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Drivers of Disinfection Byproduct Cytotoxicity in U.S. Drinking Water: Should Other DBPs Be Considered for Regulation?

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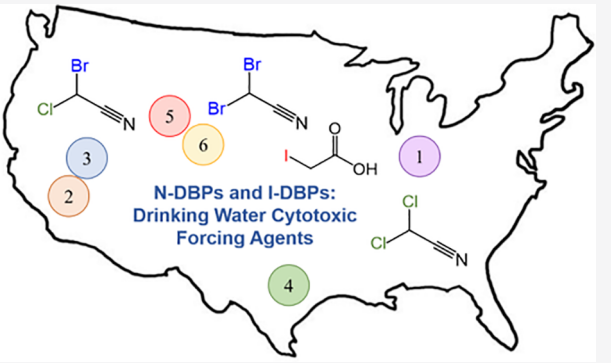
ABSTRACT: This study reveals key disinfection byproduct (DBP) toxicity drivers in drinking water across the United States. DBPs, which are ubiquitous in drinking water, form by the reaction of disinfectants, organic matter, bromide, and iodide and are generally present at 100−1000× higher concentrations than other contaminants. DBPs are linked to bladder cancer, miscarriage, and birth defects in human epidemiologic studies, but it is not known as to which DBPs are responsible. We report the most comprehensive investigation of drinking water toxicity to date, with measurements of extracted whole-water mammalian cell chronic cytotoxicity, over 70 regulated and priority unregulated DBPs, and total organic chlorine, bromine, and iodine, revealing a more complete picture of toxicity drivers. A variety of impacted waters were investigated, including those impacted by wastewater, agriculture, and seawater. The results revealed that unregulated haloacetonitriles, particularly dihaloacetonitriles, are important toxicity drivers. In seawater-impacted water treated with chloramine, toxicity was driven by iodinated DBPs, particularly iodoacetic acids. In chlorinated waters, the combined total organic chlorine and bromine was highly and significantly correlated with toxicity (r = 0.94, P < 0.01); in chloraminated waters, total organic iodine was highly and significantly correlated with toxicity (r = 0.80, P < 0.001). These results indicate that haloacetonitriles and iodoacetic acids should be prioritized in future research for potential regulation consideration.



KEYWORDS: *disinfection byproducts, cytotoxicity, drinking water, total organic halogen*

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INTRODUCTION

Water disinfection is often cited as the greatest public health achievement of the 20th century.[1] By inactivating waterborne pathogens, disinfection significantly reduced waterborne disease. However, chemical disinfection also raised a public health issue: the potential for cancer induction and reproductive/developmental effects associated with chemical disinfection byproducts (DBPs) formed by the reaction of disinfectants with organic matter (natural or anthropogenic), bromide, and iodide.[2] While 11 DBPs (including four trihalomethanes [THMs] and five haloacetic acids [HAAs]) are currently regulated in the U.S., none produces the same health effects in animal studies that are observed in human epidemiology studies (e.g., bladder cancer, colon cancer).[3,4] Moreover, regulated DBP toxicity does not account for health effects in epidemiology studies. As a result, most scientists believe the critical DBPs driving toxicity in humans are not adequately identified, regulated, or controlled in drinking water.[5,6]

Recent research indicates nitrogenous DBPs (N-DBPs) are of a higher health concern than regulated carbonaceous DBPs (C-DBPs),[7,8] yet no N-DBPs are currently regulated. These include haloacetonitriles (HANs), haloacetamides (HAMs), and halonitromethanes (HNMs). Bromine- and iodine-containing DBPs (Br- and I-DBPs) are more toxic in mammalian cells than their chlorine-containing analogues.[9−12] I-DBPs also induce adverse developmental impacts under in vitro and in vivo conditions.[13−18] With limited water resources, utilities are increasingly using poorer quality water supplies impacted by wastewater, algae, seawater intrusion, and energy-related wastes, which are sources of precursors for N-DBPs, Br-DBPs, and I-DBPs.[10,19−22]

Although the last two decades have experienced increased interdisciplinary collaborations among chemists, toxicologists,

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Table 1. Description of Plants Sampled

water utility	disinfectants	treatment	dates collected	source water impact	raw TOC (mg/L)	sucralose (μg/L)	Br^- (μg/L)	I^- (μg/L)
plant 1	Cl_2, UV	GAC[a]	5/7/2018 11/6/2018 3/5/2019	minimal	2.0−4.2	ND to 0.8	20−44	<10
plant 2	Cl_2	GAC	12/14/2017 2/20/2019 9/17/2019	saltwater intrusion	3.2−4.7	0.6−1.1	145−334	<10−28
plant 3	O_3, NH_2Cl	biofiltration	6/12/2018 1/28/2019	saltwater intrusion	2.7−3.0	0.6−0.8	159−184	<10−27
plant 4	Cl_2,[e] NH_2Cl	conventional	2/26/2018 12/10/2018 7/15/2019	connate water	4.0−4.3	ND[f]	120−146	<10−32
plant 5	Cl_2, NH_2Cl	ultrafiltration membranes	10/10/2017 7/10/2018	wastewater	3.6−5.1	2.8−8.0	92−174	<10−11
plant 6A	Cl_2, NH_2Cl	RBF,[b] SAT,[c] UV/H_2O_2, BAC,[d] GAC	1/9/2018 8/6/2018	wastewater	2.6−2.9	12.4−21.0	261−291	<10−22
plant 6B	Cl_2, NH_2Cl	conventional, BAC	9/23/2019	minimal	3.0−4.2	0.2−0.3	51−59	<10

[a]Granular activated carbon. [b]Riverbank filtration. [c]Soil aquifer treatment. [d]Biologically activated carbon. [e]Short free-chlorine contact time before NH_3 addition. [f]ND = not detected.

epidemiologists, and engineers,[5,22,23] it has become obvious that to resolve the chronic risks associated with disinfected drinking water, research is needed to identify the toxicity forcing factors. The critical question is which DBPs account for the majority of the observed toxicity?

To answer this question, we collected drinking water from different regions in the U.S. with a range of disinfection/treatment processes and source water impacts, including wastewater, agriculture, and seawater impacts. We analyzed mammalian cell chronic cytotoxicity, 70 regulated and priority unregulated DBPs (including N-DBPs, Br-DBPs, and I-DBPs) with newly developed analytical methods,[24] and total organic chlorine (TOCl), bromine (TOBr), and iodine (TOI) to comprehensively capture the forcing factors of toxicity. TOCl, TOBr, and TOI capture not only known DBPs but also unknown DBPs not yet identified.[12]

Recent studies investigated a similar suite of regulated and priority, unregulated DBPs, and TOX species in advanced water distribution systems, including full-scale drinking water plants utilizing granular activated carbon (GAC) and Cl_2 disinfection, and others using biologically activated carbon (BAC) and NH_2Cl disinfection.[24−27] Cytotoxic and genotoxic effects were evaluated using calculated toxicity, which is found by multiplying individual molar DBP concentrations by their associated cyto- and genotoxicity index value.[25,27,28] This approach is useful when analytical biology is not available and takes advantage of the recently confirmed assumption that DBP toxicity is additive,[29,30] but the current study serves to examine whether these DBPs (and TOX) are toxicity drivers of representative whole-water mixtures in real drinking water samples. Another study, referred to as the Health Impacts of long-term exposure to disinfection byproducts in drinking WATEr (HIWATE) project,[31] combined the quantitative DBP analysis of 21 regulated and unregulated DBPs and cytotoxicity in various European cities and used only relative concentrations (chromatographic peak areas) for some other unregulated DBPs. Utilizing newly developed quantitative methods, this new study provides analytical concentrations of 72 DBPs, as well as TOX (including TOCl, TOBr, and TOI), to help better understand drivers of cytotoxicity in drinking water.

MATERIALS AND METHODS

Chemical Reagents. General reagents were of ACS reagent grade and were purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Waltham, MA). DBP standards were purchased or custom synthesized from Sigma-Aldrich, CanSyn Chem. Corp. (Toronto, ON), Aldlab Chemicals (Woburn, MA), and TCI America (Waltham, MA) at the highest level of purity. Fluorobenzaldehyde and 1,2-dibromopropane, used as the surrogate standard and the internal standard, respectively, *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA), used as the derivatizing agent for mono- and dihaloacetaldehydes,[24,32] and Diazald, used as the methylating agent for halo-acids,[24] were purchased from Sigma-Aldrich. All solvents (acetonitrile, hexanes, methyl *tert*-butyl ether (MTBE), methanol, and ethyl acetate) were of the highest purity and were purchased from Sigma-Aldrich, VWR International (Radnor, PA), or Fisher Scientific.

Source and Treated Waters Studied. Plant 5 and plant 6 treat water from a common wastewater-dominated (>50% wastewater-impacted) source.[33] Plant 6 combines water from two raw water sources in two parallel treatment trains, designated as plants 6A and 6B. The effluents of the two trains are combined at the end of the plant. The intake of Plant 5 is fed by a canal that is upstream of plant 6A, where wastewater impact is greater. Because of the greater wastewater impact, plant 6A built a state-of-the-art treatment plant to reliably treat this source of water using riverbank filtration (RBF)[34] and soil aquifer treatment (SAT) to remove much of the chemicals of emerging concern (CECs) from this water[35] and an ultraviolet (UV) advanced oxidation process to further remove CECs.[36] Additionally, plant 6A blends this water source with water from a nearby reservoir (plant 6B), further diminishing the wastewater impact. Thus, plants 5 and 6 have

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relatively similar wastewater impacts, where they have each approached treatment in different ways (Table 1).

The four other plants studied utilized conventional or advanced treatment processes. Both plant 1 and plant 2 treat water with granular activated carbon (GAC) and use Cl_2 (plus UV for plant 1) as their disinfectant, but plant 2 treats water with much higher halides (particularly bromide) due to saltwater intrusion. GAC removes total organic carbon (TOC) but not bromide, which results in an increase in the bromide/TOC ratio and more bromine incorporation into DBPs.[25,27,37−39] Plant 4 and plant 3 also treat water with moderately high levels of bromide and iodide due to saltwater intrusion or connate water. Plant 3 uses the same source water as plant 2, but with ozone (O_3), conventional treatment, biofiltration, and NH_2Cl. Typically, NH_2Cl is added after O_3, but when NH_2Cl is added before O_3, the NH_2Cl addition helps control bromate formation, which is a regulated inorganic DBP.[40] Moreover, plants 2 and 3 provide a contrast to plant 4, which treats similar water (in terms of TOC and bromide) but with conventional treatment and NH_2Cl disinfection.

Survey of Plants. When possible, plants were sampled at different times of the year to observe seasonal effects on DBP formation (e.g., warm/dry season [summer/fall], wet/cold season [winter/spring]), as weather trends, water quality, and plant operational practices can change over the course of the year. For example, wastewater impacts can be higher in summer when there is less river flow.[41] For each plant, raw water (before any treatment or addition of disinfectant), plant effluent (at the entry point to the distribution system), and two locations in the distribution system (at average and maximum detention times) were sampled. Some DBPs increase in concentration in the distribution system, whereas others degrade over time.[42,43]

Each sampling point was analyzed for TOX surrogates, whereas individual unregulated DBPs and cytotoxicity were only analyzed in the raw water (background levels) and the distribution system at average detention time (average exposure to consumers) because of the extensive analyses required. However, the plant effluent and both distribution system samples were analyzed for trihalomethanes (THMs) and bromo-/chlorohaloacetic acids (Br/Cl-HAAs). Raw water was analyzed for water quality, including sucralose (indicating wastewater impacts), TOC, UV at 254 nm (UV_{254}), and total ammonia, bromide, and iodide. The plant effluent was also analyzed for TOC and UV_{254} to determine the effect of the treatment processes on removing precursors. Sucralose was measured in the plant effluent and distribution system of plants 6A and 6B to determine the percent of the finished water from each plant. The same was done for plant 5, as its distribution system could also be fed by a second plant that was not wastewater impacted. Likewise, this was done for plant 2 in the first year, as its distribution system could also be fed by a groundwater source.

Sample Collection. Samples for priority, unregulated DBP quantification and TOX were collected headspace-free in two 1-L amber glass bottles in the same manner as described previously.[24−26,44] Briefly, for plants using Cl_2, NH_4Cl was added to one bottle to convert Cl_2 to NH_2Cl for DBP analysis, and ascorbic acid was added to another bottle to quench residual Cl_2 for TOX analysis following a previously established method.[25,26] For chloraminated plants, no quencher was added to one bottle for DBP analysis, whereas ascorbic acid was added to the other for TOX analysis. Quenchers were added based on a Cl_2-to-quencher molar ratio of 1:1.3 (i.e., a slight excess of quencher). Samples for TOC, UV_{254}, and sucralose were collected in 125 mL amber glass bottles, and a 250 mL plastic bottle was used to collect samples for ammonia. Samples for regulated THM4 and HAA9 were collected in 60 mL amber glass bottles (quenched with NH_4Cl).

Water samples for cytotoxicity (20 or 40 L) were collected in 2-L Teflon bottles or poly(tetrafluoroethylene)-lined carboys. Bottles and carboys were rinsed at least three times with the sample before collection, and no quencher or acid was added. For plant 6, which treats a blend of two water sources, raw water was collected from each source and blended in the laboratory based on blending ratios from the plant on the collected date (Table S28). All samples were shipped overnight on ice and analyzed immediately or within 2 days of storage at 4 °C.

Analytical Methods. *Water Quality and Regulated DBPs.* The analytical methods for TOC, UV_{254}, ammonia, bromide, and iodide are summarized in Table S1. Sucralose was measured using liquid chromatography (LC)-tandem mass spectrometry (MS/MS) in negative electrospray ionization (ESI) mode using an isotopically labeled internal standard (sucralose-d_6) and a C_{18} reverse-phase column.[41] The method reporting limit (MRL) was 0.20 μg/L. THM4 and HAA9 were measured using the U.S. Environmental Protection Agency (EPA) Method 551.1 and 552.2, respectively.[45,46] The method reporting limit (MRL) was 0.5 μg/L for THM4 and 1.0 μg/L for HAA9, except for chloroacetic acid and bromoacetic acid (2 μg/L).

Unregulated DBPs. The remaining 59 priority, unregulated DBPs were measured using two liquid−liquid extraction methods and gas chromatography (GC)-electron ionization (EI)-mass spectrometry (MS) as previously published,[24−26,47] including 53 DBPs (haloacetonitriles [HANs], haloacetamides [HAMs], halonitromethanes [HNMs], iodinated trihalomethanes [I-THMs], iodinated haloacetic acids [I-HAAs], haloketones [HKs], and trihaloacetaldehydes [tri-HALs]) in one single extraction method. This extraction method is described in detail in the Supporting Information (SI) (Text S1). One portion of the final organic extract was analyzed for HANs, HAMs, HNMs, I-THMs, iodoacetic acids (IAAs), HKs, and tri-HALs using an Agilent 7890 GC coupled to an Agilent 5977A MS with EI (Agilent Technologies, Santa Clara, CA) in selected ion monitoring (SIM) mode. A separate portion of the extract underwent diazomethane derivatization for the analysis of four I-HAAs using freshly generated diazomethane according to a U.S. EPA Standard Operating Procedure.[48] The I-HAAs were analyzed using GC-MS/MS with a Quantum GC triple quadrupole MS coupled to a TRACE GC Ultra GC (Thermo Scientific, Waltham, MA). Multiple reaction monitoring (MRM) was used to quantify I-HAAs, with two MS/MS transitions (one quantitative and one qualitative) being used for each of the I-HAAs, along with a 1,2-dibromopropane internal standard. A specialty-phase GC column (Restek Rtx-200) was used for GC analysis[24] and was essential for effectively separating DBPs with such a dynamic range of polarities and volatilities.

Six mono- and di-HALs were measured using pentafluorobenzylhydroxylamine (PFBHA) derivatization, liquid−liquid extraction, and GC-EI-MS analysis based on a previously published procedure.[25,26,32,47] The mono- and di-HALs were measured in ascorbic acid-quenched samples using PFBHA

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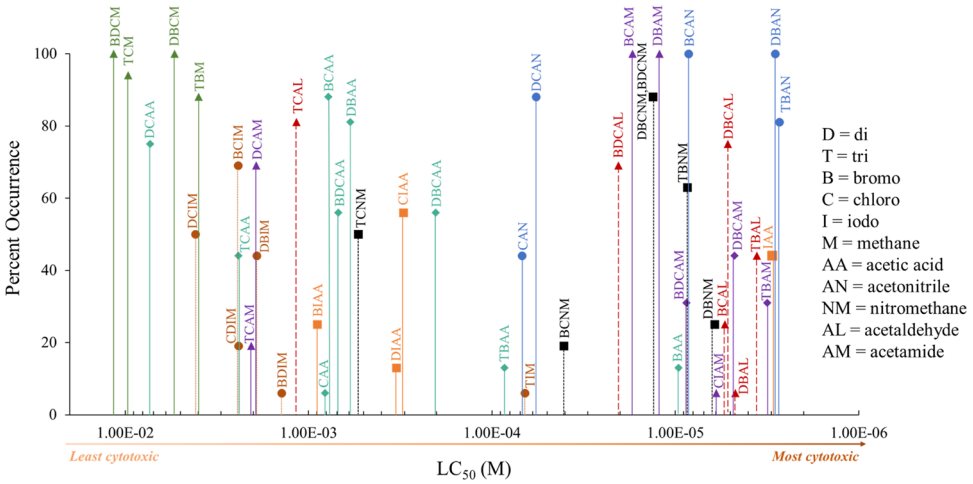

Figure 1. Summary of DBP occurrence in drinking water samples and mammalian cell cytotoxicity[9] for DBPs quantified above their respective method reporting limit.

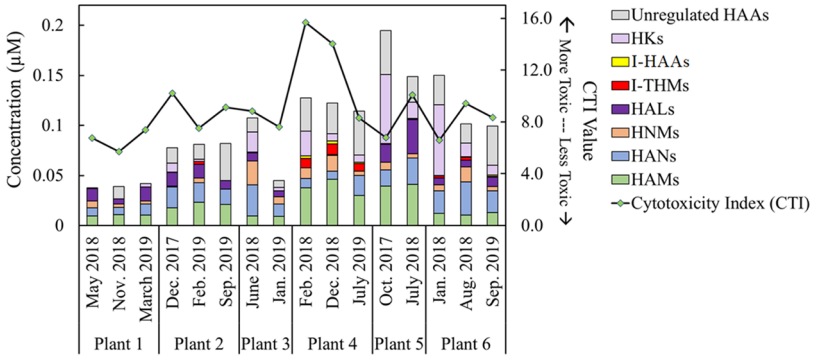

Figure 2. Stacked plot of unregulated DBPs for each drinking water plant sampled, along with a contiguous line plot of mammalian cell cytotoxicity index (CTI) values. CTI values are defined as the LC_{50}^{-1} (10^3).

derivatization, liquid−liquid extraction, and GC-EI-MS analysis. Further details, including instrumental parameters, DBP quantifier and qualifier ions, and method detection limits (MDLs), can also be found elsewhere.[24,25] MRL for most unregulated DBPs in this study was 0.1 μg/L, whereas MRLs for the four IAAs ranged from 0.010 to 0.025 μg/L.

TOX. Ascorbic acid-quenched samples were measured for TOCl, TOBr, and TOI by sorption on activated carbon columns, followed by combustion and then detection of Cl^-, Br^-, and I^- using ion chromatography as outlined previously.[25,47,49,50] Further details on the TOX procedure and instrumentation can be found in the SI (Text S2).

XAD Resin Extraction. XAD resin extractions of raw and distribution system/average water samples (20 or 40 L) were carried out according to a previously published procedure,[47,51,52] and this method has been employed to study the toxicity of disinfected water samples in many previous studies.[10,12,31,32,47] Briefly, water samples were acidified to pH < 1 to ensure protonation of haloacetic acids, passed over a bed of DAX-8 and XAD-2 resins, and ethyl acetate was used to elute organics from the resin column. To minimize the potential for acid-catalyzed hydrolysis, the samples were extracted immediately after acidification. The ethyl acetate extract was dried with Na_2SO_4 and concentrated under N_2 using a TurboVap® (Biotage).

Chinese Hamster Ovary (CHO) Cells. Chinese hamster ovary (CHO) K1 cells, line AS52, clone 11-4-8, were used for cytotoxicity analyses[53] and have been employed in many previous DBP toxicity studies.[10,12,47,54−58] The CHO cells were maintained in Ham's F12 medium containing 5% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% CO_2.

