OmniSolv Grade (Fisher)
- Concentrated H_2SO_4 (Sigma-Aldrich, St. Louis, MO)
- Sodium Sulfate anhydrous form(EMD)
- Sodium Bicarbonate(Sigma-Aldrich, St. Louis, MO)
- Helium gas (Praxair, 99.999% purity)
- Nitrogen gas (Praxair, 99.999% purity)

F. QC SAMPLES

7. ***Primary stocks.***
   EPA 552.2 Haloacetic Acids Mix (2000µg/mL in MTBE) is used as primary stock for nine chlorinated and brominated HAAs.
   Prepare separate stock solutions of diiodo-,chloroiodo-, and bromoiodo-acetic acids in MTBE at approximately 2000µg/mL.

8. ***Secondary Stocks.***
   150µl of the following four primary stocks are added to 10ml volumetric flask and filled up with MTBE: EPA 552.2 Haloacetic Acids Mix and 2000µg/ml diiodo-,chloroiodo-, and bromoiodo-acetic acids in MTBE. The target concentration of Haloacetic acids in secondary stocks should be 30mg/L.

9. ***Calibration standards.***
   Calibration standards are made fresh daily by diluting secondary stocks with DI water. At least five levels of calibration standards should be prepared. Typical concentration range for each is 0-80µg/L (0, 5, 10, 20, 30, 50, 80). For example, 5µL secondary stocks in 10ml DI water lead to an aqueous concentration of 5µg/L HAAs.

10. ***Internal Standard***
    A 100 µg/L 1, 2-dibromopropane solution is prepared for use as internal standard with ethyl acetate.

11. ***Surrogate Solutions.***
    100µl of surrogate stock (2,3-Dibromopropionic acid solution 1000µg/ml in MTBE) are diluted to 10ml in volumetric flask, and leads to a concentration of 10mg/L.

12. ***Matrix Spikes.***
    One sample per batch will be spiked in duplicate at 5µg/L. Secondary stocks are used to spike the sample to a final concentration of 5µg/L.

Ex. 30 mL of sample will be spiked with a 5µL of secondary stock and end up with 5 ug/L.

13. ***Blank samples.***
    - Lab Blank: Fresh DI water
    - Field Blank: DI water shipped along with samples

H. SAMPLE COLLECTION and PREPARATION

- After collection, samples are quenched with excess amount of L-ascorbic acid and can keep up to 14 days in 4 degree refrigerator until extraction.
- 30ml samples are poured in to a clean 60ml glass vial using a clean graduated cylinder.
- Add 20ul of surrogate solution into the vial.
- Add 2mL of concentrated nitric acid to adjust pH<0.5.
- Add approximately 12g anhydrous sodium sulfate (Na_2SO_4).
- Add 3ml of MTBE with internal standard using pipette.'
- Samples are vortexed for 1 min.
- Remove 1ml of first extract and place it into a 10ml test tube.
- Make solution of methanol+10% sulfuric acid. Add 2ml of this solution into the test tube.
- Place the test tube in 50 degree C heating block for 2hrs. Remove the test tube from the heating block, and wait for 5-10minutes for cooling down.
- Make a saturated sodium sulfate solution (about 150g/L). Add 4ml to the test tube.
- Add 1ml of fresh MTBE (without IS).
- Samples are vortexed for 1 min.
- 1 mL of organic layer is placed in a GC auto-sampler vial for analysis using a disposable glass Pasteur pipette.
- 1 mL of organic layer is stored for back up at -10°C.
- To check if water is left in the GC vial after freezing for at least 6 hours. Transfer organic layer into another GC vial if there is any water in the original GC vial.

I. GC ANALYSIS

3. Load the samples on the autosampler of the GC/ECD.
4. Load a method into software with the following
    - Injection volume: 1uL
    - Column parameters:
      Type: HP1-MS column; Length: 30 m; Internal Diameter: 0.25 mm; Thickness: 1µm
      Carrier gas: Helium with a total flow of 43.8 mL/min.
    - Oven Temperature: Initial: 37°C for 21 minute,
      Temperature ramp: 5 °C per minute for 19.8 min
      Hold at 136 °C for 3minutes
      Ramp to 250 °C at 20 °C/min in 5.7 minutes
      Final temperature: 250°C for 3 minutes
      Total run: 52.5 minutes

- The injector should be in split less mode with 0.50 minutes delay. Injection temperature is 180 ºC with a split flow of 40 ml/min. Injection volume is 1uL.
- The detector temperature should be maintained at 300 °C with a make-up gas (Nitrogen) flow of 1 mL/min

J. Detection of Chemicals

Table A3.1 Retention times and MDLs of HAAs

Chemicals	Retention Time (min)	MDL (µg/L)	MDLx (µg/L)
MCAA	20.33	1.1	10.8
MBAA	27.64	0.1	1.0
DCAA	28.76	0.4	4.3
BCAA	32.43	0.0	0.1
TCAA	33.73	0.0	0.2
DBAA	36.99	0.3	2.5
BDCAA	37.64	0.1	0.5
DBCAA	41.01	0.1	0.7
TBAA	44.64	0.0	0.3
CIAA	38.19	0.2	1.7
BIAA	41.08	0.0	0.3
DIAA	45.09	0.1	1.0

MDL: method detection limits for dilutions
MDLx: method detection limits for 142X RO concentrates corrected for dilution factors. HAAs concentration in 142X RO Concentrates were all quantified based on 1:10 dilutions.

K. REFERENCES

Domino, M. M., et al. *EPA Method 552.3, Revision 1.0: Determination of haloacetic acids and Dalapon in drinking water by liquid-liquid microextraction, derivatization, and gas chromatography with electron capture detection*. EPA/815/B-03/002, 2003.

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Appendix IV SOP for analysis of 28 DBPs in drinking water

A. PURPOSE AND SCOPE

This Standard Operating Procedure (SOP) describes the extraction and analysis of twenty-eight DBPs in finished drinking water by solid phase extraction and gas chromatography with mass spectrometry (GC-MS) analysis. The 28 DBPs include twelve halogenated ketones (haloketones), four regulated trihalomethanes(THM4), six iodinated trihalomethanes (iodo-THMs), one haloacetaldhyde(HA), and one halonitromethane(HNM). The twelve haloketones are : chloropropanone, 1,1-dichloropropanone, 1,3-dichloropropanone, 1,1-dibromopropanone, 1,1,1-trichloropropanone, 1,1,3-trichloropropanone, 1-bromo-1,1-dichloropropanone, 1,1,1-tribromopropanone, 1,1,3-tribromopropanone, 1,1,1,3-tetrachlropropanone, 1,1,3,3-tetrachloropropanone, and 1,1,3,3-tetrabromopropanone). THM4 are chloroform, bromodichloromethane, chlorodibromomethane and bromoform. Iodo-THMs included in this study are dichloroiodomethane, bromochloroiodomethane, dibromoiodomethane, chlorodiiodomethane, bromodiiodomethane, and iodoform. Chloral hydrate is included as a type of haloacetaldhydes, and chloropicrin is included as a type of halonitromethane. This method is applicable when determining these DBPs in concentrations greater than 0.1μg/L.