CHO Cell Chronic Cytotoxicity Assay. This assay measures the reduction in cell density after exposure to a drinking water concentrate over a period of 72 h and is published elsewhere.[53,57,59] XAD ethyl acetate extracts are first solvent-exchanged into dimethyl sulfoxide (DMSO). The general protocol involves the use of a 96-well flat-bottomed microplate to evaluate a series of concentrations of the concentrated water sample (CWS) for each experimental group. This assay is described in detail in the SI (Text S3).

CHO Cell Cytotoxicity Statistical Analyses. For each CWS, one-way analysis of variance (ANOVA) tests were conducted to determine the lowest concentration factor that induced a statistically significant level of cytotoxicity as compared to their concurrent negative control ($P \leq 0.05$). To determine whether a statistically significant difference existed among different samples, LC_{50} values were determined through multiple regression analyses of each concentration−response curve. Using a bootstrap statistical approach,[9,60−63] the LC_{50} values were converted into mean cytotoxicity index (CTI) values ($CTI = LC_{50}^{-1} \times 10^3$) such that cytotoxic potency could be easily ranked (higher CTI, higher cytotoxicity) and allowed for ANOVA statistical tests among the different samples. The

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power of the test was maintained at >0.8 at α = 0.05. A detailed discussion of the statistical methods was published.[9]

DBP Correlation Statistics. Regression analyses were carried out to determine the Pearson product-moment (r) and the corresponding P value, where $P < 0.05$, <0.01, and <0.001 were considered significantly correlated, highly significantly correlated, and very highly significantly correlated, respectively. An $r > 0.90$ was considered to have a strong, positive relationship between DBP concentration and cytotoxicity, while an $r < 0.50$ was considered to have a weak, positive relationship.

RESULTS AND DISCUSSION

DBP Occurrence and Cytotoxicity. DBP occurrence and individual DBP mammalian cell chronic cytotoxicity[9] are summarized in Figure 1, which indicates the percentage of samples in which the DBP was detected above its respective MRL in the study. Summed concentrations for each unregulated DBP class with respective mammalian cell chronic cytotoxicity index values for each sampling event are shown in Figure 2, and individual DBP concentrations (μg/L and nM) are found in the Supporting Information (SI) (pp S21−S48). Complete cytotoxicity concentration−response curves and statistical data (Figures S2−S17; Tables S2 and S29) are found in the SI.

Figure 1 demonstrates that many highly cytotoxic, unregulated DBPs occur just as frequently, if not more, as those that are currently regulated. Notably, dibromoacetonitrile (DBAN), the fourth most cytotoxic DBP[9,64] and a carcinogen in rats and mice,[65] was detected in every drinking water, ranging from 0.1 to 2.1 μg/L (0.7−11 nM). Further, three other highly cytotoxic brominated N-DBPs, dibromoacetamide, bromochloroacetonitrile (BCAN), and bromochloroacetamide, were detected in all waters at concentrations of 0.3−2.7 μg/L (1.4−13 nM), 0.3−1.4 μg/L (1.9−8.8 nM), and 0.6−2.6 μg/L (3.3−15 nM), respectively. Regulated THMs were detected in at least 88% of samples, and individual concentrations ranged from 0.8 to 23 μg/L (4.0−160 nM). While generally found at lower concentrations, brominated N-DBPs are on average 750× more cytotoxic than regulated THMs.[9] It is worth noting that two regulated HAAs, chloroacetic acid and bromoacetic acid, were only detected in 2 of 16 water samples.

Table 1 summarizes drinking water plant information (disinfectants, other treatments, impacts, and water quality parameters), and other details such as disinfectant doses, raw water and effluent TOC, total ammonia, and SUVA can be found in the SI (Tables S4, S8, S12, S16, S20, S24, and S28).

Granular Activated Carbon (GAC)/Cl_2 Waters. Of all drinking water plants, plant 1 had the lowest unregulated DBP formation (Figure 2). This plant uses GAC, UV, and chlorine for treatment. In contrast, plant 2, which also uses GAC and chlorine, had higher bromide concentrations (31 vs 213 μg/L on average, respectively), explaining the dramatic difference in Br-DBP and TOBr formation (Figures 3 and S1). Greatest differences in unregulated DBP formation were seen in haloacetonitriles (HANs) and haloacetamides (HAMs) (Figure 2), with more than double molar concentrations of Br-HANs and Br-HAMs in plant 2 vs plant 1 (27, 32 vs 11, 9.2 nM, respectively), which agrees with the higher observed TOBr. This is important because N-DBPs are more toxic than C-DBPs and Br-DBPs are more toxic than Cl-DBPs.[6,9] Increased levels of TOBr and toxic Br-N-DBPs account for higher cytotoxicity in plant 2 than in plant 1 (average

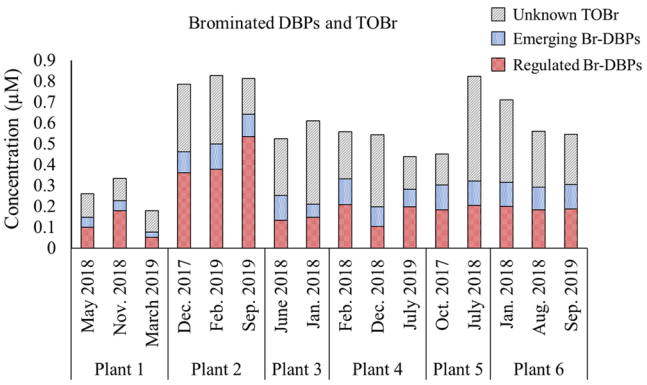

Figure 3. Summed Br-DBPs and total organic bromine (TOBr) for each drinking water plant, calculated by multiplying the individual μM Br-DBP concentrations by the number of bromine atoms.

cytotoxicity index values of 8.9 ± 1.4 and 6.6 ± 0.9, respectively) (Figure 2).[6] However, plants 1 and 2 had among the lowest average DBP concentrations of all plants studied, indicative of GAC removing DBP precursors. This aligns with a previous study where GAC treatment controlled TOBr and calculated cytotoxicity.[25]

Wastewater-Impacted Waters. Heavily wastewater-impacted drinking waters, plant 5 and plant 6, had the three highest unregulated DBP levels (Figure 2). Haloacetaldehyde (HAL) formation in plant 5 was much higher than that in plant 6 and every other plant sampled, consistent with more HAL precursors in wastewater-impacted waters;[66] these precursors were likely removed during advanced treatment processes utilized by plant 6 (GAC, riverbank filtration, soil aquifer treatment). Summed N-DBP formation was also higher in plant 5, driven by much higher concentrations of haloacetamides (Figure 2). However, summed haloacetonitriles were highest in plant 6.

Figure 2 illustrates seasonal N-DBP trends in plants 5 and 6, with higher N-DBPs formed during warmer months (July−August). During summer, river flow is often lower, resulting in higher wastewater impacts, which were evidenced by higher sucralose levels used as markers of wastewater contamination (Tables S24 and S28).[41] Additionally, cytotoxicity was typically highest when N-DBPs were the highest (Figure 2). This pattern was not observed with regulated or unregulated C-DBPs, indicating that N-DBPs could be a good indicator of drinking water cytotoxicity, particularly for heavily wastewater-impacted waters.

High Halide, Low Wastewater-Impacted Waters. Plants 2, 3, and 4 each treat water with elevated bromide but lower wastewater impacts. Iodide was also detectable at least once (>10 μg/L) in their source waters. Regulated and unregulated Br-DBPs were highest in plant 2, with levels ranging from 0.46 to 0.64 μM (36−51 μg/L) (Figure 3), likely due (in part) to a higher bromide-to-total organic carbon ratio because GAC removes organic matter but not bromide.[25,27,37] Plant 4 had lower Br-DBP formation, ranging from 0.20 to 0.33 μM (16−27 μg/L), due to a short (~2 min) free-chlorine contact time before NH_2Cl formation, which reduces the reaction time for HOBr with organic matter.[67]

Plant 3 uses O_3 and NH_2Cl, which form fewer DBPs compared to chlorine,[67] and Figure 3 illustrates the effectiveness of O_3/NH_2Cl in limiting Br-DBPs. Plants 2 and 3 treat water from the same watershed, providing a direct

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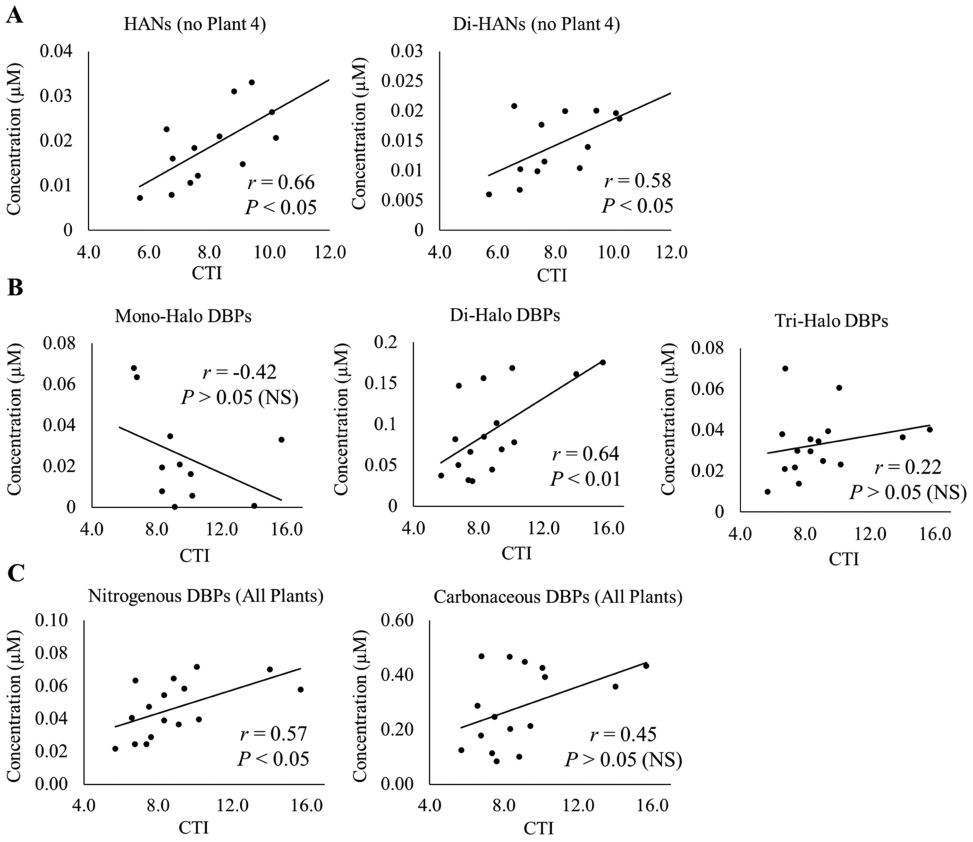

Figure 4. Correlation of mammalian cell cytotoxicity with (A) summed HANs (excluding plant 4 data); (B) unregulated DBPs classified by the number of halogens. (C) Summed N-DBPs (HAMs, HANs, HNMs) and summed C-DBPs. NS = not significant. Mammalian cell cytotoxicity index (CTI) values are defined as the LC_{50}^{-1} (10^3).

comparison between each of their treatment processes. Br-DBP formation was lower in plant 3 than in plant 2, which uses GAC/Cl_2, despite having similar levels of bromide on average (172 vs 213 μg/L, respectively). While overall Br-DBPs were limited, plant 3's June 2018 event had the highest summed halonitromethanes (HNMs) of all plants (Figure 2). Pre-O_3 can increase HNM formation during postdisinfection.[2] Furthermore, the three brominated tri-HNMs (bromodichloronitromethane, dibromochloronitromethane, and tribromonitromethane) were dominant species and higher in concentration than any other sample. HNMs are concerning not only because of their cytotoxic potency but they are also among the most genotoxic DBPs.[68] For example, dibromonitromethane is the third most genotoxic DBP.[9]

I-DBPs and TOI were the highest in plant 4's drinking water (Figures 2 and S1). I-THMs and iodo-haloacetic acids (I-HAAs) reached 10 and 3.0 nM (2.7 and 0.7 μg/L), respectively. Additionally, two iodinated N-DBPs, iodoacetonitrile and chloroiodoacetamide, were detected (up to 7.5 nM). This is significant, as they are among the most cytotoxic DBPs.[9] Only iodoacetic acid and a few I-THMs were detected in plants 2 and 3, despite iodide levels similar to plant 4 (Table 1 and Figure 2). Greater prevalence of I-DBPs can be explained by the disinfection processes used. While plant 4's short free-chlorine contact time is helpful for limiting regulated Cl- and Br-DBPs, the opposite is true for I-DBP formation.[69,70] Alternatively, Cl_2 and O_3 at plants 2 and 3 controlled I-DBP formation.[69]

DBP and Cytotoxicity Correlations. *I-DBPs.* I-HAAs showed a highly significant correlation to cytotoxicity (r = 0.88, P < 0.001) in five plants where detections were quantifiable (10 of 16 samples) (Table S3). I-HAAs were particularly higher in plant 4, where samples also had the highest cytotoxicity. Interestingly, in the third plant 4 sampling when I-HAA levels were lower, cytotoxicity decreased (Figure 2). Iodoacetic acid (IAA), the most genotoxic DBP,[9,54,70] was tumorigenic in mice,[71] and an endocrine system disruptor[14,15] was detected in each plant 4 sample, ranging from 0.3 to 0.7 nM (0.051−0.099 μg/L), twice the concentration of any other location. Chloroiodoacetic acid was detected more than IAA and accounted for the largest percentage of I-HAAs, but it is 100× less cytotoxic than IAA.[9]

It should be noted, however, that I-HAA correlations with cytotoxicity become nonsignificant when removing plant 4. Lack of correlation is mostly due to inconsistent formation and low concentrations in other plants. Therefore, it is likely that there are other chemical factors (i.e., other DBP classes) driving the toxicity in the other plants. Considering IAA's cytotoxic potency and its concentration in plant 4 water samples, it is a key driver of observed cytotoxicity in this plant. I-THMs, generally present when I-HAAs were detected, less significantly correlated to cytotoxicity (r = 0.70, P < 0.05). However, like I-HAAs, this correlation was only significant when including plant 4. Although I-THMs are cytotoxic, they are much less so than I-HAAs,[9,70] explaining their weaker correlation.

N-DBP Classes. Plant 4 consistently had the highest haloacetamide (HAM) levels overall, but a lower haloacetonitrile (HAN) formation was observed. Excluding plant 4, HAN/cytotoxicity correlations were significant (r = 0.66, P < 0.05)

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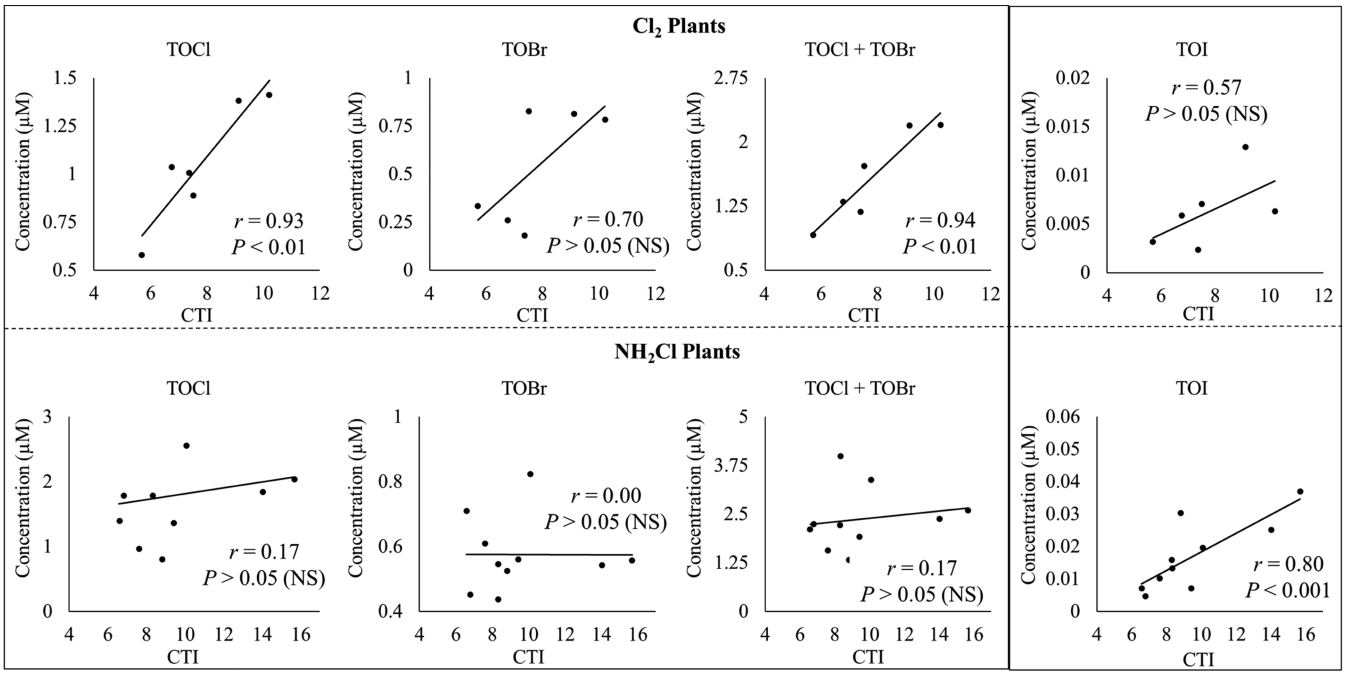

Figure 5. Relationships between TOCl, TOBr, TOI, and mammalian cell cytotoxicity of distribution system/average water for Cl_2 plants and NH_2Cl plants. NS = not significant. Mammalian cell cytotoxicity index (CTI) values are defined as the LC_{50}^{-1} (10^3).

(Figure 4A), with di-HANs (dichloro-, bromochloro-, dibromoacetonitrile) most influencing cytotoxicity (r = 0.58, P < 0.05) (Figure 4A). Bromochloroacetonitrile (BCAN) and dibromoacetonitrile (DBAN) were ubiquitous, and dichloroacetonitrile formed in 90% of waters (Figure 1). Because DBAN is 3× more cytotoxic than BCAN,[9,55,64] DBAN likely contributed more to measured cytotoxicity. DBAN was previously observed as a toxicity driving agent in GAC-treated waters.[25,27] While tribromoacetonitrile is the most cytotoxic HAN,[9,55,64] it formed at much lower concentrations than BCAN and DBAN, thus contributing less to cytotoxicity.

HAMs formed the only N-DBP class with significant cytotoxicity correlations (r = 0.61, P < 0.05) when considering data from each plant (Table S3). Like I-DBPs, HAM/cytotoxicity correlations were heavily influenced by plant 4, which had high HAM formation and cytotoxicity. Without plant 4, correlations were not significant. Three di-HAMs, dichloro-, bromochloro-, and dibromoacetamide, were ubiquitous and cytotoxicity drivers (r = 0.67, P < 0.01). In fact, Figure 4B illustrates that all unregulated dihalo DBPs combined had a significant correlation to cytotoxicity (r = 0.64, P < 0.01), whereas mono- and trihalo unregulated DBPs did not. Although tri-HAMs are generally more cytotoxic, they formed at much lower concentrations. Higher di-HAMs vs tri-HAMs were also observed in a large U.S. survey.[42] No mono-HAMs were detected in any sample for this study and likely do not contribute to cytotoxicity as much as di- and tri-HAMs. The other N-DBP class, halonitromethanes, did not correlate with cytotoxicity and was at much lower levels (Table S3).

Together, the three N-DBP classes significantly correlated with toxicity (r = 0.57, P < 0.05) (Figure 4C). This supports the recent finding that individual DBP cytotoxicity is additive in water.[29,30] Conversely, C-DBPs did not significantly correlate with cytotoxicity (Figure 4C), further illustrating that N-DBPs are more important toxicity drivers than C-DBPs, except for I-DBPs.

HKs and HALs. Two other unregulated C-DBP classes, HKs and HALs, showed no significant cytotoxicity correlations (Table S3). HKs were prominent in plant 5 and plant 6, but toxicity was lowest when HKs were the highest. Unlike other DBP classes, HK chemical standards have not yet been studied for cytotoxicity. The only HAL detected in plant 4 (where cytotoxicity was the highest) was trichloroacetaldehyde, the least cytotoxic HAL.[9,32]

Regulated DBPs. As expected, the regulated THMs, among the least cytotoxic DBPs,[9] showed poor correlation to cytotoxicity and was not significant when including and excluding Plant 4 data (r = 0.36, P > 0.05 and r = 0.48, P > 0.05, respectively) (Table S3). However, the five regulated HAAs (HAA5) are more cytotoxic and significantly correlated (r = 0.59, P < 0.05), but not when plant 4 (with higher HAA5 concentrations) was removed. While more cytotoxic than HAA5, the four unregulated Br/Cl-HAAs did not correlate with cytotoxicity (Table S3), likely because of lower concentrations. The combination of all nine Br/Cl-HAAs (HAA9) significantly correlated with cytotoxicity (r = 0.54, P < 0.05), mostly due to HAA5, where dichloro- and dibromoacetic acid were important drivers (r = 0.63, P < 0.05). Regulated di-HAAs and bromochloroacetic acid were the most prevalent HAAs (Figure 1).

TOX and Cytotoxicity. Considering all sampling events, including Cl_2 and NH_2Cl disinfection, TOCl and TOBr individually had no significant correlation with cytotoxicity. TOI showed a highly significant correlation (r = 0.80, P < 0.001) in chloraminated waters (Figure 5), mostly influenced by plant 4 with the highest TOI, I-DBPs, and toxicity; without these data, correlations were not significant. The opposite was true for TOBr, as correlations greatly strengthened (with significance) when removing plant 4 (r = 0.58, P < 0.05). These data suggest that for waters with high I-DBP formation and high TOI, TOI could be the best indicator of toxicity even

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when TOCl and TOBr are present at much higher levels (Figure S1).

On the other hand, for chlorinated waters, TOCl and TOBr (together) is an accurate predictor of cytotoxicity. For example, combining TOCl and TOBr for plants 1 and 2 results in a strong correlation ($r = 0.94$, $P < 0.01$) (Figure 5). Cytotoxicity correlations with TOCl were also strong in chlorinated waters ($r = 0.93$, $P < 0.01$). Similar positive correlations between TOCl/TOBr and toxicity in disinfected water samples has been observed.[72,73]

Raw Water Cytotoxicity. Surprisingly, raw water from the plant influents before disinfection, where no DBPs were present, was higher in cytotoxicity than the disinfected water in many cases (Figure S18). Contaminants from wastewater are likely sources of raw water cytotoxicity for plant 6 and plant 5. The dramatic cytotoxic decrease in plant 6's disinfected water for August 2018 (29.12−9.53 CTI) and September 2019 (48.13−8.34 CTI) demonstrates the effectiveness of the plant's advanced treatment system, and although DBPs were occasionally among the highest in plant 6, the decrease from raw to finished water is noteworthy. The highly cytotoxic raw waters (>29 CTI) for plants 2, 4, and 6 typically occurred during warm months (July−September), when there is typically low river flow and, thus, wastewater-derived contaminants were likely more concentrated. Around the time of the December 2018 plant 4 sampling, flooding resulting from Hurricane Harvey likely introduced contaminants into the raw source water that contributed to cytotoxicity. In addition to wastewater-derived contaminants, there are contaminants from industrial discharges and spills. Although it is unclear what specific compounds are driving raw water cytotoxicity, these data highlight the importance of protecting source waters and the need for water treatment procedures to remove cytotoxic contaminants. Moreover, the raw water cytotoxicity puts the toxicity of DBPs in perspective, as the overall impact of treatment and disinfection often produced a finished water lower in cytotoxicity than the raw water.

Implications. Drinking water is an extremely complex mixture comprised of hundreds of chemicals, with new DBPs and other contaminants increasingly discovered.[74] Therefore, it is nearly impossible to attribute drinking water cytotoxicity to any one chemical or chemical class. However, this study has important and clear findings for DBP toxicity drivers in drinking water.

First, it is clear that the four regulated THMs are not drivers of cytotoxicity in drinking water. Rather, N-DBPs, particularly dihaloacetonitriles (dichloroacetonitrile, bromochloroacetonitrile, and dibromoacetonitrile), have the greatest influence on drinking water cytotoxicity based on their ubiquity in drinking water and significant correlation to cytotoxicity. Because the analytical method used for haloacetonitrile detection in this study involves only small modifications of an existing EPA method,[45] and analytical standards of dihaloacetonitriles are inexpensive and readily available, widespread measurement in drinking water and future regulations are possible. We also show that combined TOBr and TOCl are promising metrics for evaluating drinking water cytotoxicity in chlorinated waters.

Iodoacetic acids are also important cytotoxicity drivers in chloraminated waters impacted by iodide. While HANs and IAAs are clear drivers of mammalian cell chronic cytotoxicity in this study, it is important to recognize that toxicity ranking orders of DBPs might be different for different bioassays. Different bioassays may express dissimilar levels of sensitivity and may measure different types of adverse biological effects. For example, chloroacetaldehyde and iodoacetic acid have CHO cell cytotoxicity LC_{50} values on the same order of magnitude (10^{-6}),[9] yet iodoacetic acid is 164× more genotoxic based on the CHO single cell gel electrophoresis genotoxicity assay.

Further research on, and implementation of, halide mitigation strategies such as ion-exchange resins and membrane capacitive deionization could be beneficial for reducing the formation of toxic I-DBPs and Br-DBPs. However, these technologies are typically expensive and may not be practical for many drinking water plants. Although GAC cannot remove halides, the removal of total organic carbon makes it a desirable option for removing precursors that would otherwise contribute to DBP formation. The increased DBP formation in plant 4 compared to other plants is due, in part, to plant 4's lack of advanced treatment processes such as GAC and BAC, as these are effective for removing NOM precursors. Plant 4 was much higher in I-DBP formation because of the high iodide concentrations in its source water in combination with the short free-chlorine contact time, in which it was clear that these I-DBPs were likely driving the statistical significance. Moreover, preoxidation with Cl_2 and/or O_3 can be readily used to minimize toxic I-DBPs because each of these can fully oxidize iodide to iodate, as iodate serves as a sink for iodide.[69] For wastewater-impacted waters, advanced treatment processes, including GAC, riverbank filtration, and soil aquifer treatment, can help to limit cytotoxicity and DBPs.

Because dihaloacetonitriles and iodoacetic acids are the most important drivers of cytotoxicity in these diverse drinking waters, it begs the following question: Should they be considered for regulation? Dibromoacetonitrile is the fourth most cytotoxic DBP studied to date;[9] it is a carcinogen in rats and mice[65] and was detected in every drinking water sample in this study. Iodoacetic acids are also highly toxic, with iodoacetic acid being the most genotoxic DBP studied to date[75] and the third most cytotoxic DBP;[9] it alters gene expression and has adverse effects on mouse ovarian follicles[13,15] and is tumorigenic in mice.[71] While iodoacetic acids require a source of iodine (e.g., iodide) to form in drinking water, they could be a significant concern for coastal cities that use chloramination for disinfection. Based on their occurrence and cytotoxic potencies, we suggest that these two groups of DBPs should be considered for regulation in the U.S., but future epidemiological studies would be beneficial for understanding long-term exposure of these DBPs. Currently, epidemiologists employ THMs as a metric for DBP exposure based on the assumption that THM concentrations are proportional to concentrations of other DBP classes. However, toxicological data have repeatedly indicated that THMs are less potent toxins than unregulated DBP classes, such as the HANs. Since THMs are not proportional to the DBPs driving toxicity, the use of THMs to measure exposure introduces exposure misclassification bias in epidemiologic studies.[76]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c07998.

Additional details on materials and methods, raw DBP data, water quality parameters, cytotoxicity concen-

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tration−response curves, with accompanying tables and figures (PDF)

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Notes

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REFERENCES

(1) Otterstetter, H.; Craun, G. Disinfection in the Americas: A Necessity. *J. − Am. Water Works Assoc.* **1997**, *89*, 8−10.

(2) Richardson, S. D.; Plewa, M. J.; Wagner, E. D.; Schoeny, R.; Demarini, D. M. Occurrence, Genotoxicity, and Carcinogenicity of Regulated and Emerging Disinfection by-Products in Drinking Water: A Review and Roadmap for Research. *Mutat. Res., Rev. Mutat. Res.* **2007**, *636*, 178−242.

(3) Villanueva, C. M.; Cantor, K. P.; Cordier, S.; Jaakkola, J. J.; King, W. D.; Lynch, C. F.; Porru, S.; Kogevinas, M. Disinfection Byproducts and Bladder cancer: A Pooled Analysis. *Epidemiology* **2004**, *15*, 357−367.

(4) Rahman, M. B.; Cowie, C.; Driscoll, T.; Summerhayes, R. J.; Armstrong, B. K.; Clements, M. S. Colon and Rectal Cancer Incidence and Water Trihalomethane Concentrations in New South Wales, Australia. *BMC Cancer* **2014**, *14*, No. 445.

(5) Richardson, S. D.; Plewa, M. J. To Regulate or Not to Regulate? What to Do with More Toxic Disinfection by-Products? *J. Environ. Chem. Eng.* **2020**, *8*, No. 103939.

(6) Plewa, M. J.; Wagner, E. D.; Muellner, M. G.; Hsu, K.-M.; Richardson, S. D. Comparative Mammalian Cell Toxicity of N-DBPs and C-DBPs. *Disinfection By-Products in Drinking Water: Occurrence, Formation, Health Effects, and Control*; ACS Symposium Series; American Chemical Society, 2008; Vol. *995*, pp 36−50.

(7) Krasner, S. W.; Mitch, W. A.; Westerhoff, P.; Dotson, A. Formation and Control of Emerging C- and N-DBPs in Drinking Water. *J. − Am. Water Works Assoc.* **2012**, *104*, E582−E595.

(8) Le Roux, J.; Plewa, M. J.; Wagner, E. D.; Nihemaiti, M.; Dad, A.; Croué, J. P. Chloramination of Wastewater Effluent: Toxicity and Formation of Disinfection Byproducts. *J. Environ. Sci.* **2017**, *58*, 135−145.

(9) Wagner, E. D.; Plewa, M. J. CHO Cell Cytotoxicity and Genotoxicity Analyses of Disinfection By-Products: An Updated Review. *J. Environ. Sci.* **2017**, *58*, 64−76.

(10) Dong, S.; Page, M. A.; Massalha, N.; Hur, A.; Hur, K.; Bokenkamp, K.; Wagner, E. D.; Plewa, M. J. Toxicological Comparison of Water, Wastewaters, and Processed Wastewaters. *Environ. Sci. Technol.* **2019**, *53*, 9139−9147.

(11) Zhang, Z.; Zhu, Q.; Huang, C.; Yang, M.; Li, J.; Chen, Y.; Yang, B.; Zhao, X. Comparative Cytotoxicity of Halogenated Aromatic DBPs and Implications of the Corresponding Developed QSAR Model to Toxicity Mechanisms of Those DBPs: Binding Interactions between Aromatic DBPs and Catalase Play an Important Role. *Water Res.* **2020**, *170*, No. 115283.
(12) Yang, Y.; Komaki, Y.; Kimura, S. Y.; Hu, H. Y.; Wagner, E. D.; Mariñas, B. J.; Plewa, M. J. Toxic Impact of Bromide and Iodide on Drinking Water Disinfected with Chlorine or Chloramines. *Environ. Sci. Technol.* **2014**, *48*, 12362−12369.
(13) Jeong, C. H.; Gao, L.; Dettro, T.; Wagner, E. D.; Ricke, W. A.; Plewa, M. J.; Flaws, J. A. Monohaloacetic Acid Drinking Water Disinfection By-Products Inhibit Follicle Growth and Steroidogenesis in Mouse Ovarian Antral Follicles in Vitro. *Reprod. Toxicol.* **2016**, *62*, 71−76.
(14) Xia, Y.; Mo, Y.; Yang, Q.; Yu, Y.; Jiang, M.; Wei, S.; Lu, D.; Wu, H.; Lu, G.; Zou, Y.; Zhang, Z.; Wei, X. Iodoacetic Acid Disrupting the Thyroid Endocrine System in Vitro and in Vivo. *Environ. Sci. Technol.* **2018**, *52*, 7545−7552.
(15) Gonsioroski, A.; Meling, D. D.; Gao, L.; Plewa, M. J.; Flaws, J. A. Iodoacetic Acid Inhibits Follicle Growth and Alters Expression of Genes That Regulate Apoptosis, the Cell Cycle, Estrogen Receptors, and Ovarian Steroidogenesis in Mouse Ovarian Follicles. *Reprod. Toxicol.* **2020**, *91*, 101−108.
(16) Richard, A. M.; Hunter, E. S. Quantitative Structure-Activity Relationships for the Developmental Toxicity of Haloacetic Acids in Mammalian Whole Embryo Culture. *Teratology* **1996**, *53*, 352−360.
(17) Yang, M.; Zhang, X. Comparative Developmental Toxicity of New Aromatic Halogenated DBPs in a Chlorinated Saline Sewage Effluent to the Marine Polychaete Platynereis Dumerilii. *Environ. Sci. Technol.* **2013**, *47*, 10868−10876.
(18) Yang, M.; Zhang, X. Halopyrroles: A New Group of Highly Toxic Disinfection Byproducts Formed in Chlorinated Saline Wastewater. *Environ. Sci. Technol.* **2014**, *48*, 11846−11852.
(19) Rice, J.; Wutich, A.; Westerhoff, P. Assessment of de Facto Wastewater Reuse across the U.S.: Trends between 1980 and 2008. *Environ. Sci. Technol.* **2013**, *47*, 11099−11105.
(20) Wilson, J. M.; Van Briesen, J. M. Source Water Changes and Energy Extraction Activities in the Monongahela River, 2009-2012. *Environ. Sci. Technol.* **2013**, *47*, 12575−12582.
(21) Krasner, S. W.; Sclimenti, M. J.; Means, E. G. Quality Degradation. Implications for DBP Formation. *J. − Am. Water Works Assoc.* **1994**, *86*, 34−47.
(22) Li, X. F.; Mitch, W. A. Drinking Water Disinfection Byproducts (DBPs) and Human Health Effects: Multidisciplinary Challenges and Opportunities. *Environ. Sci. Technol.* **2018**, *52*, 1681−1689.
(23) Plewa, M. J.; Wagner, E. D. Charting a New Path to Resolve the Adverse Health Effects of DBPs. *Recent Advances in Disinfection By-Products*; ACS Symposium Series; American Chemical Society, 2015; Vol. *1190*, pp 3−23.
(24) Cuthbertson, A. A.; Liberatore, H. K.; Kimura, S. Y.; Allen, J. M.; Bensussan, A. V.; Richardson, S. D. Trace Analysis of 61 Emerging Br-, Cl-, and I-DBPs: New Methods to Achieve Part-Per-Trillion Quantification in Drinking Water. *Anal. Chem.* **2020**, *92*, 3058−3068.
(25) Cuthbertson, A. A.; Kimura, S. Y.; Liberatore, H. K.; Summers, R. S.; Knappe, D. R. U. U.; Stanford, B. D.; Maness, J. C.; Mulhern, R. E.; Selbes, M.; Richardson, S. D. Does Granular Activated Carbon with Chlorination Produce Safer Drinking Water? From Disinfection Byproducts and Total Organic Halogen to Calculated Toxicity. *Environ. Sci. Technol.* **2019**, *53*, 5987−5999.
(26) Cuthbertson, A. A.; Kimura, S. Y.; Liberatore, H. K.; Knappe, D. R. U.; Stanford, B.; Summers, R. S.; Dickenson, E. R.; Maness, J. C.; Glover, C.; Selbes, M.; Richardson, S. D. GAC to BAC: Does It Make Chloraminated Drinking Water Safer? *Water Res.* **2020**, *172*, No. 115432.
(27) Krasner, S. W.; Lee, T. C. F.; Westerhoff, P.; Fischer, N.; Hanigan, D.; Karanfil, T.; Beita-Sandí, W.; Taylor-Edmonds, L.; Andrews, R. C. Granular Activated Carbon Treatment May Result in Higher Predicted Genotoxicity in the Presence of Bromide. *Environ. Sci. Technol.* **2016**, *50*, 9583−9591.
(28) Plewa, M. J.; Wagner, E. D.; Richardson, S. D. TIC-Tox: A Preliminary Discussion on Identifying the Forcing Agents of DBP-Mediated Toxicity of Disinfected Water. *J. Environ. Sci.* **2017**, *58*, 208−216.
(29) Lau, S. S.; Wei, X.; Bokenkamp, K.; Wagner, E. D.; Plewa, M. J.; Mitch, W. A. Assessing Additivity of Cytotoxicity Associated with Disinfection Byproducts in Potable Reuse and Conventional Drinking Waters. *Environ. Sci. Technol.* **2020**, *54*, 5729−5736.
(30) Stalter, D.; O'Malley, E.; Von Gunten, U.; Escher, B. I. Mixture Effects of Drinking Water Disinfection By-Products: Implications for Risk Assessment. *Environ. Sci. Water Res. Technol.* **2020**, *6*, 2341−2351.
(31) Jeong, C. H.; Wagner, E. D.; Siebert, V. R.; Anduri, S.; Richardson, S. D.; Daiber, E. J.; McKague, A. B.; Kogevinas, M.; Villanueva, C. M.; Goslan, E. H.; Wentai, L.; Isabelle, L. M.; Pankow, J. F.; Grazuleviciene, R.; Cordier, S.; Edwards, S. C.; Righi, E.; Nieuwenhuijsen, M. J.; Plewa, M. J. Occurrence and Toxicity of Disinfection Byproducts in European Drinking Waters in Relation with the HIWATE Epidemiology Study. *Environ. Sci. Technol.* **2012**, *46*, 12120−12128.
(32) Jeong, C. H.; Postigo, C.; Richardson, S. D.; Simmons, J. E.; Kimura, S. Y.; Mariñas, B. J.; Barcelo, D.; Liang, P.; Wagner, E. D.; Plewa, M. J. Occurrence and Comparative Toxicity of Haloacetaldehyde Disinfection Byproducts in Drinking Water. *Environ. Sci. Technol.* **2015**, *49*, 13749−13759.
(33) Krasner, S. W.; Westerhoff, P.; Chen, B.; Amy, G.; Nam, S. N. In *Contribution of Wastewater to DBP Formation: Case Study of an Effluent-Impacted River*, Water Quality Technology Conference Proceedings, 2005.
(34) Drewes, J. E.; Summers, R. S. Natural Organic Matter Removal During Riverbank Filtration: Current Knowledge and Research Needs. *Riverbank Filtration*; Springer, 2006; pp 303−309.
(35) Sharma, S. K.; Kennedy, M. D. Soil Aquifer Treatment for Wastewater Treatment and Reuse. *Int. Biodeterior. Biodegrad.* **2017**, *119*, 671−677.
(36) Swaim, P.; Royce, A.; Smith, T.; Maloney, T.; Ehlen, D.; Carter, B. Effectiveness of UV Advanced Oxidation for Destruction of Micro-Pollutants, in. *Ozone: Sci. Eng.* **2008**, *30*, 34−42.
(37) Summers, R. S.; Benz, M. A.; Shukairy, H. M.; Cummings, L. Effect of Separation Processes on the Formation of Brominated THMs. *J. − Am. Water Works Assoc.* **1993**, *85*, 88−95.
(38) Chili, C.-A.; Westerhoff, P.; Ghosh, A. GAC Removal of Organic Nitrogen and Other DBP Precursors. *J. − Am. Water Works Assoc.* **2012**, *104*, E406−E415.
(39) Symons, J. M.; Krasner, S. W.; Simms, L. A.; Sclimenti, M. Measurement of THM and Precursor Concentrations Revisited: The Effect of Bromide Ion. *J. − Am. Water Works Assoc.* **1993**, *85*, 51−62.
(40) Yun, T. I.; Liang, S.; Yates, R. S.; Krasner, S. W.; Dale, M. In *Full-Scale Evaluation of the Ammonia-Chlorine Process as a Bromate Control Strategy*, American Water Works Association Annual Conference and Exposition, 2011; pp 2402−2435.
(41) Prescott, M.; Krasner, S. W.; Guo, Y. C. Estimation of NDMA Precursor Loading in Source Water via Artificial Sweetener Monitoring. Removal of Organic Nitrogen and Other DBP Precursors. *J. − Am. Water Works Assoc.* **2017**, *109*, E243−E251.
(42) Krasner, S. W.; Weinberg, H. S.; Richardson, S. D.; Pastor, S. J.; Chinn, R.; Sclimenti, M. J.; Onstad, G. D.; Thruston, A. D. Occurrence of a New Generation of Disinfection Byproducts. *Environ. Sci. Technol.* **2006**, *40*, 7175−7185.
(43) Stevens, A. A.; Moore, L. A.; Miltner, R. J. Formation and Control of Non-Trihalomethane Disinfection by-Products. *J. − Am. Water Works Assoc.* **1989**, *81*, 54−60.
(44) Allen, J. M.; Cuthbertson, A. A.; Liberatore, H. K.; Kimura, S. Y.; Mantha, A.; Edwards, M. A.; Richardson, S. D. Showering in Flint, MI: Is There a DBP Problem? *J. Environ. Sci.* **2017**, *58*, 271−284.
(45) *Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides*