B. SUMMARY

This SOP describes a solid phase extraction and GC-MS direct injection analysis of 28 halogenated disinfection by-products (DBPs) in finished drinking water using solid phase extraction and separated by GC on an HP-1 column.

C. SAFETY AND SAMPLE HANDLING

Exposure to chemicals should be kept to a minimum. All procedures should be carried out under a functional fume hood. Safety Data Sheets should be accessible to all lab workers for proper chemical handling. Personal protective equipment including lab coat, gloves, and goggles must be worn at all times. Standard solutions and extracts are stored at -20°C. Raw samples and working solutions are stored at 4°C.

D. APPARATUS AND MATERIALS

- 40 mL glass screw cap sample vials with polytetrafluoroethylene (PFTE)-lined screw caps.
- Glass volumetric flasks with glass stoppers
- 20 mL graduated glass pipettes and bulbs
- Vortexer
- 0.45μm, 25mm ID syringe filters
- 12 port Visiprep vacuum manifold
- Positive displacement pipettes with glass capillary tips (VWR Scientific)
- GC autosampler vials
- GC-ECD (Shimadzu) with GC Solution Software
- GC Column: 30-m, 0.25 mm ID, 1 μm film thickness HP-1-MS ultra inert; 7in cage (Agilent, Folsom, CA) P/N: 19091S-733UI
- 40 mL glass screw cap sample vials with polytetrafluoroethylene (PFTE)-lined screw caps.
- Varian Bond Elut PPL solid phase extraction cartridges, 200 mg

E. REAGENTS AND CHEMICALS

- Chloropopanone (Sigma-Aldrich, St Louis, MO) P/N: 167479-5G
- 1,1-Dichloropropanone (Sigma-Aldrich, St Louis, MO) P/N: 442210
- 1,3-Dichloropropanone (Sigma-Aldrich, St Louis, MO) P/N: 168548-10G
- 1,1-Dibromopropanone (CanSyn Chem Corp, Toronto, Ontario) P/N: HK001
- 1,1,1-Trichloropropanone (Sigma-Aldrich, St Louis, MO) P/N: S439940-250MG
- 1,1,3-Trichloropropanone (Sigma-Aldrich, St Louis, MO) P/N: 10879-1KG
- 1-Bromo-1,1-dichloropropanone (CanSyn Chem Corp, Toronto, Ontario)
- 1,1,1-Tribromopropanone (CanSyn Chem Corp, Toronto, Ontario) P/N: HK002
- 1,1,3-Tribromopropanone (CanSyn Chem Corp, Toronto, Ontario) P/N: HK003
- 1,1,1,3-Tetrachloropropanone (CanSyn Chem Corp, Toronto, Ontario) P/N: HK009
- 1,1,3,3-Tetrachloropropanone (CanSyn Chem Corp, Toronto, Ontario) P/N: HK008
- 1,1,3,3-Tetrabromopropanone (Sigma-Aldrich, St Louis, MO) P/N: T295833-1EA
- EPA 551A mix (Sigma-Aldrich, St Louis, MO) P/N: 4M8140-U
- EPA 551B mix (Sigma-Aldrich, St Louis, MO) P/N: 48046
- Dichloroiodomethane (CanSyn Chem Corp, Toronto, Ontario) P/N: HM006
- Bromochloroiodomethane (CanSyn Chem Corp, Toronto, Ontario) P/N: HM003
- Dibromoiodomethane (CanSyn Chem Corp, Toronto, Ontario) P/N: HM005
- Chlorodiiodomethane (CanSyn Chem Corp, Toronto, Ontario) P/N: HM004
- Bromodiiodomethane (CanSyn Chem Corp, Toronto, Ontario) P/N: HM002
- Iodoform (Sigma Aldrich, St Louis, MO) P/N: 109452-5G
- 1,2-Dibromopropane (97%)(Sigma Aldrich, St Louis, MO)
- Acetonitrile (Fisher Scientific, Fair Lawn, NJ) P/N: A955-4, Optima LC/MS
- Methanol (Fisher Scientific, Fair Lawn, NJ) P/N: A456-4, Optima LC/MS or Optima 0.2mm grade
- Sulfuric acid (95 – 98%) (JT Baker, Phillipsburg, NJ) P/N: 9675-02, HPLC or analytical grade
- Organic-free deionized water or also referred to as MilliQ water (Barnstead Easypure, Dubuke, IA)
- Sodium sulfite (90 – 100%) (Mallinckrodt Chemicals, Phillipsburg, NJ)
- Methyl tertiary-butyl ether (EMD, Gibbstown, NJ) P/N: , OmniSolv® grade
- Helium gas (Praxair, 99.999% purity)
- 5% Methane/Argon gas (Praxair, 99.999% purity)

F. QC SAMPLES

- Primary stocks
  Individual compound of the 28DBPs was prepared at a concentration of about 4000 mg/L in acetonitrile.

- Secondary Stocks
  A mixture of the 28DBPs was prepared in acetonitrile (CH_3CN) at a concentration of 40 mg/L by diluting each individual compound.

- External calibration standards
  Diluting the secondary stock solution (40 mg/L 28DBPs) into methyl tertiary-butyl ether for a total of five calibration standards with concentration range is 22.5-900µg/L. (36/1.6=22.5, 22.5 times the method calibration sample concentration)

- Internal standard
  Fresh internal standard solution of 200µg/L 1,2-dibromopropane is prepared each analytical day in methyl tertiary-butyl ether (MtBE).

- Matrix Spikes
  Secondary stocks are used to spike the sample to a final concentration of 11.1 µg/L (10 µL of 40 mg/L 28DBPs in 36 mL sample).

- Blank samples.
  - Lab Blank: MilliQ (MQ) water
  - Field Blank: MQ water shipped along with samples

H. SAMPLE COLLECTION AND PREPARATION

- Samples were diluted as required with MQ water in the lab.
- After proper dilution, pH of diluted samples were adjusted to be below 3.5 (2.5-3.5) with 5M hydrochloric acid (HCl).
- pH-adjusted diluted samples were kept head space free in the 40 mL glass vial before SPE.

I. SOLID PHASE EXTRACTION

- First condition SPE cartridges with a total 8 mL of methanol (MeOH) at a flow rate of 10 mL/min under vacuum; stop the flow when ethanol just reaches the top of SPE cartridge resin.
- Follow MeOH with 36 mL of acidified (pH below 3.5) and diluted water samples at a flow rate of 5 mL/min; for matrix spiked samples, spike 10 µL of secondary standard (40 mg/L 29DBPs) at this step; stop the flow when sample just stopped dripping (no forced drying).
- Add 1.6 mL MtBE with 200 µg/L 1,2-dibromopropane; control the flow rate to be 1 mL/min; collect eluted samples in 2mL GC vials.
- Allow collected samples to stay in freezer overnight; then collect only the upper level (MtBE layer) of samples to clean GC vials. Save GC vials in freezer for future GC-MS analysis.