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in Drinking Water by Liquid−Liquid Extraction and Gas Chromatography with Electron-Capture Detection; U.S. EPA: Cincinnati, OH, 1992.
(46) *Method 552.2: Determination of Haloacetic Acids and Dalapon in Drinking Water by Liquid−Liquid Extraction, Derivatization and Gas Chromatography with Electron Capture Detection*; U.S. EPA: Cincinnati, OH, 1995.
(47) Allen, J. M.; Plewa, M. J.; Wagner, E. D.; Wei, X.; Bollar, G. E.; Quirk, L. E.; Liberatore, H. K.; Richardson, S. D. Making Swimming Pools Safer: Does Copper−Silver Ionization with Chlorine Lower the Toxicity and Disinfection Byproduct Formation? *Environ. Sci. Technol.* **2021**, *55*, 2908−2918.
(48) Richardson, S. D. *Diazomethane Generation Using Sigma-Aldrich Diazald and Diazomethane Generator and Methylation of Carboxylic Acid Compounds*; U.S. EPA: Athens, GA, 2009.
(49) Kimura, S. Y.; Zheng, W.; Hipp, T. N.; M Allen, J. M.; Richardson, S. D. Total Organic Halogen (TOX) in Human Urine: A Halogen-Specific Method for Human Exposure Studies. *J. Environ. Sci.* **2017**, *58*, 285−295.
(50) Echigo, S.; Zhang, X.; Minear, R. A.; Plewa, M. J. Differentiation of Total Organic Brominated and Chlorinated Compounds in Total Organic Halide Measurement: A New Approach with an Ion-Chromatographic Technique. *Natural Organic Matter and Disinfection By-Products*; ACS Symposium Series; American Chemical Society, 2000; Vol. *761*, pp 330−342.
(51) Richardson, S. D. *XAD Resin Extraction of Disinfection By-Products in Drinking Water: SOP—RSB-003.1—Revision No. 1*; U.S. Environmental Protection Agency: Athens, GA, 2011.
(52) Richardson, S. D.; Thruston, A. D.; Collette, T. W.; Patterson, K. S.; Lyklns, B. W.; Majetich, G.; Zhang, Y. Multispectral Identification of Chlorine Dioxide Disinfection Byproducts in Drinking Water. *Environ. Sci. Technol.* **1994**, *28*, 592−599.
(53) Wagner, E. D.; Rayburn, A. L.; Anderson, D.; Plewa, M. J. Analysis of Mutagens with Single Cell Gel Electrophoresis, Flow Cytometry, and Forward Mutation Assays in an Isolated Clone of Chinese Hamster Ovary Cells. *Environ. Mol. Mutagen.* **1998**, *32*, 360−368.
(54) Attene-Ramos, M. S.; Wagner, E. D.; Plewa, M. J. Comparative Human Cell Toxicogenomic Analysis of Monohaloacetic Acid Drinking Water Disinfection Byproducts. *Environ. Sci. Technol.* **2010**, *44*, 7206−7212.
(55) Muellner, M. G.; Wagner, E. D.; Mccalla, K.; Richardson, S. D.; Woo, Y. T.; Plewa, M. J. Haloacetonitriles vs. Regulated Haloacetic Acids: Are Nitrogen-Containing DBFs More Toxic? *Environ. Sci. Technol.* **2007**, *41*, 645−651.
(56) Liberatore, H. K.; Plewa, M. J.; Wagner, E. D.; Vanbriesen, J. M.; Burnett, D. B.; Cizmas, L. H.; Richardson, S. D. Identification and Comparative Mammalian Cell Cytotoxicity of New Iodo-Phenolic Disinfection Byproducts in Chloraminated Oil and Gas Wastewaters. *Environ. Sci. Technol. Lett.* **2017**, *4*, 475−480.
(57) Plewa, M. J.; Kargalioglu, Y.; Vankerk, D.; Minear, R. A.; Wagner, E. D. Mammalian Cell Cytotoxicity and Genotoxicity Analysis of Drinking Water Disinfection By-Products. *Environ. Mol. Mutagen.* **2002**, *40*, 134−142.
(58) Plewa, M. J.; Muellner, M. G.; Richardson, S. D.; Fasano, F.; Buettner, K. M.; Woo, Y. T.; Mckague, A. B.; Wagner, E. D. Occurrence, Synthesis, and Mammalian Cell Cytotoxicity and Genotoxicity of Haloacetamides: An Emerging Class of Nitrogenous Drinking Water Disinfection Byproducts. *Environ. Sci. Technol.* **2008**, *42*, 955−961.
(59) Wagner, E. D.; Rayburn, A. L.; Anderson, D.; Plewa, M. J. Calibration of the Single Cell Gel Electrophoresis Assay, Flow Cytometry Analysis and Forward Mutation in Chinese Hamster Ovary Cells. *Mutagenesis* **1998**, *13*, 81−84.
(60) Singh, K.; Xie, M. *Bootstrap: A Statistical Method*; Rutgers University: New Brunswick, NJ, 2008.
(61) Canty, A. J.; Davison, A. C.; Hinkley, D. V.; Ventura, V. Bootstrap Diagnostics and Remedies. *Can. J. Stat.* **2006**, *34*, 5−27.
(62) Qin, L. T.; Zhang, X.; Chen, Y. H.; Mo, L. Y.; Zeng, H. H.; Liang, Y. P.; Lin, H.; Wang, D. Q. Predicting the Cytotoxicity of Disinfection By-Products to Chinese Hamster Ovary by Using Linear Quantitative Structure−Activity Relationship Models. *Environ. Sci. Pollut. Res.* **2019**, *26*, 16606−16615.
(63) Efron, B. Better Bootstrap Confidence Intervals. *J. Am. Stat. Assoc.* **1987**, *82*, 171−185.
(64) Wei, X.; Yang, M.; Zhu, Q.; Wagner, E. D.; Plewa, M. J. Comparative Quantitative Toxicology and QSAR Modeling of the Haloacetonitriles: Forcing Agents of Water Disinfection Byproduct Toxicity. *Environ. Sci. Technol.* **2020**, *54*, 8909−8918.
(65) National Toxicology Program (NTP). *Toxicologyand Carcinogenesis Studies of Dibromoacetonitrile (CAS No. 3252-43-5) in F344/Nrats and B6C3F1 Mice (Drinking Water Studies)*; Publication 10-5886; NTP, 2010.
(66) Krasner, S. W.; Westerhoff, P.; Chen, B.; Rittmann, B. E.; Nam, S. N.; Amy, G. Impact of Wastewater Treatment Processes on Organic Carbon, Organic Nitrogen, and DBP Precursors in Effluent Organic Matter. *Environ. Sci. Technol.* **2009**, *43*, 2911−2918.
(67) Krasner, S. W. Halogenated DBPs and Emerging Issues. *Disinfection By-Products and Human Health*; IWA Publishing, 2012; pp 59−71.
(68) Plewa, M. J.; Wagner, E. D.; Jazwierska, P.; Richardson, S. D.; Chen, P. H.; McKague, A. B. Halonitromethane Drinking Water Disinfection Byproducts: Chemical Characterization and Mammalian Cell Cytotoxicity and Genotoxicity. *Environ. Sci. Technol.* **2004**, *38*, 62−68.
(69) Bichsel, Y.; Von Gunten, U. Oxidation of Iodide and Hypoiodous Acid in the Disinfection of Natural Waters. *Environ. Sci. Technol.* **1999**, *33*, 4040−4045.
(70) Richardson, S. D.; Fasano, F.; Ellington, J. J.; Crumley, F. G.; Buettner, K. M.; Evans, J. J.; Blount, B. C.; Silva, L. K.; Waite, T. J.; Luther, G. W.; McKague, B.; Miltner, R. J.; Wagner, E. D.; Plewa, M. J. Occurrence and Mammalian Cell Toxicity of Iodinated Disinfection Byproducts in Drinking Water. *Environ. Sci. Technol.* **2008**, *42*, 8330−8338.
(71) Wei, X.; Wang, S.; Zheng, W.; Wang, X.; Liu, X.; Jiang, S.; Pi, J.; Zheng, Y.; He, G.; Qu, W. Drinking Water Disinfection Byproduct Iodoacetic Acid Induces Tumorigenic Transformation of NIH3T3 Cells. *Environ. Sci. Technol.* **2013**, *47*, 5913−5920.
(72) Sun, H.; Liu, H.; Han, J.; Zhang, X.; Cheng, F.; Liu, Y. Chemical Cleaning-Associated Generation of Dissolved Organic Matter and Halogenated Byproducts in Ceramic MBR: Ozone versus Hypochlorite. *Water Res.* **2018**, *140*, 243−250.
(73) Jiang, J.; Zhang, X.; Zhu, X.; Li, Y. Removal of Intermediate Aromatic Halogenated DBPs by Activated Carbon Adsorption: A New Approach to Controlling Halogenated DBPs in Chlorinated Drinking Water. *Environ. Sci. Technol.* **2017**, *51*, 3435−3444.
(74) Richardson, S. D.; Kimura, S. Y. Water Analysis: Emerging Contaminants and Current Issues. *Anal. Chem.* **2020**, *92*, 473−505.
(75) Pals, J.; Attene-Ramos, M. S.; Xia, M.; Wagner, E. D.; Plewa, M. J. Human Cell Toxicogenomic Analysis Linking Reactive Oxygen Species to the Toxicity of Monohaloacetic Acid Drinking Water Disinfection Byproducts. *Environ. Sci. Technol.* **2013**, *47*, 12514−12523.
(76) Furst, K. E.; Bolorinos, J.; Mitch, W. A. Use of trihalomethanes as a surrogate for haloacetonitrile exposure introduces misclassification bias. *Water Res. X* **2021**, *11*, No. 100089.

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SUPPORTING INFORMATION

for

Disinfection By-Product Drivers of Cytotoxicity in U.S. Drinking Water: Should Other DBPs Be Regulated?

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Text S1. Unregulated disinfection by-product extraction procedure

Raw (untreated) and distribution system at average detention (Dist. Avg.) samples were extracted in duplicate. The 53 priority, unregulated haloacetonitriles (HANs), haloacetamides (HAMs), halonitromethanes (HNMs), iodinated trihalomethanes (I-THMs), iodinated haloacetic acids (I-HAAs), haloketones (HKs), and tri-haloacetaldehydes (tri-HALs) were extracted in a single method.[1–5] For this procedure, 100 mL samples were adjusted to pH < 1.0 with concentrated H_2SO_4, spiked with 30 g of sodium sulfate and 5 mL of methyl *tert*-butyl ether (MTBE), and shaken for 15 min on a mechanical shaker. This was done three times, with a 10-minute wait in between each shake for phase separation before removing the organic layer into a separate container. After passing the 15 mL of final extract over dried Na_2SO_4 to remove excess water, the extract was concentrated under nitrogen to a final volume of 200 µL and spiked with internal standard (1,2-dibromopropane). Half of this extract was used for analysis of HANs, HAMs, HNMs, I-THMs, IAAs, HKs, and tri-HALs, and the second 100 µL extract underwent diazomethane derivatization for analysis of the four I-HAAs.

Text S2. Total organic halogen procedure

First, 50 mL of sample was adjusted to pH 2 with concentrated HNO_3 and passed through two activated carbon (AC) columns, then the columns were washed with 10 mL of 5,000 mg/L of KNO_3 adjusted to pH 2. Each AC was then loaded onto a ceramic boat and automatically loaded into a quick furnace (AQF-2100H) using an automatic solid sampler (ASC-240S). The ACs were pyrolyzed inside the furnace at 1000°C, and the produced gasses were bubbled into centrifuge tubes that contained 5 mL of adsorption

solution (0.003% H_2O_2, 0.01 mM phosphate) using a gas absorption unit (AU-250). The adsorption solution was analyzed for chloride, bromide, and iodide with a 1600 ion chromatography (IC) system (Dionex, Sunnyvale, CA). For low iodide concentrations (<10 μg/L), a Finnigan ELEMENT XR double focusing magnetic sector field ICP-MS instrument (Thermo Electron Corporation) instrument was used for quantification.[4,6]

Text S3. Chinese Hamster Ovary (CHO) cell chronic cytotoxicity assay

After XAD ethyl acetate extracts were solvent-exchanged into dimethyl sulfoxide (DMSO), a 96-well flat-bottomed microplate was used to evaluate a series of concentrations of the concentrated water sample (CWS) for each experimental group. One column of eight microplate wells served as the blank control consisting of 200 μL of F12 + 5% fetal bovine serum (FBS) medium only. The concurrent negative control column consisted of wells with 3×10^3 CHO cells plus F12 + FBS medium. The remaining wells within the experiment contained 3×10^3 CHO cells, F12 + FBS and a known volume of the CWS for a total of 200 μL. The wells were covered with a sheet of sterile Alumna Seal™ to prevent volatile cross contamination of adjacent wells. The microplate was placed on a rocking platform at 37°C for two 5 min-periods (turning the plate 90° after the first 5 min). This step is important to ensure an even distribution of cells across the bottom of the microplate wells. The cells were incubated for 72 h at 37°C under 5% CO_2.

After the treatment time, the medium from each well was aspirated, the cells fixed in methanol for 5 min and stained for 10 min with a 1% crystal violet solution in 50% methanol. The microplate was washed in tap water and patted dry and 50 μL of dimethyl sulfoxide (DMSO)/methanol (3:1 v/v) was added to each well; the plate was incubated at room temperature for 10 min. The microplate was analyzed at 595 nm with a

SpectraMax™ microplate reader. This assay was calibrated and there was a direct relationship between the absorbance of the crystal violet dye associated with cell density and the number of viable cells.[7] The averaged absorbance of the blank wells was subtracted from the absorbance data from each microplate well. The mean blank-corrected absorbance value of the negative control was set at 100%. The absorbance for each treatment group well was converted into a percentage of the negative control. This procedure normalized the data, maintained the variance and allowed the combination of data from multiple microplates. For each experiment, a series of concentrations (generally 10 concentration factors) are constructed by diluting the DMSO concentrate, and then mixed with culture medium just prior to the experiment. A median lethal concentration (LC_{50}) ± standard error (SE) value could be generated, which is the concentration of the water sample, determined from a bootstrap multiple regression analysis of the data,[8–11] that induced a cell density of 50% as compared to the concurrent negative control. A cytotoxicity index (CTI) could then be found using the LC_{50}, which is defined as the $LC_{50}^{-1}(10^3)$, such that cytotoxic potency could be easily ranked (higher CTI, higher cytotoxicity).

Literature Cited

(1) Cuthbertson, A. A.; Liberatore, H. K.; Kimura, S. Y.; Allen, J. M.; Bensussan, A. V.; Richardson, S. D. Trace Analysis of 61 Emerging Br-, Cl-, and I-DBPs: New Methods to Achieve Part-Per-Trillion Quantification in Drinking Water. *Anal. Chem.* **2020**, *92*, 3058–3068.

(2) Cuthbertson, A. A.; Kimura, S. Y.; Liberatore, H. K.; Summers, R. S.; Knappe, D. R. U. U.; Stanford, B. D.; Maness, J. C.; Mulhern, R. E.; Selbes, M.; Richardson, S. D. Does Granular Activated Carbon with Chlorination Produce Safer Drinking Water? From Disinfection Byproducts and Total Organic Halogen to Calculated Toxicity. *Environ. Sci. Technol.* **2019**, *53*, 5987–5999.

(3) Cuthbertson, A. A.; Kimura, S. Y.; Liberatore, H. K.; Knappe, D. R. U.; Stanford, B.; Summers, R. S.; Dickenson, E. R.; Maness, J. C.; Glover, C.; Selbes, M.;

Richardson, S. D. GAC to BAC: Does It Make Chloraminated Drinking Water Safer? *Water Res.* **2020**, *172*, 115432.

(4) Alllen, J.M., Plewa, M.J., Wagner, E.D., Wei, X., Bollar, G.E., Quirk, L.E., Liberatore, H.K., Richardson, S. D. Making Swimming Pools Safer: Does Copper–Silver Ionization with Chlorine Lower the Toxicity and Disinfection Byproduct Formation? *Environ. Sci. Technol.* **2021**, *55*, 2908–2918.

(5) Allen, J. M.; Cuthbertson, A. A.; Liberatore, H. K.; Kimura, S. Y.; Mantha, A.; Edwards, M. A.; Richardson, S. D. Showering in Flint, MI: Is There a DBP Problem? *J. Environ. Sci.* **2017**, *58*, 271–284.

(6) Kimura, S. Y.; Zheng, W.; Hipp, T. N.; M Allen, J. M.; Richardson, S.D. Total Organic Halogen (TOX) in Human Urine: A Halogen-Specific Method for Human Exposure Studies. *J. Environ. Sci.* **2017**, *58*, 285–295.

(7) Wagner, E. D.; Rayburn, A. L.; Anderson, D.; Plewa, M. J. Calibration of the Single Cell Gel Electrophoresis Assay, Flow Cytometry Analysis and Forward Mutation in Chinese Hamster Ovary Cells. *Mutagenesis* **1998**, *13*, 81–84.

(8) Wagner, E. D.; Plewa, M. J. CHO Cell Cytotoxicity and Genotoxicity Analyses of Disinfection By-Products: An Updated Review. *J. Environ. Sci.* **2017**, *58*, 64–76.

(9) Efron, B. Better Bootstrap Confidence Intervals. *J. Am. Stat. Assoc*. **1987**, *82*, 171–185.

(10) Canty, A. J.; Davison, A. C.; Hinkley, D. V.; Ventura, V. Bootstrap Diagnostics and Remedies. *Can. J. Stat*. **2006**, *34*, 5–27.

(11) Singh, K.; Xie, M. Bootstrap: A Statistical Method; Rutgers University: New Brunswick, NJ, 2008.

Tables and Figures

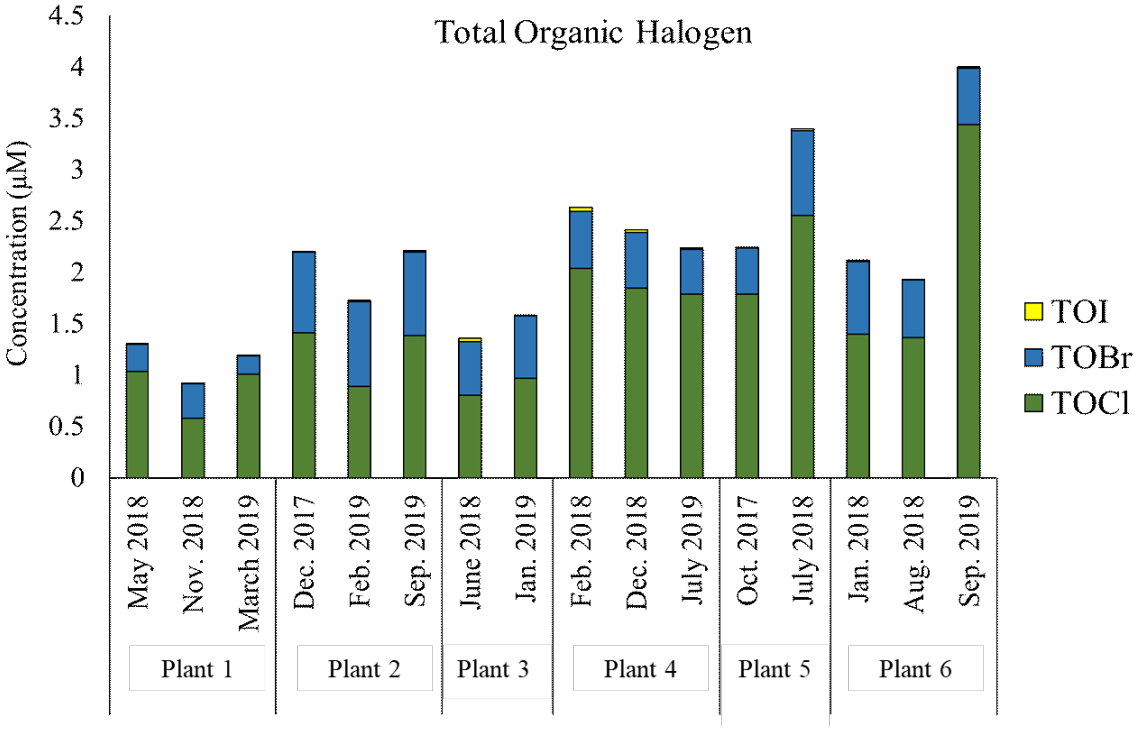

Figure S1. TOX data for all plants (distribution system/average).