J. GC ANALYSIS

- Load the samples on the autosampler of the GC/ECD.
- Load a method into software with the following
  - Inject 1.9µL at rate of 0.7µL/sec
  - Column parameters:

  Type: HP-1-MS ultra inert column; Length: 30 m; Internal Diameter: 0.25 mm; Thickness: 1µm with a 7in cage
  - Oven Temperature Program:

    - 35°C for 1min,
    - 4°C/min to 103°C with no hold time,
    - 27°C/min to 292°C, hold for 2min
    - Total run time: 57.0min
- Injector Program: 0.77min splitless injection with a split ratio of 19:1
- Carrier gas: Helium with a 1.7 mL/min flow
- Make-up gas: 5% Methane/Argon with a total flow rate of 38.7mL/min

K. Detection of Chemicals

Table A4.1 Ion monitoring and retention times for 28 DBPs in GC/MS and MDLs

Compound	Acronym	Quant ion m/z	Qual ion m/z	RT (min)	MDL (μg/L)	MDLx (μg/L)		
						A	B	C
Chloroform	TCM	83	49	5.16	3.8	38	38/380[#]	380
Chloropropanone	CP	43	49	7.00	3.0	30	6.0	6.0
Trichloroacetonitrile	TCAN	108	49	7.57	2.4	4.8	4.8	4.8
Dichloroacetonitrile	DCAN	74	49	8.79	2.2	4.4	4.4	4.4
Bromodichloromethane	BDCM	83	79	9.02	1.7	17	17/170[#]	170
Chloral Hydrate	TCA	82	145.9	9.78	2.3	4.6	4.6	4.6
1,1-Dichloropropanone	11DCP	43	93	10.90	1.9	19	3.8	3.8
Chloropicrin	TCNM	117	49	17.00	1.6	3.2	3.2	3.2
Dibromochloromethane	DBCM	127	91	17.69	1.4	2.8	14	140
Bromochloroacetonitrile	BCAN	74	none	18.40	1.1	2.2	2.2	2.2
Dichloroiodomethane	DCIM	83	127	21.71	1.4	14	14	2.8
1,1,1-Trichloropropanone	111TCP	43	83	26.43	2.0	4.0	4.0	4.0
1,2-Dibromopropane	ISTD*	121	none	27.33	/	/	/	/
1,3-Dichloropropanone	13DCP	77	49	28.21	1.3	2.6	2.6	2.6
Bromoform	TBM	173	91	29.94	1.6	3.2	16	160
Dibromoacetonitrile	DBAN	118	79	30.98	2.0	4.0	4.0	4.0
1,1-Dibromopropanone	11DBP	43	173	31.94	1.5	3.0	3.0	3.0
Bromochloroiodomethane	BCIM	127	175	32.16	1.3	2.6	13	2.6
1-Bromo-1,1-dichloropropanone	1B11DCP	43	127	34.63	1.6	3.2	3.2	3.2
1,1,3-Trichloropropanone	113TCP	77	83	35.48	1.2	2.4	2.4	2.4
Dibromoiodomethane	DBIM	173	127	38.17	3.7	7.4	7.4	7.4
Chlorodiiodomethane	CDIM	175	127	39.56	1.3	2.6	2.6	2.6
1,1,3,3,-Tetrachloropropanone	1133TeCP	83	none	40.10	2.9	5.8	5.8	5.8
1,1,1,3-Tetrachloropropanone	1113TeCP	77	49	40.63	1.8	3.6	3.6	3.6
Bromodiiodomethane	BDIM	219	127	44.11	2.6	5.2	5.2	5.2
1,1,1-Tribromopropanone	111TBP	43	251	44.67	2.2	4.4	4.4	4.4
1,1,3-Tribromopropanone	113TBP	121	93	47.94	0.9	1.8	1.8	1.8
Iodoform	TIM	127	267	49.15	9.8	19.6	19.6	19.6
1,1,3,3-Tetrabromopropanone	1133TeBP	120	173	51.99	8.2	16.4	16.4	16.4

MDL: method detection limits for dilutions
MDLx: method detection limits for 142X RO concentrates corrected for dilution factors.
Chloroform and bromodichloromethane were quantified based on two different dilutions, and therefore two different MDLx values were listed here.
* ISTD = Internal standard used for relative area quantification

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Appendix V DBP concentrations in 142X chloraminated RO concentrates

Table A5.1 DBP categories concentration in 142X chloraminated RO concentrates

DBP Categories Concentration (µg/L)	THM_4	Iodo-THMs	HKs	HANs	Chloropicrin	Chloral Hydrate	HAA_9	Iodo-HAAs	HAMs
Batch A	486.8	55.1	115.1	17.9	3.9	10.5	640.2	1.4	317.41
	441.5	48.8	117.2	17.0	3.8	9.0	801.8	2.3	300.23
	385.7	44.5	102.9	12.2	2.6	6.5	626.6	1.1	308.18
Batch B	4138.2	509.5	150.9	129.4	15.2	47.5	1673.8	27.0	599.99
	4050.4	511.8	139.1	127.9	14.6	55.9	1440.6	19.7	629.72
	3535.2*	443.7	137.5	128.8	14.9	48.5	1529.0	19.3	580.75
Batch C	5183.6	304.1	136.9	232.9	22.3	82.1	2905.4	3.7	822.75
	5514.7	350.7	131.1	221.5	22.6	81.4	3277.3	8.2	753.71
	5423.7	327.2	136.1	244.4	24.5	93.1	3199.8	4.6	758.96
Batch A Average	438.0	49.5	111.7	15.7	3.4	8.7	689.5	1.6	308.6
Batch B Average	4094.3	488.3	142.5	128.7	14.9	50.7	1547.8	22.0	603.5
Batch C Average	5374.0	327.3	134.7	232.9	23.1	85.5	3127.5	5.5	778.5
Batch A SAMPLE SD	50.66	5.30	7.75	3.08	0.75	2.05	97.49	0.61	8.60
Batch B SAMPLE SD	62.07	38.67	7.31	0.76	0.31	4.58	117.70	4.31	24.67
Batch C SAMPLE SD	171.04	23.33	3.15	11.48	1.16	6.59	196.19	2.36	38.44

* Not included in average and SD calculation because it is quantified in another sample dilution

Table A5.2 THM_4 concentration in 142X chloraminated RO concentrates

THM_4 Concentration (µg/L)	TCM	BDCM	DBCM	TBM
Batch A	451.8	32.8	2.2	N.D.
	406.9	32.0	2.6	N.D.
	353.4	30.4	1.9	N.D.
Batch B	2059.5	1436.5	523.8	118.5
	1924.9	1404.8	583.2	137.5
	1506.1*	1272.8*	607.3	149.0
Batch C	1725.5	1788.5	1187.8	481.8
	1911.2	1909.2	1235.1	459.2
	1828.3	1870.9	1223.5	500.9
Batch A average	404.0	31.7	2.2	N.D.
Batch B average	1992.2	1371.4	571.4	135.0
Batch C average	1821.7	1856.2	1215.5	480.6
Batch A SAMPLE sd	49.23	1.27	0.37	N.A.
Batch B SAMPLE sd	95.15	86.81	42.98	15.39
Batch C SAMPLE sd	93.03	61.67	24.69	20.91