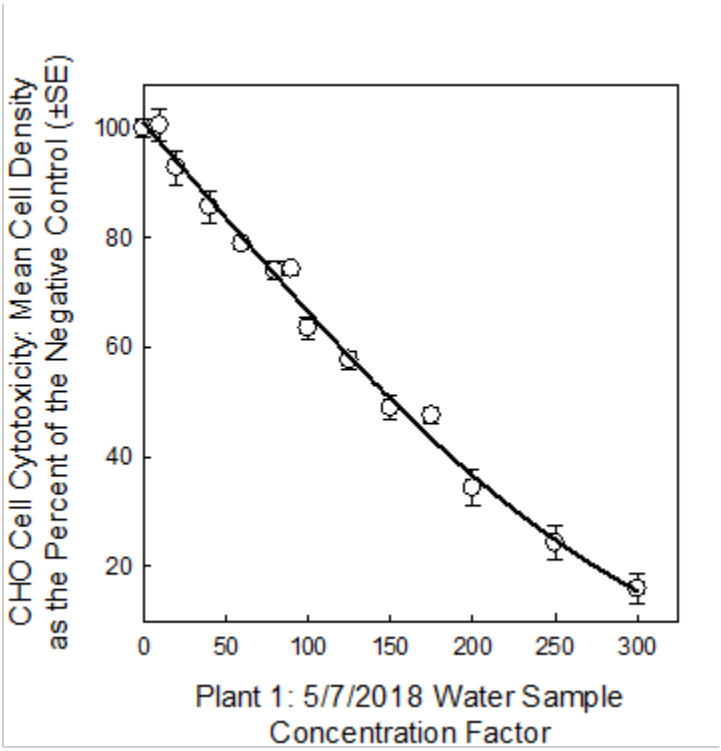

Figure S2. CHO cell cytotoxicity concentration-response curve for Plant 1 sample 5/7/2018.

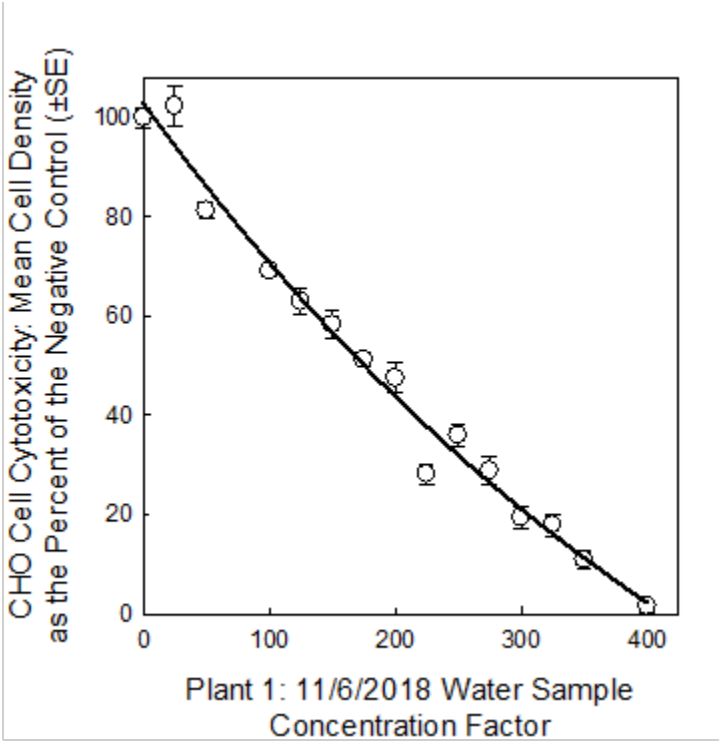

Figure S3. CHO cell cytotoxicity concentration-response curve for Plant 1 sample 11/6/2018.

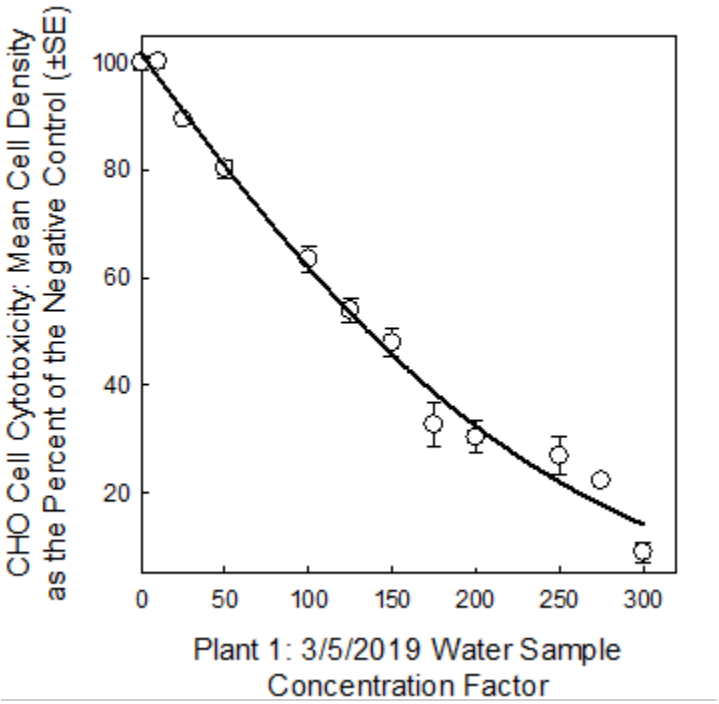

Figure S4. CHO cell cytotoxicity concentration-response curve for Plant 1 sample 3/5/2019.

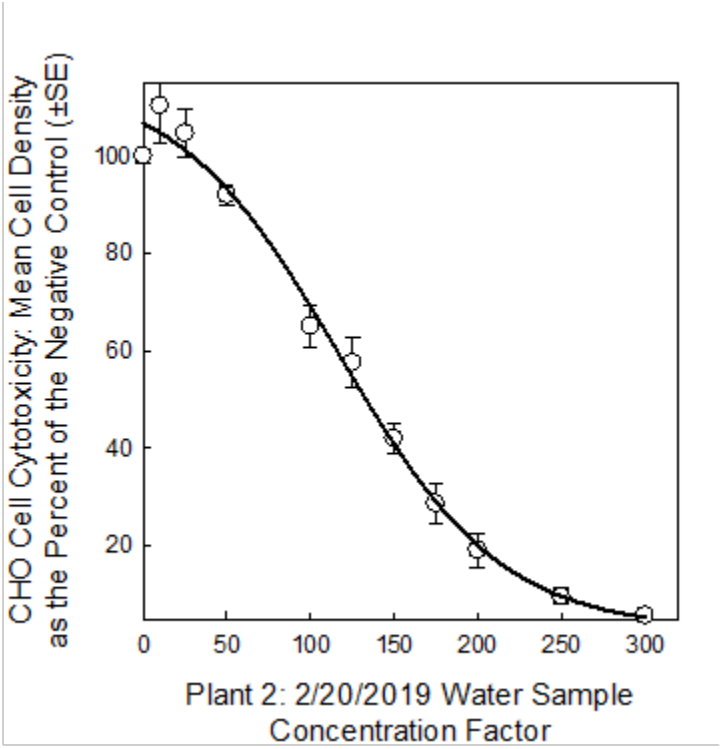

Figure S5. CHO cell cytotoxicity concentration-response curve for Plant 2 sample 12/14/2017.

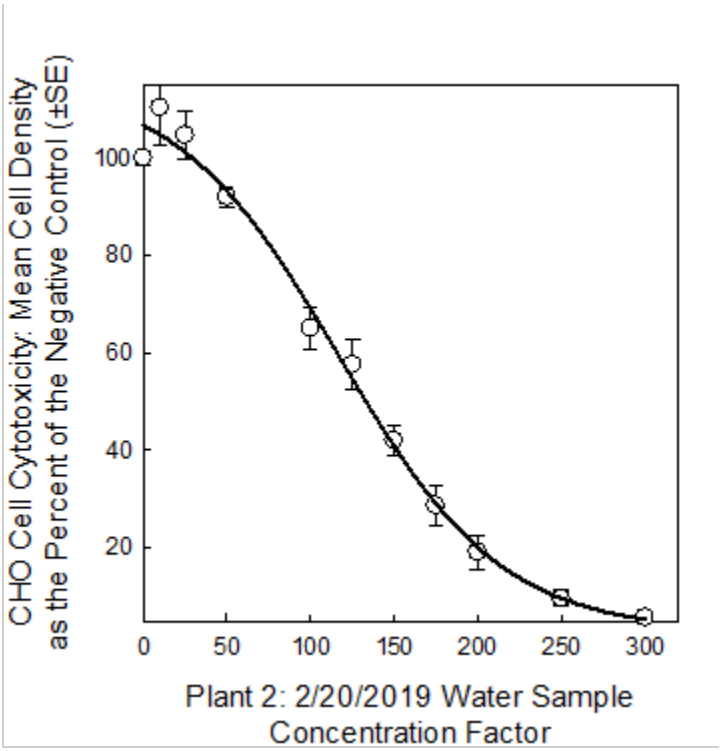

Figure S6. CHO cell cytotoxicity concentration-response curve for Plant 2 sample 2/20/2019.

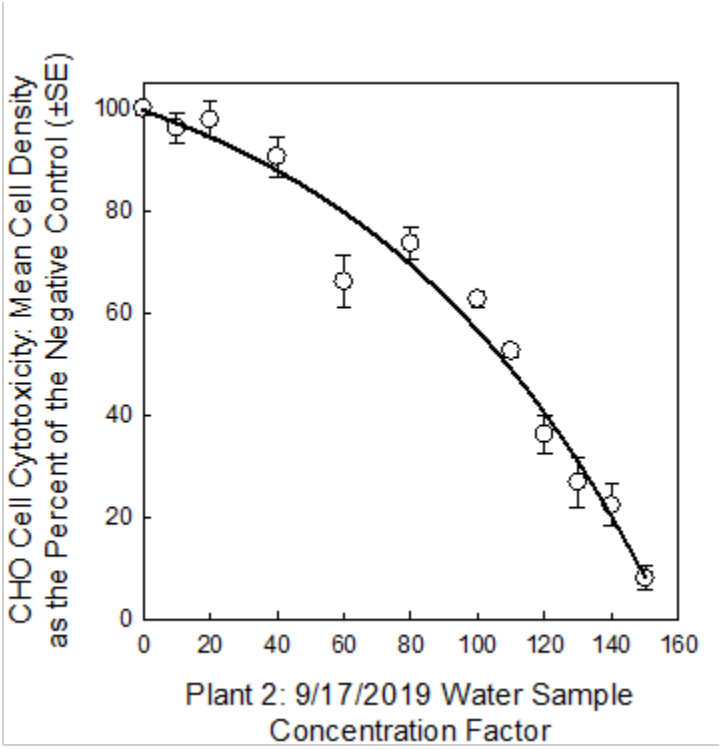

Figure S7. CHO cell cytotoxicity concentration-response curve for Plant 2 sample 9/17/2019.

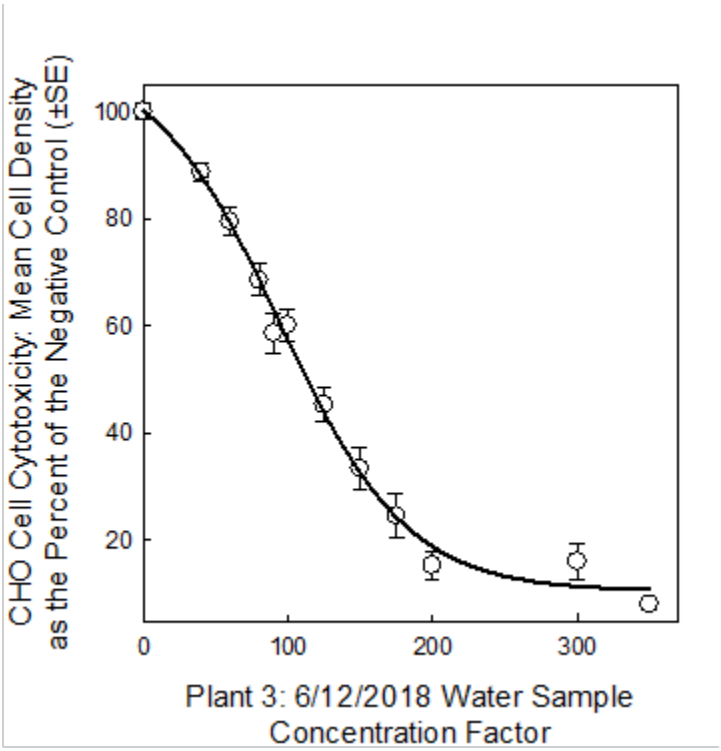

Figure S8. CHO cell cytotoxicity concentration-response curve for Plant 3 sample 6/12/2018.

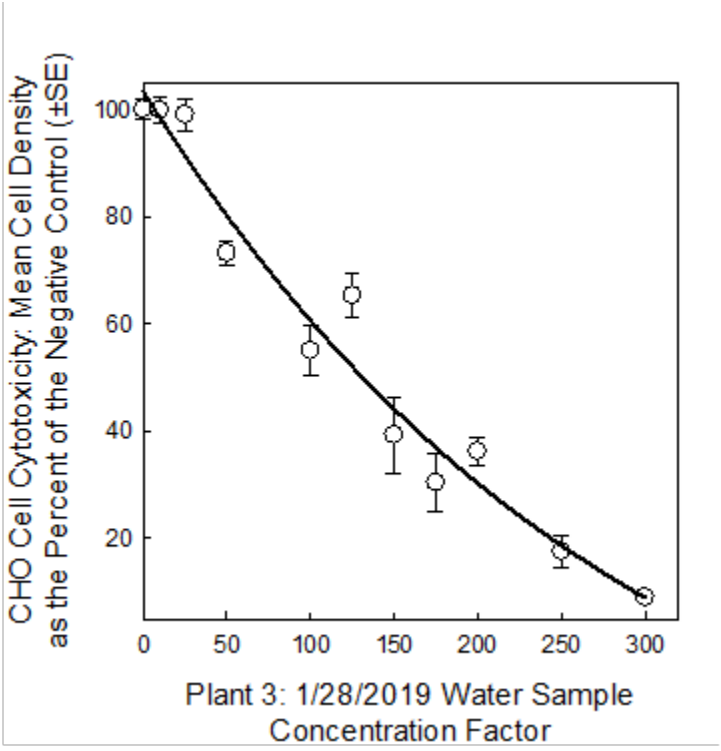

Figure S9. CHO cell cytotoxicity concentration-response curve for Plant 3 sample 1/28/2019.

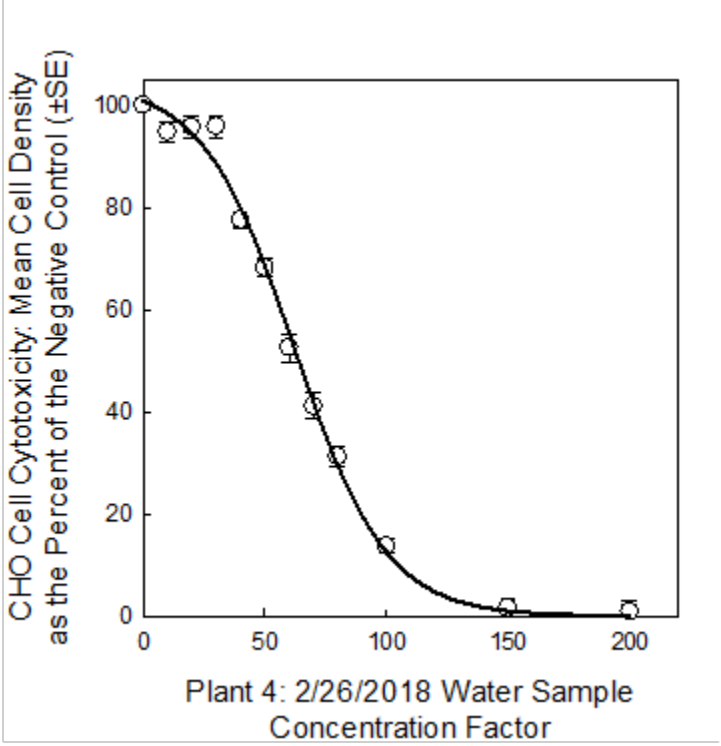

Figure S10. CHO cell cytotoxicity concentration-response curve for Plant 4 sample 2/26/2018.

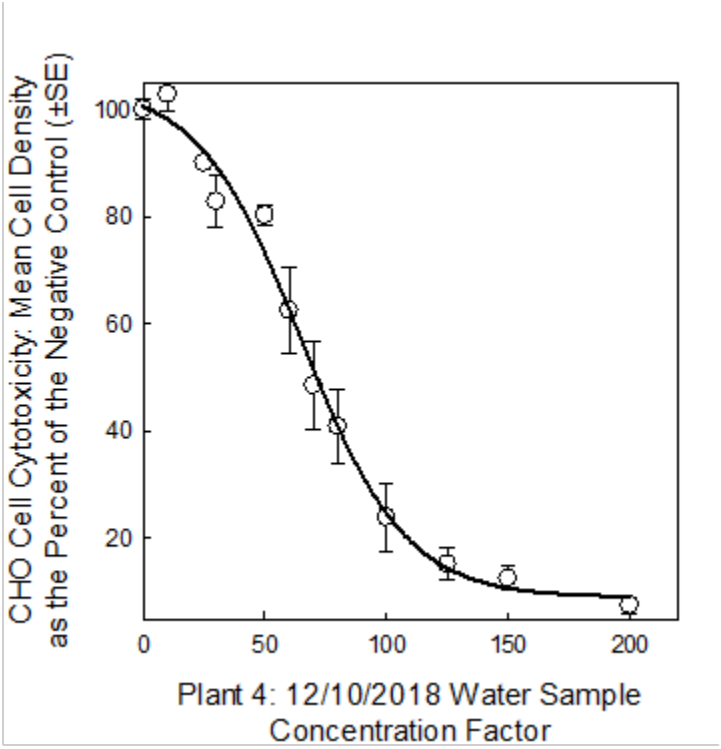

Figure S11. CHO cell cytotoxicity concentration-response curve for Plant 4 sample 12/10/2018.

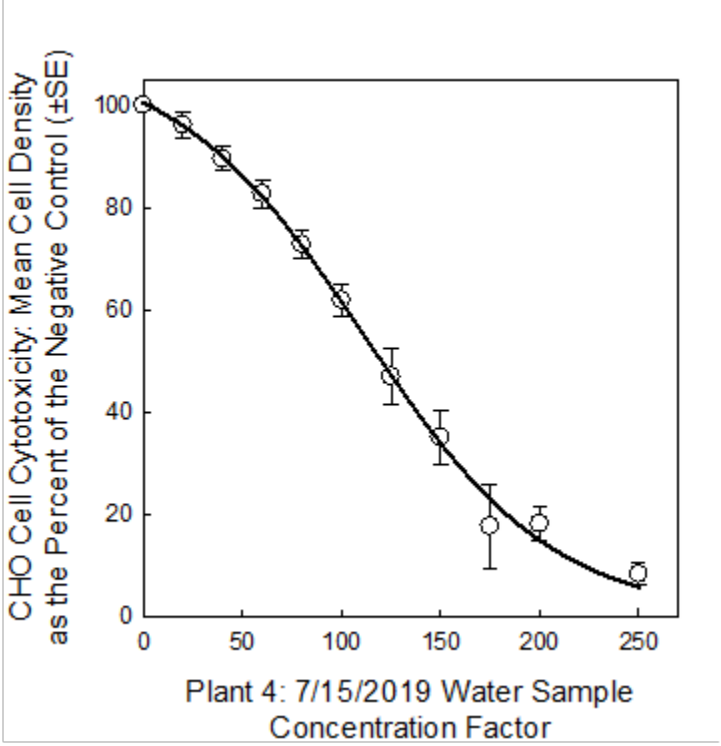

Figure S12. CHO cell cytotoxicity concentration-response curve for Plant 4 sample 7/15/2019.