* Not included in average and SD calculation because it is quantified in another sample dilution
N.D.: Not detected.
N.A.: Not Applicable

Table A5.3 Iodo-THMs concentration in 142X chloraminated RO concentrates

Iodo-THMs Concentration (µg/L)	DCIM	BCIM	DBIM	CDIM	BDIM	TIM
Batch A	40.3	3.7	N.D.	6.1	N.D.	5.1
	39.8	3.7	N.D.	5.3	N.D.	N.D.
	36.8	3.5	N.D.	4.2	N.D.	N.D.
Batch B	162.8	100.5	37.4	73.0	23.5	112.3
	169.7	110.4	40.3	71.7	23.5	96.2
	157.0	106.6	35.1	58.8	19.5	66.7
Batch C	132.9	105.2	38.9	18.0	9.1	N.D.
	160.6	118.4	40.8	20.3	10.6	N.D.
	150.4	112.2	37.6	17.3	9.7	N.D.
Batch A Average	39.0	3.6	N.D.	5.2	N.D.	N.D.
Batch B Average	163.2	105.8	37.6	67.8	22.2	91.7
Batch C Average	148.0	111.9	39.1	18.6	9.8	N.D.
Batch A SAMPLE SD	1.86	0.13	N.A.	0.91	N.A.	N.A.
Batch B SAMPLE SD	6.37	5.01	2.62	7.82	2.31	23.16
Batch C SAMPLE SD	14.01	6.61	1.64	1.58	0.75	N.A.

N.D.: Not detected.
N.A.: Not Applicable

Table A5.4 HAA_9 concentration in 142X chloraminated RO concentrates

HAA9 Concentration (µg/L)	MCAA	MBAA	DCAA	BCAA	TCAA	DBAA	BDCAA	DBCAA	TBAA
Batch A	N.D.	N.D.	554.2	67.7	4.4	8.0	N.D.	4.5	1.4
	N.D.	N.D.	685.0	84.6	5.8	14.6	N.D.	11.4	0.5
	N.D.	N.D.	542.8	69.0	4.5	9.1	N.D.	0.6	0.7
Batch B	93.4	12.2	616.2	335.0	239.7	103.0	205.7	60.2	8.4
	N.D.	N.D.	569.0	312.9	211.1	100.6	184.1	52.5	10.3
	N.D.	N.D.	593.5	347.2	214.0	110.9	192.7	59.4	11.3
Batch C	118.6	51.2	959.7	568.8	327.7	222.8	427.0	193.1	36.6
	111.4	45.3	989.6	622.7	423.6	249.2	535.6	248.4	51.5
	120.1	50.1	1004.6	626.4	389.6	252.3	489.9	222.4	44.4
Batch A Average	N.D.	N.D.	594.0	73.8	4.9	10.5	N.D.	5.5	0.9
Batch B Average	N.D.	N.D.	592.9	331.7	221.6	104.8	194.2	57.4	10.0
Batch C Average	116.7	48.9	984.6	606.0	380.3	241.4	484.2	221.3	44.2
Batch A SAMPLE SD	N.A.	N.A.	79.01	9.36	0.78	3.57	N.A.	5.48	0.49
Batch B SAMPLE SD	N.A.	N.A.	23.61	17.39	15.70	5.38	10.86	4.22	1.45
Batch C SAMPLE SD	4.69	3.10	22.87	32.22	48.62	16.21	54.57	27.67	7.43

N.D.: Not detected.
N.A.: Not Applicable

Table A5.5 Iodo-HAAs concentration in 142X chloraminated RO concentrates

Iodo-HAAs Concentration (µg/L)	CIAA	BIAA	DIAA
Batch A	N.D.	N.D.	N.D.
	N.D.	N.D.	N.D.
	N.D.	N.D.	N.D.
Batch B	27.0	N.D.	N.D.
	19.7	N.D.	N.D.
	19.3	N.D.	N.D.
Batch C	3.7	N.D.	N.D.
	8.2	N.D.	N.D.
	4.6	N.D.	N.D.
Batch A Average	1.0	N.D.	0.6
Batch B Average	22.0	N.D.	N.D.
Batch C Average	5.5	N.D.	N.D.
Batch A SAMPLE SD	0.40	N.A.	0.24
Batch B SAMPLE SD	4.31	N.A.	N.A.
Batch C SAMPLE SD	2.36	N.A.	N.A.

N.D.: Not detected.
N.A.: Not Applicable

Table A5.6 HAMs concentration in 142X chloraminated RO concentrates

HAMs Concentration (µg/L)	DCAM	BCAM	TCAM	DBAM	DCBAM	DBCAM	TBAM
Batch A	259.5	27.4	BDL	30.5	BDL	BDL	BDL
	249.8	28.6	BDL	21.8	BDL	BDL	BDL
	257.9	29.4	BDL	20.9	BDL	BDL	BDL
Batch B	276.4	109.9	25.9	102.1	34.6	BDL	51.1
	278.0	108.8	25.1	104.6	33.9	27.4	51.9
	239.7	96.5	24.8	103.3	30.7	35.1	50.6
Batch C	273.0	162.4	30.2	229.1	29.4	37.6	61.0
	264.5	141.2	28.4	196.0	26.2	36.1	61.3
	254.3	125.2	28.5	219.8	26.2	41.6	63.4
Batch A Average	255.7	28.5	N.D.	24.4	N.D.	N.D.	N.D.
Batch B Average	264.7	105.1	25.3	103.3	33.1	31.2	51.2
Batch C Average	263.9	142.9	29.0	215.0	27.2	38.5	61.9
Batch A SAMPLE SD	5.19	1.02	N.A.	5.31	N.A.	N.A.	N.A.
Batch B SAMPLE SD	21.68	7.41	0.56	1.27	2.07	5.43	0.67
Batch C SAMPLE SD	9.40	18.65	1.01	17.08	1.84	2.85	1.33

N.D.: Not detected.
N.A.: Not Applicable

Table A5.7 HKs concentration in 142X chloraminated RO concentrates (part 1)

HKs Concentration (µg/L)	Chloropropanone	1,1-Dichloropropanone	1,3-Dichloropropanone	1,1-Dibromopropanone	1,1,1-Trichloropropanone	1,1,3-Trichloropropanone	1-Bromo-1,1-dichloropropanone
Batch A	51.6	56.9	2.4	N.D.	N.D.	4.2	N.D.
	54.6	55.8	2.5	N.D.	N.D.	4.3	N.D.
	49.0	47.4	2.4	N.D.	N.D.	4.1	N.D.
Batch B	58.3	57.4	4.5	6.7	9.1	7.6	N.D.
	55.3	51.1	4.4	6.8	7.2	7.3	N.D.
	52.6	51.3	4.5	6.7	8.8	7.4	N.D.
Batch C	44.3	51.5	4.4	5.7	9.2	6.4	4.9
	40.4	50.6	4.3	5.6	8.9	6.6	4.7
	42.7	51.0	4.6	5.9	10.5	6.9	4.8
Batch A average	51.7	53.4	2.5	N.D.	N.D.	4.2	N.D.
Batch B average	55.4	53.3	4.5	6.8	8.4	7.4	N.D.
Batch C average	42.5	51.0	4.4	5.7	9.5	6.6	4.8
Batch A SAMPLE sd	2.80	5.17	0.07	N.A.	N.A.	0.13	N.A.
Batch B SAMPLE sd	2.87	3.58	0.06	0.08	1.00	0.16	N.A.
Batch C SAMPLE sd	1.98	0.45	0.14	0.18	0.83	0.24	0.10

N.D.: Not detected.

N.A.: Not Applicable

Table A5.8 HKs concentration in 142X chloraminated RO concentrates (part 2)

HKs Concentration (µg/L) cont.	1,1,1-Tribromopropanone	1,1,3-Tribromopropanone	1,1,1,3-Tetrachloropropanone	1,1,3,3-Tetrachloropropanone	1,1,3,3-Tetrabromopropanone
Batch A	N.D.	N.D.	N.D.	N.D.	N.D.
	N.D.	N.D.	N.D.	N.D.	N.D.
	N.D.	N.D.	N.D.	N.D.	N.D.
Batch B	N.D.	N.D.	N.D.	7.3	N.D.
	N.D.	N.D.	N.D.	6.9	N.D.
	N.D.	N.D.	N.D.	6.3	N.D.
Batch C	4.5	N.D.	N.D.	5.9	N.D.
	4.3	N.D.	N.D.	5.7	N.D.
	4.0	N.D.	N.D.	5.7	N.D.
Batch A Average	N.D.	N.D.	N.D.	N.D.	N.D.
Batch B Average	N.D.	N.D.	N.D.	6.8	N.D.
Batch C Average	4.3	N.D.	N.D.	5.8	N.D.
Batch A SAMPLE SD	N.A.	N.A.	N.A.	N.A.	N.A.
Batch B SAMPLE SD	N.A.	N.A.	N.A.	0.51	N.A.
Batch C SAMPLE SD	0.28	N.A.	N.A.	0.12	N.A.

N.D.: Not detected.

N.A.: Not Applicable.

Table A5.9 HANs, HNM and HA concentration in 142X chloraminated RO concentrates

Other DBPs Concentration (µg/L)	DCAN	BCAN	DBAN	TCAN	Chloropicrin	Chloral Hydrate
Batch A	13.3	4.6	N.D.	N.D.	3.9	10.5
	12.4	4.6	N.D.	N.D.	3.8	9.0
	8.4	3.8	N.D.	N.D.	2.6	6.5
Batch B	65.3	43.2	20.9	N.D.	15.2	47.5
	59.5	44.4	24.0	N.D.	14.6	55.9
	60.6	45.1	23.1	N.D.	14.9	48.5
Batch C	83.9	91.7	57.2	N.D.	22.3	82.1
	82.3	87.0	52.1	N.D.	22.6	81.4
	89.0	96.9	58.5	N.D.	24.5	93.1
Batch A Average	11.4	4.3	N.D.	N.D.	3.4	3.4
Batch B Average	61.8	44.2	22.7	N.D.	14.9	14.9
Batch C Average	85.1	91.9	56.0	N.D.	23.1	23.1
Batch A SAMPLE SD	2.62	0.47	NA	N.A.	0.75	0.75
Batch B SAMPLE SD	3.10	0.98	1.62	N.A.	0.31	0.31
Batch C SAMPLE SD	3.50	4.95	3.37	N.A.	1.16	1.16

N.D.: Not detected.

N.A.: Not Applicable.

Appendix VI Toxicological review for 17 DBPs

Table A6.1 17 DBPs reproductive/developmental effect [a]

Chemical	Literature NOAEL[b](mg/kg/d)	Reproductive/developmental studies effect [c]
Chloroform	50	Reduced fetal weight
Bromoform	100	Full-litter resorption
Bromodichloromethane	25	Full-litter resorption
Chlorodibromomethane	40	No detected effects
Monochloroacetic acid	70	Heart
Dichloroacetic acid	14	Embryo resorption
Trichloroacetic acid	33	Resorption LOAEL=330mg/kg/d [d]
Monobromoacetic acid	50	Fetal effects
Dibromoacetic acid	13	Mice, delayed parturition= 25mg/kg/d
Bromochloroacetic acid	20	Pup viability
Tribromoacetic acid	39	No detected effects
Dibromochloroacetic acid	89[42]	Decrease of sperm velocity and ALH max[42]
Dichloroacetonitrile	55	Increased fetal resorption
Bromochloroacetonitrile	5.5	Fetal weight LOAEL=55 mg/kg/d [d]
Dibromoacetonitrile	5	Fetal weight LOAEL=50 mg/kg/d [d]
Trichloroacetonitrile	1	Increased fetal resorption
1,1,3,3-tetrachloropropanone	4.8[43]	Mice, delayed ossification=5mg/kg/d[43]

a Source of information in the table is Rice etal. 2008[16] unless noted
b Dose conversion, d(rat)=d(species)*[weight(species)/weight(rat)]0.25, rat=0.3 kg, mouse =0.25kg
c Studies based on rat bioassay unless noted
d If NOAEL is no available, a factor of 10 is used to get NOAEL from LOAEL

Table A6.2 17 DBPs Kow values

Chemical	Log Kow			
	Experimental Value [a]	Estimated value by XlogP3-AA method [b]	Estimated value by EPA EPISUITE [a]	Estimated value by ACD method [a]
Chloroform	1.97	2.3	1.52	1.75
Bromoform	2.4	2.8	1.79	2.29
Bromodichloromethane	2	2.4	1.61	2.02
Dibromochloromethane	2.16	2.6	1.7	2.2
Monochloroacetic acid	0.22	0.2	0.34	-0.05
Dichloroacetic acid	0.92	0.9	0.52	0.54
Trichloroacetic acid	1.33	1.3	1.44	1.67
Monobromoacetic acid	0.41	0.4	0.43	0.51
Dibromoacetic acid	N.A.	1.5	0.7	1.65
Bromochloroacetic acid	N.A.	1.3	0.61	1.14
Tribromoacetic acid	N.A.	2.2	1.71	3.33
Dibromochloroacetic acid	N.A.	2	1.62	2.86
Dichloroacetonitrile	N.A.	1.3	0.29	1.07
Bromochloroacetonitrile	N.A.	1.5	0.38	1.23
Dibromoacetonitrile	N.A.	1.7	0.47	1.3
Trichloroacetonitrile	2.09	2.1	1.21	2.54
1,1,3,3-tetrachloropropanone	N.A.	2.6	0.63	4.02

N.A.: Not available
a: information obtained from http://www.chemspider.com/
b: information obtained from https://pubchem.ncbi.nlm.nih.gov/

Appendix VII Dose-Response Curve: Benchmark Dose Software Output

Data for BDCM's dose-response curve was obtained from Narotsky et al. 1997 [41].