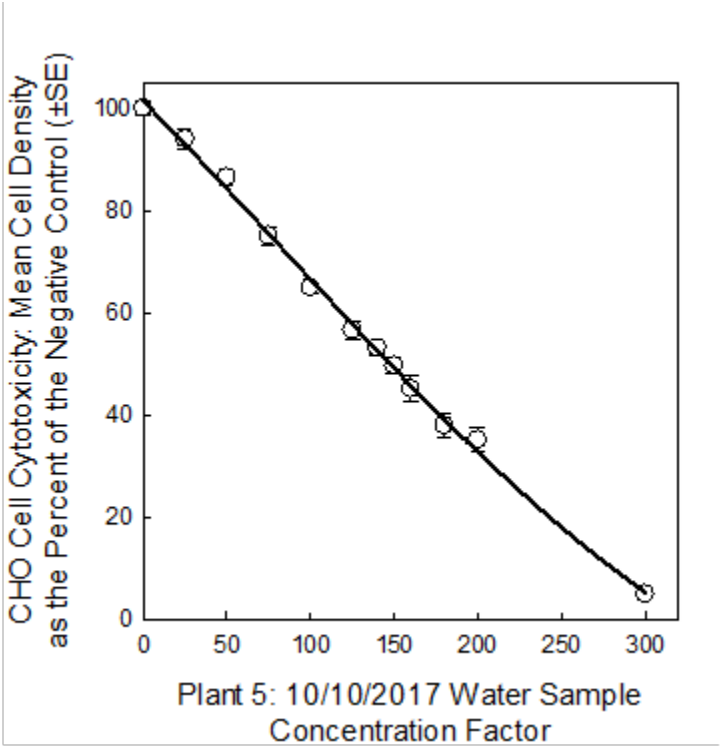

Figure S13. CHO cell cytotoxicity concentration-response curve for Plant 5 sample 10/10/2017.

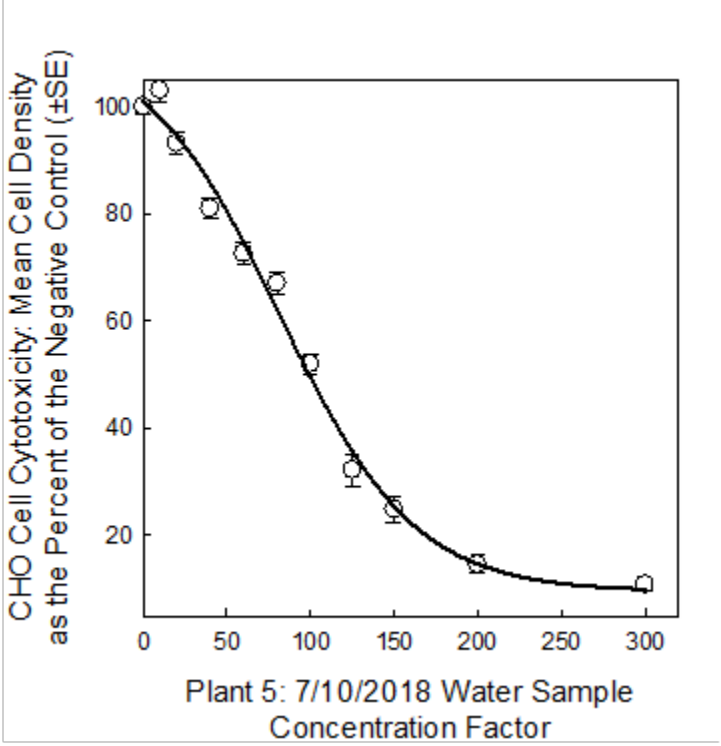

Figure S14. CHO cell cytotoxicity concentration-response curve for Plant 5 sample 7/10/2018.

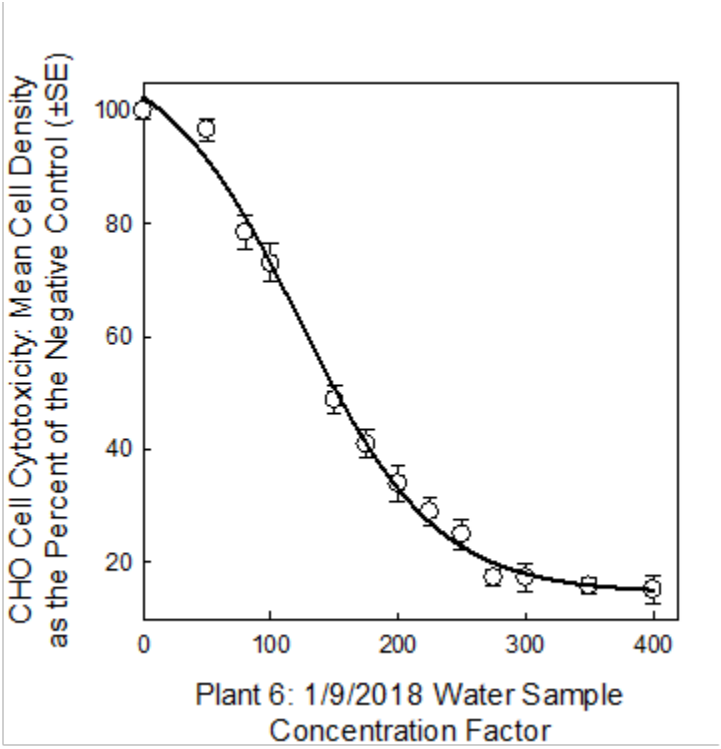

Figure S15. CHO cell cytotoxicity concentration-response curve for Plant 6 sample 1/9/2018.

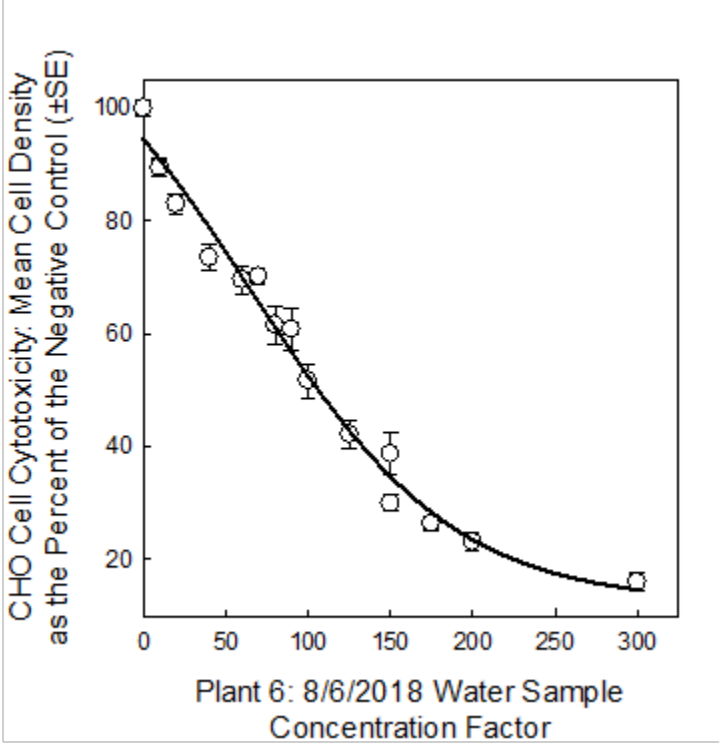

Figure S16. CHO cell cytotoxicity concentration-response curve for Plant 6 sample 8/6/2018.

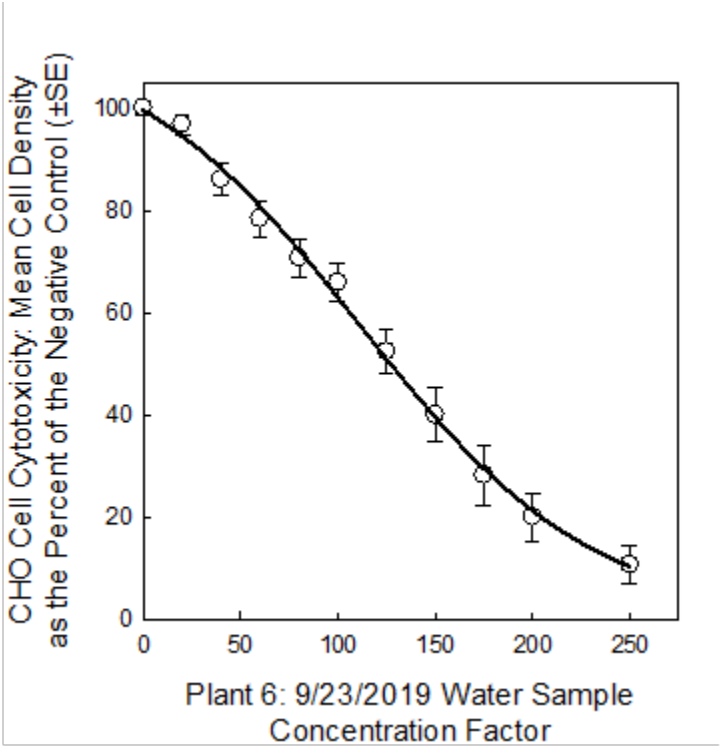

Figure S17. CHO cell cytotoxicity concentration-response curve for Plant 6 sample 9/23/2019.

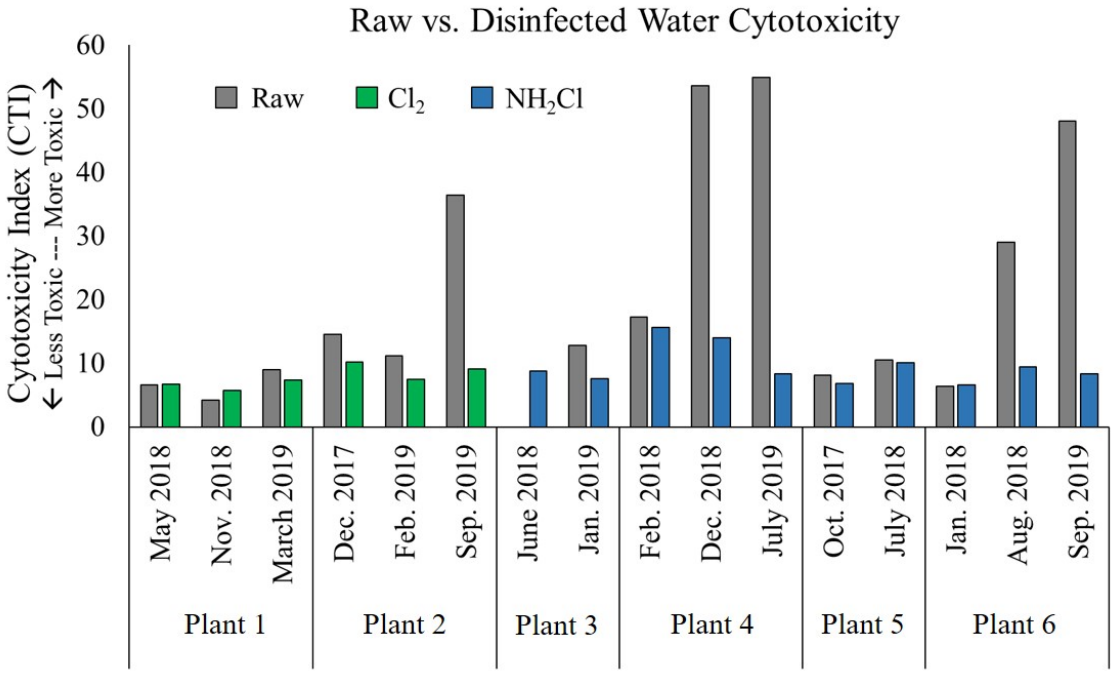

Figure S18. Raw vs. distribution system/average cytotoxicity index values for each plant.

Table S1. Summary of analytical methods.

Parameter	Method
Total organic carbon	Standard Methods[a]
Bromide, iodide	Direct analysis of raw water after 0.45 μm filtration; ion chromatography with conductivity detector
TOCl, TOBr, TOI	GAC sorption, combustion, measurement of Cl^-, Br^-, and I^- with ion chromatography and conductivity detector or inductively coupled plasma MS
THM4	EPA 551.1
HAA9	EPA 552.2
HAMs, HANs, HNMs, HALs, HKs, I-THMs	Liquid-liquid extraction, GC-MS analysis (and PFBHA derivatization for mono- and di-HALs)
IAAs	Liquid-liquid extraction, diazomethane derivatization, GC-MS/MS analysis
Sucralose	Direct injection, LC-MS/MS analysis
Total ammonia	Standard Methods[a]
UV_{254}	Standard Methods[a]

[a]American Public Health Association, American Water Works Association, and Water Environment Federation, *Standard Methods for the Examination of Water and Wastewater*, 23rd ed., American Public Health Association, Washington, D.C., 2017.

Table S2. CHO cell cytotoxicity data for all plants.

Sample	Date	LC_{50}[a] (CF)	CTI[b]
Plant 1 Raw	May 2018	151.32 ± 3.3	6.64 ± 0.14
Plant 1 Distribution Average		149.43 ± 2.58	6.71 ± 0.12
Plant 1 Raw	Nov. 2018	238.53 ± 8.96	4.25 ± 0.16
Plant 1 Distribution Average		176.75 ± 3.98	5.68 ± 0.13
Plant 1 Raw	March 2019	111.68 ± 0.80	8.96 ± 0.06
Plant 1 Distribution Average		137.41 ± 5.05	7.38 ± 0.27
Plant 2 Raw	Dec. 2017	70.26 ± 3.14	14.50 ± 0.66
Plant 2 Distribution Average		98.48 ± 2.27	10.21 ± 0.26
Plant 2 Raw	Sep. 2019	27.49 ± 0.23	36.4 ± 0.30
Plant 2 Distribution Average		109.90 ± 1.32	9.12 ± 0.11
Plant 2 Raw	Feb. 2019	88.84 ± 1.63	11.30 ± 0.22
Plant 2 Distribution Average		131.68 ± 2.86	7.64 ± 0.18
Plant 3 Raw	June 2018	33.88 ± 1.95	30.58 ± 1.87
Plant 3 Distribution Average		116.33 ± 5.80	8.81 ± 0.43
Plant 3 Raw	Jan. 2019	79.07 ± 2.41	12.79 ± 0.38
Plant 3 Distribution Average		128.48 ± 6.40	8.01 ± 0.39
Plant 4 Raw	Feb. 2018	57.70 ± 0.70	17.36 ± 0.22
Plant 4 Distribution Average		63.79 ± 1.02	15.72 ± 0.25
Plant 4 Raw	Dec. 2018	18.56 ± 0.53	54.43 ± 1.59
Plant 4 Distribution Average		72.35 ± 3.78	14.28 ± 0.75
Plant 4 Raw	July 2019	18.26 ± 0.31	54.96 ± 0.95
Plant 4 Distribution Average		123.67 ± 5.97	8.32 ± 0.42
Plant 5 Raw	Oct. 2017	123.44 ± 3.18	8.15 ± 0.22
Plant 5 Distribution Average		148.01 ± 3.35	6.79 ± 0.15
Plant 5 Raw	July 2018	96.66 ± 1.37	10.37 ± 0.14
Plant 5 Distribution Average		101.29 ± 2.38	9.93 ± 0.24
Plant 6 Raw	Jan. 2018	157.01 ± 4.59	6.42 ± 0.18
Plant 6 Distribution Average		152.97 ± 3.41	6.57 ± 0.15
Plant 6 Raw	Aug. 2018	34.44 ± 0.55	29.12 ± 0.51
Plant 6 Distribution Average		105.25 ± 1.85	9.53 ± 0.17
Plant 6 Raw	Sep. 2019	20.79 ± 0.13	48.13 ± 0.30
Plant 6 Distribution Average		128.41 ± 8.34	8.34 ± 0.64

[a] The mean LC_{50} ± (standard error) SE value is the concentration of the water sample, determined from a bootstrap multiple regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative controls. [b]Cytotoxicity index (CTI) is defined as $(LC_{50}^{-1})(10)^3$ ± SE; a higher CTI indicating higher cytotoxicity.

Table S3. Pearson Product Moment Correlation statistical analyses for DBP and CHO cell cytotoxicity.

	All Sampling Events		No Plant 4	
DBP Class	**Pearson's *r***	***P* value**	**Pearson's *r***	***P* value**
THM-4	0.36	0.17	0.48	0.09
HAA-9	0.53	**0.03**	0.21	0.50
Unregulated HAAs	0.30	0.26	0.16	0.59
HAMs	0.61	**0.01**	0.25	0.41
HANs	0.08	0.76	0.66	**0.01**
HNMs	0.37	0.18	0.17	0.60
I-THMs	0.70	**0.02**	0.31	0.46
I-HAAs	0.88	**0.0007**	0.23	0.62
HALs	0.05	0.86	0.39	0.19
HKS	0.18	0.53	0.37	0.26
Mono-Halo DBPs	-0.42	0.19		
Di-Halo DBPs	0.64	**0.008**		
Tri-Halo DBPs	0.39	0.13		
Summed N-DBPs	0.56	**0.02**		
Summed C-DBPs	0.44	0.09		

*Bold *P* value indicates a significant correlation.

Table S4. Summary of chemical doses applied for each plant.

Plant	Date	O_3 (mg/L)	UV (mJ/cm^2)	H_2O_2 (mg/L)	Chlorine (mg/L)
Plant 1	5/7/18	NA	~25	NA	1.6
Plant 1	11/6/2018	NA	~25	NA	1.4
Plant 1	3/5/2019	NA	~25	NA	1.3
Plant 2	12/12/17	NA	NA	NA	3.4
Plant 2	2/19/2019	NA	NA	NA	4.0
Plant 2	9/17/2019	NA	NA	NA	3.9
Plant 3	6/11/2018	1.36	NA	NA	3.2
Plant 3	1/28/2019	1.17	NA	NA	3.1
Plant 4	2/26/18	NA	NA	NA	4.2
Plant 4	12/2018	NA	NA	NA	4.1
Plant 4	7/2019	NA	NA	NA	5.0
Plant 5	10/10/17	NA	NA	NA	17.8
Plant 5	7/10/18	NA	NA	NA	14.0
Plant 6	1/9/18	NA	>500	2.5	4.1
Plant 6	8/6/18	NA	>500	1.75	3.3
Plant 6	9/2019	NA	>500	1.60	3.2

*NA = not applicable;

Unregulated DBP Abbreviation Key for Quantitative Data Tables:

D = di
T = tri
Te = tetra
C = chloro
B = bromo
I = iodo
AL = aldehyde
P = propanone
NM = nitromethane
AN = acetonitrile
AM = acetamide
M = methane
AA = acetic acid

Table S5. Mean unregulated DBP data for Plant 1.