Table A7.1 Data for BDCM's dose-response curve

Dosage (mg/kg/d)	N (sample)	Effect (positive effect sample)
0	14	0
25	12	0
50	12	2
75	14	3

Based on the shape of the dose-response curve, the log-logistic model of Benchmark Dose Software was chosen to fit the curve. Followed is the brief summary of the software's output.

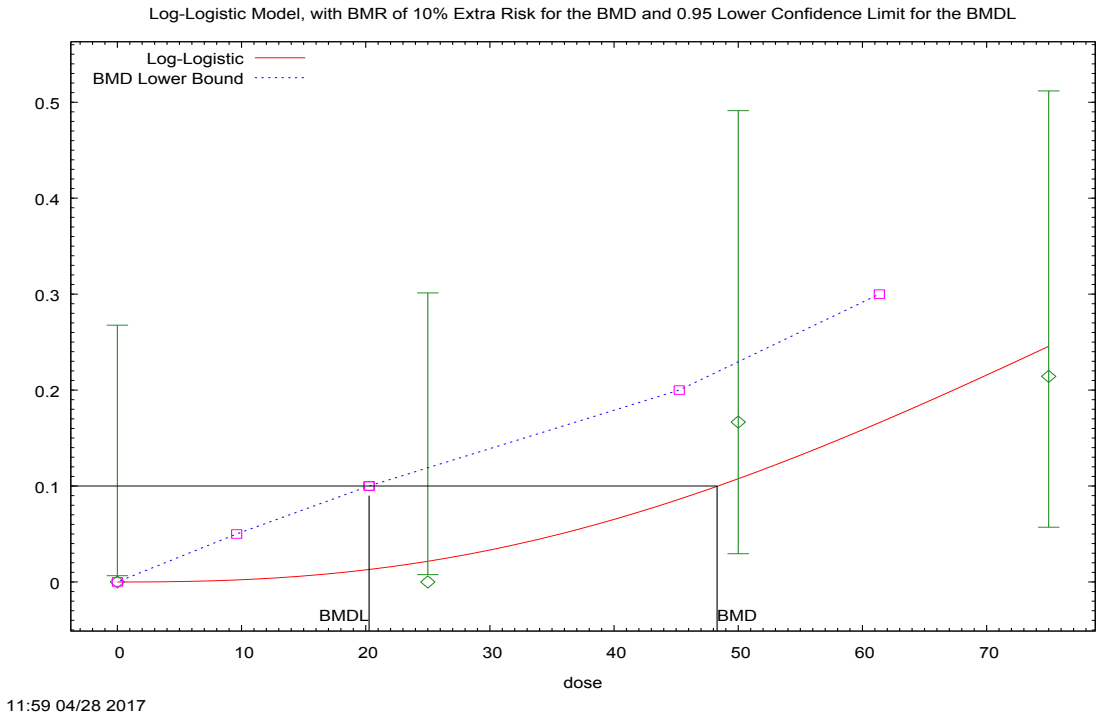

Figure A6.0.1 Log-logistic model fitness for BDCM's dose-response curve

BMDS_Model_Run
The form of the probability function is:
P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
Dependent variable = Effect
Independent variable = Dose
Slope parameter is restricted as slope >= 1
Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Table A7.2 Model estimates for BDCM's dose-response curve

	Parameter Estimates		**95.0% Wald Confidence Interval**	
Variable	**Estimate**	**Std. Error**	**Lower Confidence Limit**	**Upper Confidence Limit**
Background	0	NA	NA	NA
Intercept	-11.689	6.92646	-25.2646	1.88663
Slope	2.44801	1.67242	-0.82987	5.72589

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Appendix VIII RPF-based health risk estimation calculation for 142X RO concentrates

Table A8.1 Health risk calculation for 142X chloraminated RO concentrates: Batch A

Batch A Chemical	Literature NOAEL (mg/kg/d)	RPF	Concentration in 142X RO Concentrates (mg/L)	Water consumption (Lwater/kgbody weight/d)	ICED (mg/kg/d) =Concentration×RPF ×Water Consumption
Chloroform	50	0.5	4.04E-01	0.1	2.02E-02
Bromoform	100	0.3	0.00E+00	0.1	0.00E+00
Bromodichloromethane	25	1.0	3.17E-02	0.1	3.17E-03
Chlorodibromomethane	40	0.6	2.20E-03	0.1	1.32E-04
Monochloracetic acid	70	0.4	0.00E+00	0.1	0.00E+00
Dichloroacetic acid	14	1.8	5.94E-01	0.1	1.07E-01
Trichloroacetic acid	33	0.8	4.90E-03	0.1	3.92E-04
Monobromoacetic acid	50	0.5	0.00E+00	0.1	0.00E+00
Dibromoacetic acid	13	1.9	1.05E-02	0.1	2.00E-03
Bromochloroacetic acid	20	1.3	7.38E-02	0.1	9.59E-03
Tribromoacetic acid	39	0.6	9.00E-04	0.1	5.40E-05
Dibromochloroacetic acid	89	13.0	5.50E-03	0.1	1.65E-04
Dichloroacetonitrile	55	0.5	1.14E-02	0.1	5.70E-04
Bromochloroacetonitrile	5.5	4.5	4.30E-03	0.1	1.94E-03
Dibromoacetonitrile	5	5.0	0.00E+00	0.1	0.00E+00
Trichloroacetonitrile	1	25.0	0.00E+00	0.1	0.00E+00
1,1,3,3-tetrachloropropanone	4.8	5.2	0.00E+00	0.1	0.00E+00
Total ICED in Batch A (mg/kg/d)					1.45E-01
$P(effect) = 1/[1 + \exp(11.7 - 2.5\times \ln(dosage))]$					7.33E-08

Table A8.2 Health risk calculation for 142X chloraminated RO concentrates: Batch B

Batch B Chemical	Literature NOAEL (mg/kg/d)	RPF	Concentration in 142X RO Concentrates (mg/L)	Water consumption (Lwater/kgbody weight/d)	ICED (mg/kg/d) =Concentration×RPF ×Water Consumption
Chloroform	50	0.5	1.99E+00	0.1	9.96E-02
Bromoform	100	0.3	1.35E-01	0.1	4.05E-03
Bromodichloromethane	25	1.0	1.42E+00	0.1	1.42E-01
Chlorodibromomethane	40	0.6	5.71E-01	0.1	3.43E-02
Monochloracetic acid	70	0.4	0.00E+00	0.1	0.00E+00
Dichloroacetic acid	14	1.8	5.93E-01	0.1	1.07E-01
Trichloroacetic acid	33	0.8	2.22E-01	0.1	1.77E-02
Monobromoacetic acid	50	0.5	0.00E+00	0.1	0.00E+00
Dibromoacetic acid	13	1.9	1.05E-01	0.1	1.99E-02
Bromochloroacetic acid	20	1.3	3.32E-01	0.1	4.31E-02
Tribromoacetic acid	39	0.6	1.00E-02	0.1	6.00E-04
Dibromochloroacetic acid	89	13.0	5.74E-02	0.1	1.72E-03
Dichloroacetonitrile	55	0.5	6.18E-02	0.1	3.09E-03
Bromochloroacetonitrile	5.5	4.5	4.42E-02	0.1	1.99E-02
Dibromoacetonitrile	5	5.0	2.27E-02	0.1	1.14E-02
Trichloroacetonitrile	1	25.0	0.00E+00	0.1	0.00E+00
1,1,3,3-tetrachloropropanone	4.8	5.2	6.80E-03	0.1	3.54E-03
Total ICED in Batch B (mg/kg/d)					4.95E-01
$P(effect) = 1/[1 + \exp(11.7 - 2.5\times \ln(dosage))]$					1.48E-06

Table A8.3 Health risk calculation for 142X chloraminated RO concentrates: Batch C

Batch C Chemical	Literature NOAEL (mg/kg/d)	RPF	Concentration in 142X RO Concentrates (mg/L)	Water consumption (Lwater/kgbody weight/d)	ICED(mg/kg/d) =Concentration×RPF ×Water Consumption
Chloroform	50	0.5	1.82E+00	0.1	9.11E-02
Bromoform	100	0.3	4.81E-01	0.1	1.44E-02
Bromodichloromethane	25	1.0	1.86E+00	0.1	1.86E-01
Chlorodibromomethane	40	0.6	1.22E+00	0.1	7.29E-02
Monochloracetic acid	70	0.4	1.17E-01	0.1	4.67E-03
Dichloroacetic acid	14	1.8	9.85E-01	0.1	1.77E-01
Trichloroacetic acid	33	0.8	3.80E-01	0.1	3.04E-02
Monobromoacetic acid	50	0.5	4.89E-02	0.1	2.45E-03
Dibromoacetic acid	13	1.9	2.41E-01	0.1	4.59E-02
Bromochloroacetic acid	20	1.3	6.06E-01	0.1	7.88E-02
Tribromoacetic acid	39	0.6	4.42E-02	0.1	2.65E-03
Dibromochloroacetic acid	89	13.0	2.21E-01	0.1	6.64E-03
Dichloroacetonitrile	55	0.5	8.51E-02	0.1	4.26E-03
Bromochloroacetonitrile	5.5	4.5	9.19E-02	0.1	4.14E-02
Dibromoacetonitrile	5	5.0	5.60E-02	0.1	2.80E-02
Trichloroacetonitrile	1	25.0	0.00E+00	0.1	0.00E+00
1,1,3,3-tetrachloropropanone	4.8	5.2	5.80E-03	0.1	3.02E-03
Total ICED in Batch C (mg/kg/d)					7.89E-01
$P(effect) = 1/[1 + \exp(11.7 - 2.5\times \ln(dosage))]$					4.65E-06

Appendix IX DOC-normalized RPF-based health risk estimation calculation

Table A9.1 DOC-normalized concentration in 142X chloraminated RO concentrates and 136X chlorinated RO concentrates

Chemical	DBP concentrations in RO concentrates normalized by DOC (µg/L DBP per mg/L DOC) [a]			
	Batch A	Batch B	Batch C	EPA 4Lab chlorination [b]
Chloroform	1.42E+00	6.98E+00	6.38E+00	2.22E+01
Bromoform	0.00E+00	4.73E-01	1.68E+00	1.67E-01
Bromodichloromethane	1.11E-01	4.98E+00	6.50E+00	1.03E+01
Chlorodibromomethane	7.71E-03	2.00E+00	4.26E+00	2.14E+00
Monochloracetic acid	0.00E+00	0.00E+00	4.09E-01	0.00E+00
Dichloroacetic acid	2.08E+00	2.08E+00	3.45E+00	1.41E+01
Trichloroacetic acid	1.72E-02	7.76E-01	1.33E+00	2.14E+01
Monobromoacetic acid	0.00E+00	0.00E+00	1.71E-01	0.00E+00
Dibromoacetic acid	3.68E-02	3.67E-01	8.46E-01	5.98E-01
Bromochloroacetic acid	2.59E-01	1.16E+00	2.12E+00	3.55E+00
Tribromoacetic acid	3.15E-03	3.50E-02	1.55E-01	0.00E+00
Dibromochloroacetic acid	1.93E-02	2.01E-01	7.75E-01	5.56E+00
Dichloroacetonitrile	3.99E-02	2.17E-01	2.98E-01	7.69E-01
Bromochloroacetonitrile	1.51E-02	1.55E-01	3.22E-01	3.16E-01
Dibromoacetonitrile	0.00E+00	7.95E-02	1.96E-01	2.82E-01
Trichloroacetonitrile	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1,1,3,3-tetrachloropropanone	0.00E+00	2.38E-02	2.03E-02	3.42E-02

a: DBP concentrations (µg/L) are divided by DOC concentration (mg/L).
b: DBP concentration in EPA 4lab study chlorinated RO concentrates was obtained from a published paper[17].

Table A9.2 DOC-normalized health risk calculation for 142X chloraminated RO concentrates: Batch A

Batch A Chemical	Literature NOAEL (mg/kg/d)	RPF	DOC-normalized concentration in 142X RO Concentrates (mg/L)	Water consumption (Lwater/kgbody weight/d)	ICED (mg/kg/d) =Concentration×RPF ×Water Consumption
Chloroform	50	0.5	1.42E+00	0.1	7.08E-05
Bromoform	100	0.3	0.00E+00	0.1	0.00E+00
Bromodichloromethane	25	1.0	1.11E-01	0.1	1.11E-05
Chlorodibromomethane	40	0.6	7.71E-03	0.1	4.62E-07
Monochloracetic acid	70	0.4	0.00E+00	0.1	0.00E+00
Dichloroacetic acid	14	1.8	2.08E+00	0.1	3.75E-04
Trichloroacetic acid	33	0.8	1.72E-02	0.1	1.37E-06
Monobromoacetic acid	50	0.5	0.00E+00	0.1	0.00E+00
Dibromoacetic acid	13	1.9	3.68E-02	0.1	6.99E-06
Bromochloroacetic acid	20	1.3	2.59E-01	0.1	3.36E-05
Tribromoacetic acid	39	0.6	3.15E-03	0.1	1.89E-07
Dibromochloroacetic acid	89	13.0	1.93E-02	0.1	5.78E-07
Dichloroacetonitrile	55	0.5	3.99E-02	0.1	2.00E-06
Bromochloroacetonitrile	5.5	4.5	1.51E-02	0.1	6.78E-06
Dibromoacetonitrile	5	5.0	0.00E+00	0.1	0.00E+00
Trichloroacetonitrile	1	25.0	0.00E+00	0.1	0.00E+00
1,1,3,3-tetrachloropropanone	4.8	5.2	0.00E+00	0.1	0.00E+00
DOC-normalized Total ICED in Batch A (mg/kg/d)					5.08E-04
$P(effect) = 1/[1 + \exp(11.7 - 2.5\times \ln(dosage))]$					7.06E-14