Compound	MRL (µg/L)	Concentration - µg/L (nM)			
		Raw	Dist. Avg. May 2018	Dist. Avg. Nov. 2018	Dist. Avg. March 2019
HNMs					
BDCNM	0.1	ND	0.2 (1.0)	0.1 (0.3)	0.2 (1.0)
DBCNM	0.1	ND	0.6 (2.6)	0.2 (0.8)	0.2 (0.8)
TBNM	0.1	ND	1.0 (3.5)	0.7 (2.4)	< 0.1
DCNM	0.1	ND	ND	ND	ND
BCNM	0.1	ND	< 0.1	ND	< 0.1
DBNM	0.1	ND	< 0.1	< 0.1	< 0.1
TCNM	0.1	ND	< 0.1	< 0.1	0.2 (1.2)
HALs					
TCAL	0.1	ND	1.5 (10.3)	0.3 (2.4)	1.3 (8.8)
BDCAL	0.1	ND	0.4 (2.0)	0.4 (1.9)	0.8 (4.2)
DBCAL	0.1	ND	< 0.1	0.1 (0.5)	0.3 (1.3)
TBAL	0.1	ND	< 0.1	< 0.1	< 0.1
CAL	0.1	ND	ND	ND	ND
DCAL	0.1	ND	< 0.1	< 0.1	< 0.1
BAL	0.1	ND	ND	ND	ND
BCAL	0.1	ND	< 0.1	< 0.1	< 0.1
IAL	0.1	ND	ND	ND	ND
DBAL	0.1	ND	ND	ND	ND
HANs					
DCAN	0.1	ND	0.3 (2.7)	< 0.1	0.5 (4.5)
BCAN	0.1	ND	0.4 (2.6)	0.4 (2.7)	0.6 (3.9)
TBAN	0.1	ND	0.3 (1.1)	0.4 (1.3)	0.2 (0.7)
TCAN	0.1	ND	< 0.1	ND	< 0.1
CAN	0.25	ND	ND	ND	ND
BAN	0.1	ND	< 0.1	ND	ND
DBAN	0.1	ND	0.3 (1.5)	0.7 (3.3)	0.3 (1.5)
IAN	0.1	ND	ND	ND	ND
BDCAN	0.1	ND	NM	ND	0.1 (0.5)
DBCAN	0.1	ND	NM	< 0.1	ND

HKs					
1,1-DCP	0.1	ND	ND	ND	ND
CP	0.1	ND	ND	ND	ND
1,1,1-TCP	0.1	ND	0.1 (0.6)	< 0.1	0.4 (2.5)
1,1-DBP	0.1	ND	ND	ND	ND
1-B-1,1-DCP	0.1	ND	ND	ND	0.1 (0.8)
1,3-DCP	0.1	ND	< 0.1	ND	ND
1,1,3-TCP	0.1	ND	ND	ND	ND
1,1,3,3-TeCP	0.1	ND	ND	< 0.1	ND
1,1,3,3-TeBP	0.1	ND	ND	ND	ND
I-THMs					
DCIM	0.1	ND	ND	ND	ND
BCIM	0.1	ND	< 0.1	< 0.1	< 0.1
DBIM	0.1	ND	< 0.1	< 0.1	< 0.1
CDIM	0.1	ND	ND	ND	ND
BDIM	0.1	ND	ND	ND	ND
TIM	0.1	ND	ND	ND	ND
HAMs					
CAM	1.0	ND	ND	ND	ND
BAM	1.0	ND	ND	ND	ND
IAM	1.0	ND	ND	ND	ND
BCAM	0.2	ND	0.6 (3.6)	0.6 (3.3)	0.6 (3.5)
TCAM	0.1	ND	ND	ND	< 0.1
DCAM	0.3	ND	0.6 (4.4)	0.3 (2.4)	0.7 (5.5)
DBAM	0.2	ND	0.4 (1.7)	1.1 (4.9)	0.3 (1.4)
CIAM	0.3	ND	ND	ND	ND
BIAM	0.5	ND	ND	ND	ND
DBCAM	0.1	ND	ND	ND	ND
TBAM	0.1	ND	< 0.1	0.1 (0.4)	ND
DIAM	0.1	ND	ND	ND	ND
BDCAM	0.1	ND	< 0.1	ND	< 0.1
IAAs					
IAA	.010	ND	< 0.010	< 0.010	ND
CIAA	.025	ND	0.069 (0.3)	< 0.025	ND
BIAA	.025	ND	ND	< 0.025	ND
DIAA	.015	ND	ND	ND	ND

Table S6. Mean THM4 and HAA9 data for Plant 1.

	Concentration - µg/L (nM)					
Compound	**Dist. Avg. May 2018**	**Dist. Max. May 2018**	**Dist. Avg. Nov. 2018**	**Dist. Max. Nov. 2018**	**Dist. Avg. March 2019**	**Dist. Max. March 2019**
THMs						
Trichloromethane	7.0 (58.6)	17 (142)	1.9 (15.9)	0.8 (6.7)	5.5 (46.1)	14 (117)
Bromodichloromethane	6.1 (37.2)	8.6 (52.5)	4.7 (28.7)	1.4 (8.5)	4.4 (26.9)	7.0 (42.7)
Dibromochloromethane	5.4 (25.9)	5.2 (25.0)	8.2 (39.4)	1.9 (9.1)	2.6 (12.5)	3.6 (17.3)
Tribromomethane	1.0 (4.0)	0.7 (2.8)	4.0 (15.8)	0.8 (3.2)	ND	ND
HAAs						
Chloroacetic acid	ND	ND	ND	ND	ND	ND
Bromoacetic acid	ND	ND	ND	ND	ND	ND
Dichloroacetic acid	2.8 (21.7)	5.9 (45.8)	ND	1.7 (13.2)	1.5 (11.6)	4.5 (34.9)
Bromochloroacetic acid	2.1 (12.1)	2.8 (16.1)	1.5 (8.7)	2.1 (12.1)	ND	1.2 (6.9)
Dibromoacetic acid	ND	ND	2.7 (12.4)	3.1 (14.2)	ND	ND
Trichloroacetic acid	1.0 (6.1)	2.5 (15.3)	ND	ND	ND	1.6 (9.8)
Bromodichloroacetic acid	ND	ND	ND	ND	ND	ND
Chlorodibromoacetic acid	ND	ND	ND	ND	ND	ND
Tribromoacetic acid	ND	ND	ND	ND	ND	ND

Table S7. Mean total organic halogen data for Plant 1 - µg/L (µM).

Date	Sample	TOCl (as Cl-)	TOBr (as Br-)	TOI (as I-)
May 2018	Raw	13.4 (0.38)	3.2 (0.04)	0.9 (0.007)
	Effluent	28.2 (0.79)	19.9 (0.25)	0.6 (0.004)
	Dist. Avg.	36.8 (1.04)	20.9 (0.26)	0.7 (0.005)
	Dist. Max	59.6 (1.68)	20.7 (0.26)	0.5 (0.004)
November 2018	Raw	7.0 (0.20)	2.3 (0.03)	1.2 (0.009)
	Effluent	14.6 (0.41)	21.5 (0.27)	0.3 (0.002)
	Dist. Avg.	20.6 (0.58)	26.7 (0.33)	0.4 (0.003)
	Dist. Max.	25.8 (0.73)	31.8 (0.40)	0.3 (0.002)
March 2019	Raw	6.7 (0.19)	1.2 (0.02)	0.4 (0.003)
	Effluent	17.4 (0.49)	10.7 (0.13)	0.3 (0.002)
	Dist. Avg.	35.7 (1.01)	14.4 (0.18)	0.3 (0.002)
	Dist. Max	60.3 (1.70)	16.7 (0.21)	0.3 (0.002)

Table S8. Water quality parameters for Plant 1.

Date	Sample	Sucralose (ug/L)	TOC (mg/L)	UV_{254} (abs/cm)	SUVA (L/mg-m)	Total Ammonia (mg/L)	Br- (µg/L)	I- (µg/L)
May 2018	Raw	0.40	2.5	0.093	3.7	0.08	30	< 10
	Effluent	--	0.8	0.017	2.1	--	--	--
	Dist. Avg.	--	--	--	--	--	--	--
	Dist. Max.	--	--	--	--	--	--	--
November 2018	Raw	0.80	4.2	0.114	2.7	0.19	44	< 10
	Effluent	--	1.0	0.008	0.8	--	--	--
	Dist. Avg.	--	--	--	--	--	--	--
	Dist. Max.	--	--	--	--	--	--	--
March 2019	Raw	ND	2.0	0.054	2.8	0.13	20	< 10
	Effluent	--	1.0	0.016	1.6	--	--	--
	Dist. Avg.	--	--	--	--	--	--	--
	Dist. Max.	--	--	--	--	--	--	--

Table S9. Mean unregulated DBP data for Plant 2.

		Concentration - µg/L (nM)			
Compound	**MRL (µg/L)**	**Raw**	**Dist. Avg. Dec. 2017**	**Dist. Avg. Feb. 2019**	**Dist. Avg. Sept. 2019**
HNMs					
BDCNM	0.1	ND	ND	0.2 (1.0)	< 0.1
DBCNM	0.1	ND	ND	0.2 (0.8)	< 0.25
TBNM	0.5	ND	ND	0.5 (1.7)	< 0.5
DCNM	0.1	ND	ND	ND	ND
BCNM	0.1	ND	0.1	ND	ND
DBNM	0.1	ND	0.1	0.3 (1.4)	< 0.1
TCNM	0.1	ND	< 0.1	< 0.1	ND
HALs					
TCAL	0.1	ND	0.4 (2.7)	0.4 (2.7)	0.5 (3.2)
BDCAL	0.1	ND	0.8 (4.2)	1.3 (6.8)	0.7 (3.9)
DBCAL	0.1	ND	1.3 (5.5)	0.7 (3.0)	0.3 (1.2)
TBAL	0.1	ND	0.3 (1.1)	0.3 (1.1)	< 0.1
CAL	0.1	ND	ND	ND	ND
DCAL	0.1	ND	< 0.1	ND	< 0.1
BAL	0.1	ND	ND	ND	ND
BCAL	0.1	ND	< 0.1	< 0.1	< 0.1
IAL	0.1	ND	ND	ND	ND
DBAL	0.1	ND	ND	ND	< 0.1
HANs					
DCAN	0.1	ND	0.4 (3.6)	< 0.1	0.1 (1.0)
BCAN	0.1	ND	1.0 (6.5)	1.1 (7.1)	0.7 (4.8)
TBAN	0.1	ND	0.2 (0.7)	0.2 (0.7)	0.2 (0.8)
TCAN	0.1	ND	ND	ND	ND
CAN	0.25	ND	0.1 (1.3)	ND	ND
BAN	0.1	ND	ND	ND	ND
DBAN	0.1	ND	1.7 (8.5)	2.1 (10.6)	1.6 (8.2)
IAN	0.1	ND	ND	ND	ND
BDCAN	0.1	ND	NM	< 0.1	ND
DBCAN	0.1	ND	NM	0.2 (0.9)	ND
HKs					
1,1-DCP	0.1	ND	0.1 (0.8)	ND	ND
CP	0.1	ND	0.4 (4.3)	ND	ND
1,1,1-TCP	0.1	ND	0.4 (2.5)	< 0.1	< 0.1
1,1-DBP	0.1	ND	0.3 (1.4)	0.3 (1.4)	< 0.1
1-B-1,1-DCP	0.1	ND	ND	ND	ND

1,3-DCP	0.1	ND	ND	ND	ND
1,1,3-TCP	0.1	ND	ND	ND	ND
1,1,3,3-TeCP	0.1	ND	< 0.1	ND	ND
1,1,3,3-TeBP	0.1	ND	ND	0.3 (0.8)	ND
I-THMs					
DCIM	0.1	ND	ND	0.4 (1.9)	< 0.1
BCIM	0.1	ND	0.1 (0.4)	0.3 (1.2)	< 0.1
DBIM	0.1	ND	ND	0.2 (0.7)	< 0.1
CDIM	0.1	ND	ND	ND	ND
BDIM	0.1	ND	ND	ND	ND
TIM	0.1	ND	ND	ND	ND
HAMs					
CAM	1.0	ND	ND	ND	ND
BAM	1.0	ND	ND	ND	ND
IAM	1.0	ND	ND	ND	ND
BCAM	0.2	ND	0.6 (3.5)	1.0 (5.8)	1.0 (5.5)
TCAM	0.1	ND	ND	ND	< 0.1
DCAM	0.3	ND	ND	0.4 (3.1)	0.5 (4.0)
DBAM	0.2	ND	2.7 (12.5)	2.3 (10.6)	2.1 (9.5)
CIAM	0.3	ND	ND	ND	ND
BIAM	0.5	ND	ND	ND	ND
DBCAM	0.1	ND	ND	0.3 (1.2)	0.3 (1.0)
TBAM	0.1	ND	ND	0.5 (1.7)	0.3 (1.1)
DIAM	0.1	ND	ND	ND	ND
BDCAM	0.1	ND	0.4 (1.9)	0.2 (1.0)	< 0.1
IAAs					
IAA	.010	ND	ND	ND	0.040 (0.2)
CIAA	.025	ND	ND	ND	ND
BIAA	.025	ND	ND	ND	< 0.025
DIAA	.015	ND	ND	ND	ND

Table S10. Mean THM4 and HAA9 data for Plant 2.

Compound	Concentration - µg/L (nM)					
	Dist. Avg. Dec. 2017	**Dist. Max. Dec. 2017**	**Dist. Avg. Feb. 2019**	**Dist. Max. Feb. 2019**	**Dist. Avg. Sep. 2019**	**Dist. Max. Sep. 2019**
THMs						
Trichloromethane	13 (109)	12 (101)	2.8 (23.5)	2.0 (16.8)	11 (92.1)	9.5 (79.6)
Bromodichloromethane	19 (116)	19 (116)	8.2 (50.1)	6.0 (36.6)	17 (104)	16 (97.7)
Dibromochloromethane	16 (76.8)	17 (81.6)	16 (76.8)	12 (57.6)	23 (110)	22 (106)
Tribromomethane	5.8 (22.9)	5.4 (21.4)	12 (47.5)	9.1 (36.0)	13 (51.4)	12 (47.5)
HAAs						
Chloroacetic acid	ND	ND	ND	ND	ND	ND
Bromoacetic acid	ND	ND	ND	ND	ND	1.1 (7.9)
Dichloroacetic acid	2.0 (15.5)	2.7 (20.9)	ND	ND	2.1 (16.3)	2.7 (20.9)
Bromochloroacetic acid	2.4 (13.8)	--	1.8 (10.4)	1.2 (6.9)	4.1 (23.6)	4.6 (26.5)
Dibromoacetic acid	2.4 (11.0)	4.3 (19.7)	3.5 (16.1)	2.2 (10.1)	6.2 (28.5)	6.7 (30.8)
Trichloroacetic acid	ND	1.0 (6.1)	ND	ND	ND	ND
Bromodichloroacetic acid	ND	--	ND	ND	1.1 (5.3)	1.2 (5.8)
Chlorodibromoacetic acid	1.1 (4.4)	--	1.1 (4.4)	ND	2.0 (7.9)	2.2 (8.7)
Tribromoacetic acid	ND	--	ND	ND	ND	ND

Table S11. Mean total organic halogen data for Plant 2 - µg/L (µM).

Date	Sample	TOCl (as Cl-)	TOBr (as Br-)	TOI (as I-)
December 2017	Raw	15.7 (0.44)	8.7 (0.11)	2.2 (0.02)
	Effluent	83.0 (2.34)	24.9 (0.31)	0.8 (0.006)
	Dist. Avg.	50.2 (1.41)	62.7 (0.78)	0.8 (0.006)
	Dist. Max	54.9 (1.55)	66.5 (0.83)	0.6 (0.005)
February 2019	Raw	13.3 (0.37)	8.2 (0.10)	3.9 (0.03)
	Effluent	20.0 (0.56)	20.0 (0.25)	0.7 (0.006)
	Dist. Avg.	31.6 (0.89)	66.2 (0.83)	0.9 (0.007)
	Dist. Max.	27.0 (0.76)	51.3 (0.64)	0.8 (0.006)
September 2019	Raw	38.8 (1.09)	7.9 (0.10)	4.1 (0.03)
	Effluent	50.3 (1.42)	19.8 (0.25)	1.0 (0.008)
	Dist. Avg.	49.2 (1.38)	65.0 (0.81)	1.6 (0.01)
	Dist. Max	81.1 (2.28)	64.9 (0.81)	1.5 (0.01)

Table S12. Water quality parameters for Plant 2.

Date	Sample	Sucralose (ug/L)	TOC (mg/L)	UV_{254} (abs/cm)	SUVA (L/mg-m)	Total Ammonia (mg/L)	Br- (µg/L)	I- (µg/L)
December 2017	Raw	0.79	4.7	0.074	1.6	ND	160	28
	Effluent	ND	1.3	0.010	0.8	--	--	--
	Dist. Avg.	ND	--	--	--	--	--	--
	Dist. Max.	ND	--	--	--	--	--	--
February 2019	Raw	1.06	3.2	0.064	2.0	ND	334	< 10
	Effluent	--	1.0	0.008	0.8	--	--	--
	Dist. Avg.	--	--	--	--	--	--	--
	Dist. Max.	--	--	--	--	--	--	--
September 2019	Raw	0.60	3.7	0.014	0.4	ND	145	< 10
	Effluent	--	0.9	< 0.004	< 0.5	--	--	--
	Dist. Avg.	--	--	--	--	--	--	--
	Dist. Max.	--	--	--	--	--	--	--

Table S13. Mean unregulated DBP data for Plant 3.

Compound	MRL µg/L	Concentration - µg/L (nM)		
		Raw	Dist. Avg. June 2018	Dist. Avg. Jan. 2019
HNMs				
BDCNM	0.1	ND	1.0 (5.0)	0.3 (1.4)
DBCNM	0.1	ND	2.6 (10.2)	0.6 (2.4)
TBNM	0.5	ND	2.6 (8.8)	0.7 (2.4)
DCNM	0.1	ND	ND	ND
BCNM	0.1	ND	< 0.1	< 0.1
DBNM	0.1	ND	< 0.1	0.1 (0.5)
TCNM	0.1	ND	< 0.1	0.1 (0.6)
HALs				
TCAL	0.1	ND	< 0.1	0.1 (0.7)
BDCAL	0.1	ND	0.4 (2.0)	ND
DBCAL	0.1	ND	0.7 (3.1)	0.6 (2.5)
TBAL	0.1	ND	0.4 (1.5)	0.4 (1.4)
CAL	0.1	ND	ND	ND
DCAL	0.1	ND	< 0.1	< 0.1
BAL	0.1	ND	ND	ND
BCAL	0.1	ND	0.1 (0.6)	0.2 (1.3)
IAL	0.1	ND	ND	ND
DBAL	0.1	ND	0.2 (1.0)	< 0.1
HANs				
DCAN	0.1	ND	0.2 (2.1)	0.3 (2.7)
BCAN	0.1	ND	0.7 (4.4)	0.5 (3.2)
TBAN	0.1	ND	0.4 (1.6)	0.2 (0.7)
TCAN	0.1	ND	ND	ND
CAN	0.25	ND	1.4 (19.0)	ND
BAN	0.5	ND	ND	ND
DBAN	0.1	ND	0.8 (3.9)	1.1 (5.5)
IAN	0.1	ND	ND	ND
BDCAN	0.1	ND	NM	ND
DBCAN	0.1	ND	NM	ND
HKs				
1,1-DCP	0.1	ND	ND	ND
CP	0.1	ND	1.4 (15.6)	ND
1,1,1-TCP	0.1	ND	< 0.1	0.1 (0.6)
1,1-DBP	0.1	ND	0.3 (1.5)	0.5 (2.3)
1-B-1,1-DCP	0.1	ND	0.1 (0.6)	< 0.1
1,3-DCP	0.1	ND	ND	ND

1,1,3-TCP	0.1	ND	ND	ND
1,1,3,3-TeCP	0.1	ND	0.1 (0.6)	ND
1,1,3,3-TeBP	0.1	ND	0.5 (1.3)	0.2 (0.5)
I-THMs				
DCIM	0.1	ND	< 0.1	ND
BCIM	0.1	ND	0.1 (0.4)	ND
DBIM	0.1	ND	0.2 (0.6)	< 0.1
CDIM	0.1	ND	ND	ND
BDIM	0.1	ND	< 0.1	ND
TIM	0.1	ND	ND	ND
HAMs				
CAM	1.0	ND	ND	ND
BAM	1.0	ND	ND	ND
IAM	1.0	ND	ND	ND
BCAM	0.2	ND	0.7 (3.8)	0.6 (3.5)
TCAM	0.1	ND	< 0.1	ND
DCAM	0.3	ND	ND	ND
DBAM	0.2	ND	1.1 (5.3)	1.0 (4.6)
CIAM	0.3	ND	ND	ND
BIAM	0.5	ND	ND	ND
DBCAM	0.1	ND	0.1 (0.4)	0.3 (1.2)
TBAM	0.1	ND	< 0.1	ND
DIAM	0.1	ND	ND	ND
BDCAM	0.1	ND	< 0.1	ND
IAAs				
IAA	.010	ND	< 0.010	< 0.010
CIAA	.025	ND	ND	ND
BIAA	.025	ND	ND	ND
DIAA	.015	ND	ND	ND

Table S14. Mean THM4 and HAA9 data for Plant 3.