Table A9.3 DOC-normalized health risk calculation for 142X chloraminated RO concentrates: Batch B

Batch B Chemical	Literature NOAEL (mg/kg/d)	RPF	DOC-normalized concentration in 142X RO Concentrates (mg/L)	Water consumption (Lwater/kgbody weight/d)	ICED (mg/kg/d) =Concentration×RPF ×Water Consumption
Chloroform	50	0.5	6.98E+00	0.1	3.49E-04
Bromoform	100	0.3	4.73E-01	0.1	1.42E-05
Bromodichloromethane	25	1.0	4.98E+00	0.1	4.98E-04
Chlorodibromomethane	40	0.6	2.00E+00	0.1	1.20E-04
Monochloracetic acid	70	0.4	0.00E+00	0.1	0.00E+00
Dichloroacetic acid	14	1.8	2.08E+00	0.1	3.74E-04
Trichloroacetic acid	33	0.8	7.76E-01	0.1	6.21E-05
Monobromoacetic acid	50	0.5	0.00E+00	0.1	0.00E+00
Dibromoacetic acid	13	1.9	3.67E-01	0.1	6.98E-05
Bromochloroacetic acid	20	1.3	1.16E+00	0.1	1.51E-04
Tribromoacetic acid	39	0.6	3.50E-02	0.1	2.10E-06
Dibromochloroacetic acid	89	13.0	2.01E-01	0.1	6.03E-06
Dichloroacetonitrile	55	0.5	2.17E-01	0.1	1.08E-05
Bromochloroacetonitrile	5.5	4.5	1.55E-01	0.1	6.97E-05
Dibromoacetonitrile	5	5.0	7.95E-02	0.1	3.98E-05
Trichloroacetonitrile	1	25.0	0.00E+00	0.1	0.00E+00
1,1,3,3-tetrachloropropanone	4.8	5.2	2.38E-02	0.1	1.24E-05
DOC-normalized Total ICED in Batch B (mg/kg/d)					1.78E-03
$P(effect) = 1/[1 + \exp(11.7 - 2.5\times \ln(dosage))]$					1.52E-12

Table A9.4 DOC-normalized health risk calculation for 142X chloraminated RO concentrates: Batch C

Batch C Chemical	Literature NOAEL (mg/kg/d)	RPF	DOC-normalized concentration in 142X RO Concentrates (mg/L)	Water consumption (Lwater/kgbody weight/d)	ICED (mg/kg/d) =Concentration×RPF ×Water Consumption
Chloroform	50	0.5	6.38E+00	0.1	3.19E-04
Bromoform	100	0.3	1.68E+00	0.1	5.05E-05
Bromodichloromethane	25	1.0	6.50E+00	0.1	6.50E-04
Chlorodibromomethane	40	0.6	4.26E+00	0.1	2.56E-04
Monochloracetic acid	70	0.4	4.09E-01	0.1	1.64E-05
Dichloroacetic acid	14	1.8	3.45E+00	0.1	6.21E-04
Trichloroacetic acid	33	0.8	1.33E+00	0.1	1.07E-04
Monobromoacetic acid	50	0.5	1.71E-01	0.1	8.57E-06
Dibromoacetic acid	13	1.9	8.46E-01	0.1	1.61E-04
Bromochloroacetic acid	20	1.3	2.12E+00	0.1	2.76E-04
Tribromoacetic acid	39	0.6	1.55E-01	0.1	9.29E-06
Dibromochloroacetic acid	89	13.0	7.75E-01	0.1	2.33E-05
Dichloroacetonitrile	55	0.5	2.98E-01	0.1	1.49E-05
Bromochloroacetonitrile	5.5	4.5	3.22E-01	0.1	1.45E-04
Dibromoacetonitrile	5	5.0	1.96E-01	0.1	9.81E-05
Trichloroacetonitrile	1	25.0	0.00E+00	0.1	0.00E+00
1,1,3,3-tetrachloropropanone	4.8	5.2	2.03E-02	0.1	1.06E-05
DOC-normalized Total ICED in Batch C (mg/kg/d)					2.77E-03
$P(effect) = 1/[1 + \exp(11.7 - 2.5\times \ln(dosage))]$					4.48E-12

Table A9.5 DOC-normalized health risk calculation for 136X chlorinated RO concentrates from EPA 4Lab study

Chemical in 136X RO concentrates	Literature NOAEL (mg/kg/d)	RPF	DOC-normalized concentration in 136X RO Concentrates (mg/L)	Water consumption (Lwater/kgbody weight/d)	ICED (mg/kg/d) =Concentration×RPF ×Water Consumption
Chloroform	50	0.5	2.22E+01	0.1	1.11E-03
Bromoform	100	0.3	1.67E-01	0.1	5.00E-06
Bromodichloromethane	25	1.0	1.03E+01	0.1	1.03E-03
Chlorodibromomethane	40	0.6	2.14E+00	0.1	1.28E-04
Monochloracetic acid	70	0.4	0.00E+00	0.1	0.00E+00
Dichloroacetic acid	14	1.8	1.41E+01	0.1	2.54E-03
Trichloroacetic acid	33	0.8	2.14E+01	0.1	1.71E-03
Monobromoacetic acid	50	0.5	0.00E+00	0.1	0.00E+00
Dibromoacetic acid	13	1.9	5.98E-01	0.1	1.14E-04
Bromochloroacetic acid	20	1.3	3.55E+00	0.1	4.61E-04
Tribromoacetic acid	39	0.6	0.00E+00	0.1	0.00E+00
Dibromochloroacetic acid	89	13.0	5.56E+00	0.1	1.67E-04
Dichloroacetonitrile	55	0.5	7.69E-01	0.1	3.85E-05
Bromochloroacetonitrile	5.5	4.5	3.16E-01	0.1	1.42E-04
Dibromoacetonitrile	5	5.0	2.82E-01	0.1	1.41E-04
Trichloroacetonitrile	1	25.0	0.00E+00	0.1	0.00E+00
1,1,3,3-tetrachloropropanone	4.8	5.2	3.42E-02	0.1	1.78E-05
DOC-normalized Total ICED in 136X chlorinated RO concentrates (mg/kg/d)					7.60E-03
$P(effect) = 1/[1 + \exp(11.7 - 2.5\times \ln(dosage))]$					5.33E-11