Compound	Concentration - µg/L (nM)			
	Dist. Avg. June 2018	Dist. Max. June 2018	Dist. Avg. Jan. 2019	Dist. Max. Jan. 2019
THMs				
Trichloromethane	ND	0.6 (5.0)	0.8 (6.7)	0.7 (5.9)
Bromodichloromethane	1.7 (10.4)	2.1 (12.8)	2.2 (13.4)	2.2 (13.4)
Dibromochloromethane	4.1 (19.7)	4.6 (22.1)	5.2 (25.0)	5.5 (26.4)
Tribromomethane	4.8 (19.0)	5.0 (19.8)	6.0 (23.7)	6.2 (24.5)
HAAs				
Chloroacetic acid	ND	ND	ND	ND
Bromoacetic acid	ND	ND	ND	ND
Dichloroacetic acid	ND	ND	ND	ND
Bromochloroacetic acid	1.5 (8.7)	1.7 (9.8)	ND	ND
Dibromoacetic acid	3.0 (13.8)	3.1 (14.2)	1.5 (6.9)	1.1 (5.0)
Trichloroacetic acid	ND	ND	ND	ND
Bromodichloroacetic acid	ND	ND	ND	ND
Chlorodibromoacetic acid	ND	ND	ND	ND
Tribromoacetic acid	ND	ND	ND	ND

Table S15. Mean total organic halogen data for Plant 3 - µg/L (µM).

Date	Sample	TOCl (as Cl-)	TOBr (as Br-)	TOI (as I-)
June 2018	Raw	25.2 (0.71)	10.2 (0.13)	5.2 (0.04)
	Effluent	41.0 (1.15)	45.0 (0.56)	3.5 (0.03)
	Dist. Avg.	28.5 (0.80)	42.0 (0.53)	3.9 (0.03)
	Dist. Max	63.1 (1.78)	42.1 (0.53)	2.8 (0.02)
January 2019	Raw	21.3 (0.60)	10.4 (0.13)	2.3 (0.02)
	Effluent	36.3 (1.02)	51.0 (0.64)	1.0 (0.008)
	Dist. Avg.	34.3 (0.97)	48.7 (0.61)	1.3 (0.01)
	Dist. Max.	37.1 (1.05)	47.9 (0.60)	1.4 (0.01)

Table S16. Water quality parameters for Plant 3.

Date	Sample	Sucralose (µg/L)	TOC (mg/L)	UV_{254} (abs/cm)	SUVA (L/mg-m)	Total Ammonia (mg/L)	Br- (µg/L)	I- (µg/L)
June 2018	Raw	0.6	3.0	0.076	2.6	ND	159	27
	Effluent	--	2.2	0.040	1.8	--	--	--
	Dist. Avg.	--	--	--		--	--	--
	Dist. Max.	--	--	--		--	--	--
January 2019	Raw	0.8	2.7	0.062	2.3	ND	184	< 10
	Effluent	--	2.0	0.038	1.9	--	--	--
	Dist. Avg.	--	--	--		--	--	--
	Dist. Max.	--	--	--		--	--	--

Table S17. Mean unregulated DPB data for Plant 4.

		Concentration - µg/L (nM)			
Compound	**MRL (µg/L)**	**Raw**	**Dist. Avg. Feb. 2018**	**Dist. Avg. Dec. 2018**	**Dist. Avg. July 2019**
HNMs					
BDCNM	0.1	ND	0.6 (2.9)	0.8 (3.8)	0.3 (1.3)
DBCNM	0.1	ND	1.0 (3.9)	1.5 (5.9)	0.3 (1.1)
TBNM	0.5	ND	1.2 (4.0)	1.4 (4.7)	0.4 (1.2)
DCNM	0.1	ND	ND	ND	ND
BCNM	0.1	ND	ND	ND	ND
DBNM	0.1	ND	< 0.1	< 0.1	< 0.1
TCNM	0.1	ND	ND	0.2 (1.2)	0.1 (0.6)
HALs					
TCAL	0.1	ND	< 0.1	0.1 (0.7)	< 0.1
BDCAL	0.1	ND	ND	< 0.1	< 0.1
DBCAL	0.1	ND	< 0.1	< 0.1	< 0.1
TBAL	0.1	ND	< 0.1	< 0.1	< 0.1
CAL	0.1	ND	ND	ND	ND
DCAL	0.1	ND	< 0.1	0.1 (0.9)	< 0.1
BAL	0.1	ND	ND	ND	ND
BCAL	0.1	ND	< 0.1	ND	ND
IAL	0.1	ND	ND	ND	ND
DBAL	0.1	ND	ND	ND	ND
HANs					
DCAN	0.1	ND	0.2 (1.8)	0.4 (3.6)	0.1 (0.9)
BCAN	0.1	ND	0.3 (1.9)	0.5 (3.2)	0.4 (2.4)
TBAN	0.1	ND	ND	ND	ND
TCAN	0.1	ND	ND	ND	< 0.1
CAN	0.25	ND	0.3 (4.0)	ND	1.2 (16.0)
BAN	0.1	ND	ND	ND	ND
DBAN	0.1	ND	0.3 (1.5)	0.3 (1.5)	0.1 (0.7)
IAN	0.1	ND	ND	ND	< 0.1
BDCAN	0.1	ND	NM	NM	ND
DBCAN	0.1	ND	NM	NM	ND
HKs					
1,1-DCP	0.1	ND	ND	ND	ND
CP	0.1	ND	1.5 (16.2)	ND	0.3 (3.1)
1,1,1-TCP	0.1	ND	< 0.1	ND	ND
1,1-DBP	0.1	ND	0.3 (1.4)	0.2 (0.9)	0.4 (1.9)
1-B-1,1-DCP	0.1	ND	ND	ND	< 0.1

1,3-DCP	0.1	ND	ND	0.7 (5.5)	ND
1,1,3-TCP	0.1	ND	ND	ND	ND
1,1,3,3-TeCP	0.1	ND	0.8 (4.1)	ND	0.3 (1.3)
1,1,3,3-TeBP	0.1	ND	1.0 (2.7)	0.4 (1.1)	0.5 (1.2)
I-THMs					
DCIM	0.1	ND	1.1 (5.2)	1.0 (4.7)	1.1 (5.0)
BCIM	0.1	ND	0.6 (2.4)	0.6 (2.4)	0.4 (1.5)
DBIM	0.1	ND	0.2 (0.7)	0.1 (0.3)	0.1 (0.4)
CDIM	0.1	ND	0.3 (1.0)	0.7 (2.3)	0.2 (0.7)
BDIM	0.1	ND	< 0.1	0.1 (0.3)	< 0.1
TIM	0.1	ND	< 0.3	0.2 (0.5)	< 0.1
HAMs					
CAM	1.0	ND	ND	ND	ND
BAM	1.0	ND	ND	ND	ND
IAM	1.0	ND	ND	ND	ND
BCAM	0.2	ND	1.7 (9.9)	1.7 (9.9)	1.3 (7.7)
TCAM	0.1	ND	ND	ND	ND
DCAM	0.3	ND	2.9 (22.3)	4.1 (32.0)	2.3 (17.9)
DBAM	0.2	ND	1.1 (5.1)	0.9 (4.2)	0.8 (3.6)
CIAM	0.2	ND	ND	ND	0.2 (0.7)
BIAM	0.5	ND	ND	ND	ND
DBCAM	0.1	ND	ND	ND	ND
TBAM	0.1	ND	ND	ND	ND
DIAM	0.1	ND	ND	ND	ND
BDCAM	0.1	ND	< 0.25	< 0.1	< 0.1
IAAs					
IAA	.010	ND	0.099 (0.5)	0.128 (0.7)	0.051 (0.3)
CIAA	.025	ND	0.430 (2.0)	0.440 (2.0)	0.152 (0.7)
BIAA	.025	ND	0.072 (0.3)	0.052 (0.2)	0.059 (0.2)
DIAA	.015	ND	0.025 (0.08)	0.030 (0.1)	ND

Table S18. Mean THM4 and HAA9 data for Plant 4.

Compound	Concentration - µg/L (nM)					
	Dist. Avg. Feb. 2018	Dist. Max. Feb. 2018	Dist. Avg. Dec. 2018	Dist. Max. Dec. 2018	Dist. Avg. July 2019	Dist. Max. July 2019
THMs						
Trichloromethane	12 (99.9)	11 (92.1)	15 (126)	17 (142)	19 (159)	18 (151)
Bromodichloromethane	12 (73.0)	10 (61.0)	10 (61.0)	7.2 (43.9)	14 (85.5)	13 (79.4)
Dibromochloromethane	9.1 (43.3)	7.6 (36.5)	4.4 (21.1)	2.8 (13.4)	8.7 (41.8)	8.2 (39.4)
Tribromomethane	1.5 (6.1)	1.4 (5.5)	ND	ND	1.0 (4.0)	1.0 (4.0)
HAAs						
Chloroacetic acid	0.9 (9.0)	0.9 (9.5)	ND	ND	ND	ND
Bromoacetic acid	0.5 (3.4)	0.4 (2.9)	ND	ND	ND	ND
Dichloroacetic acid	11 (84.6)	11 (85.3)	10 (77.6)	14 (109)	11 (85.3)	14 (109)
Bromochloroacetic acid	5.4 (30.9)	5.3 (30.6)	3.6 (20.8)	3.7 (21.3)	4.5 (26.1)	5.9 (34.0)
Dibromoacetic acid	2.9 (13.2)	2.9 (13.3)	ND	ND	1.8 (8.2)	2.3 (10.6)
Trichloroacetic acid	2.3 (14.2)	1.9 (11.6)	3.3 (20.2)	2.5 (15.3)	3.7 (22.5)	3.5 (21.4)
Bromodichloroacetic acid	2.5 (12.2)	1.9 (9.1)	2.0 (9.6)	1.2 (5.8)	2.7 (12.8)	2.5 (12.0)
Chlorodibromoacetic acid	1.9 (7.5)	1.3 (5.2)	ND	ND	1.2 (4.8)	1.1 (4.4)
Tribromoacetic acid	0.13 (0.4)	ND	ND	ND	ND	ND

Table S19. Mean total organic halogen data for Plant 4 - µg/L (µM).

Date	Sample	TOCl (as Cl-)	TOBr (as Br-)	TOI (as I-)
February 2018	Raw	18.5 (0.52)	7.2 (0.09)	4.0 (0.03)
	Effluent	73.6 (2.07)	43.4 (0.54)	3.0 (0.02)
	Dist. Avg.	72.4 (2.04)	44.5 (0.56)	4.7 (0.04)
	Dist. Max	71.2 (2.01)	42.1 (0.53)	3.3 (0.03)
December 2018	Raw	6.7 (0.19)	8.3 (0.10)	4.9 (0.04)
	Effluent	73.4 (2.07)	46.9 (0.59)	3.3 (0.03)
	Dist. Avg.	65.5 (1.85)	43.3 (0.54)	3.5 (0.03)
	Dist. Max.	67.9 (1.91)	23.1 (0.29)	3.5 (0.03)
July 2019	Raw	11.1 (0.31)	10.3 (0.13)	4.2 (0.03)
	Effluent	91.8 (2.59)	39.4 (0.49)	2.3 (0.02)
	Dist. Avg.	63.4 (1.79)	35.0 (0.44)	2.0 (0.02)
	Dist. Max	69.5 (1.96)	53.7 (0.67)	2.3 (0.02)

Table S20. Water quality parameters for Plant 4.

Date	Sample	Sucralose (µg/L)	TOC (mg/L)	UV_{254} (abs/cm)	SUVA (L/mg-m)	Total Ammonia (mg/L)	Br- (µg/L)	I- (µg/L)
February 2018	Raw	ND	4.0	0.067	1.7	ND	146	32
	Effluent	--	2.9	0.051	1.7	--	--	--
	Dist. Avg.	--	--	--	--	--	--	--
	Dist. Max.	--	--	--	--	--	--	--
December 2018	Raw	ND	4.3	0.107	2.5	0.1	120	22
	Effluent	--	3.4	0.067	2.0	--	--	--
	Dist. Avg.	--	--	--	--	--	--	--
	Dist. Max.	--	--	--	--	--	--	--
July 2019	Raw	< 0.2	4.2	0.082	2.0	0.03	126	< 10
	Effluent	--	3.0	0.054	1.8	--	--	--
	Dist. Avg.	--	--	--	--	--	--	--
	Dist. Max.	--	--	--	--	--	--	--

Table S21. Mean unregulated DBP data for Plant 5.

		Concentration - µg/L (nM)		
Compound	**MRL (µg/L)**	**Raw**	**Dist. Avg. Oct. 2017**	**Dist. Avg. July 2018**
HNMs				
BDCNM	0.1	ND	0.4 (1.9)	0.3 (1.4)
DBCNM	0.1	ND	0.7 (2.8)	0.5 (2.0)
TBNM	0.5	ND	ND	< 0.5
DCNM	0.1	ND	ND	ND
BCNM	0.1	ND	0.2 (1.1)	< 0.1
DBNM	0.1	ND	< 0.1	< 0.1
TCNM	0.1	ND	0.3 (1.8)	0.1 (0.6)
HALs				
TCAL	0.1	ND	1.9 (12.9)	3.1 (21.0)
BDCAL	0.1	ND	0.8 (4.2)	2.2 (11.5)
DBCAL	0.1	ND	0.2 (0.8)	0.4 (1.7)
TBAL	0.1	ND	< 0.1	< 0.1
CAL	0.1	ND	ND	ND
DCAL	0.1	ND	< 0.1	0.1 (0.9)
BAL	0.1	ND	ND	ND
BCAL	0.1	ND	< 0.1	0.2 (1.3)
IAL	0.1	ND	ND	ND
DBAL	0.1	ND	ND	ND
HANs				
DCAN	0.1	ND	0.6 (5.5)	1.1 (10.0)
BCAN	0.1	ND	1.0 (3.2)	1.1 (7.1)
TBAN	0.1	ND	0.5 (1.8)	0.4 (1.4)
TCAN	0.1	ND	ND	< 0.1
CAN	0.25	ND	0.3 (4.0)	0.4 (5.3)
BAN	0.5	ND	ND	ND
DBAN	0.1	ND	0.3 (1.5)	0.5 (2.5)
IAN	0.1	ND	ND	ND
BDCAN	0.1	NM	NM	NM
DBCAN	0.1	NM	NM	NM
HKs				
1,1-DCP	0.1	ND	ND	0.4 (3.2)
CP	0.1	ND	5.5 (59.4)	1.0 (10.8)
1,1,1-TCP	0.1	ND	0.3 (1.9)	0.2 (1.2)
1,1-DBP	0.1	ND	ND	< 0.1
1-B-1,1-DCP	0.1	ND	ND	0.1 (0.5)
1,3-DCP	0.1	ND	0.8 (6.3)	< 0.1

1,1,3-TCP	0.1	ND	0.1 (0.6)	< 0.1
1,1,3,3-TeCP	0.1	ND	0.1 (0.5)	< 0.1
1,1,3,3-TeBP	0.1	ND	0.1 (0.3)	0.1 (0.3)
I-THMs				
DCIM	0.1	ND	ND	0.1 (0.5)
BCIM	0.1	ND	0.1 (0.4)	0.1 (0.4)
DBIM	0.1	ND	ND	< 0.1
CDIM	0.1	ND	ND	ND
BDIM	0.1	ND	ND	ND
TIM	0.1	ND	ND	ND
HAMs				
CAM	1.0	ND	ND	ND
BAM	1.0	ND	ND	ND
IAM	1.0	ND	ND	ND
BCAM	0.1	ND	1.7 (9.9)	2.6 (14.1)
TCAM	0.1	ND	0.3 (1.8)	0.2 (1.2)
DCAM	0.3	ND	2.7 (21.1)	1.8 (14.1)
DBAM	0.2	ND	0.7 (3.2)	1.7 (7.8)
CIAM	0.3	ND	ND	ND
BIAM	0.5	ND	ND	ND
DBCAM	0.1	ND	0.4 (1.6)	0.3 (1.2)
TBAM	0.1	ND	ND	0.1 (0.3)
DIAM	0.2	ND	ND	ND
BDCAM	0.1	ND	0.4 (1.9)	0.3 (1.5)
IAAs				
IAA	.010	ND	0.023 (0.1)	< 0.010
CIAA	.015	ND	0.032 (0.1)	0.120 (0.5)
BIAA	.020	ND	ND	< 0.020
DIAA	.015	ND	ND	ND

Table S22. Mean THM4 and HAA9 data for Plant 5.

Compound	Concentration - µg/L (nM)			
	Dist. Avg. Oct. 2017	Dist. Max. Oct. 2017	Dist. Avg. July 2018	Dist. Max. July 2018
THMs				
Trichloromethane	12 (101)	11 (92.1)	12 (101)	11 (92.1)
Bromodichloromethane	13 (79.4)	12 (73.2)	12 (73.2)	16 (97.7)
Dibromochloromethane	7.3 (35.0)	7.3 (35.0)	9.0 (43.2)	15 (72.0)
Tribromomethane	1.4 (5.5)	1.6 (6.3)	1.9 (7.5)	4.3 (17.0)
HAAs				
Chloroacetic acid	ND	ND	ND	ND
Bromoacetic acid	ND	ND	ND	ND
Dichloroacetic acid	7.6 (58.9)	9.1 (70.6)	8.8 (68.2)	10 (77.6)
Bromochloroacetic acid	4.8 (27.7)	5.7 (32.9)	5.0 (28.8)	8.5 (49.0)
Dibromoacetic acid	1.8 (8.3)	2.3 (10.6)	2.5 (11.5)	4.9 (22.5)
Trichloroacetic acid	4.8 (29.4)	5.5 (33.7)	4.6 (28.2)	6.5 (39.8)
Bromodichloroacetic acid	5.9 (28.4)	3.3 (15.9)	2.9 (14.0)	5.7 (27.4)
Chlorodibromoacetic acid	1.8 (7.1)	ND	ND	3.0 (11.9)
Tribromoacetic acid	ND	ND	ND	ND

Table S23. Mean total organic halogen data for Plant 5 -µg/L (µM).

Date	Sample	TOCl (as Cl-)	TOBr (as Br-)	TOI (as I-)
October 2017	Raw	26.1 (0.74)	10.0 (0.13)	1.6 (0.01)
	Effluent	93.3 (2.63)	45.2 (0.57)	0.8 (0.006)
	Dist. Avg.	63.5 (1.79)	36.1 (0.45)	0.6 (0.005)
	Dist. Max	57.7 (1.63)	30.9 (0.39)	0.5 (0.004)
July 2018	Raw	20.9 (0.59)	9.1 (0.11)	3.9 (0.03)
	Effluent	84.3 (2.37)	77.6 (0.97)	2.4 (0.02)
	Dist. Avg.	90.8 (2.56)	65.8 (0.82)	2.5 (0.02)
	Dist. Max.	70.8 (1.99)	37.7 (0.47)	1.9 (0.01)

Table S24. Water quality parameters for Plant 5.

Date	Sample	Sucralose (µg/L)	TOC (mg/L)	UV_{254} (abs/cm)	SUVA (L/mg-m)	Total Ammonia (mg/L)	Br- (µg/L)	I- (µg/L)
October 2017	Raw	2.8	3.6	0.056	1.5	0.17	92	11
	Effluent	1.2	1.8	0.035	1.9	--	--	--
	Dist. Avg.	1.1	--	--	--	--	--	--
	Dist. Max.	0.9	--	--	--	--	--	--
July 2018	Raw	8.0	5.1	0.072	1.4	0.05	174	< 10
	Effluent	3.1	3.2	0.044	1.4	--	--	--
	Dist. Avg.	2.6	--	--	--	--	--	--
	Dist. Max.	1.2	--	--	--	--	--	--

Table S25. Mean unregulated DBP data for Plant 6.

Compound	MRL µg/L	Concentration - µg/L (nM)				
		Raw A	Raw B	Dist. Avg. Jan. 2018	Dist. Avg. Aug. 2018	Dist. Avg. Sep. 2019
HNMs						
BDCNM	0.1	ND	ND	0.3 (1.4)	0.7 (3.4)	0.2 (1.0)
DBCNM	0.1	ND	ND	0.6 (2.4)	1.4 (5.4)	0.4 (1.6)
TBNM	0.25	ND	ND	ND	1.5 (4.9)	0.5 (1.7)
DCNM	0.1	ND	ND	ND	ND	ND
BCNM	0.1	ND	ND	0.1 (0.6)	ND	ND
DBNM	0.1	ND	ND	0.2 (0.9)	< 0.1	< 0.1
TCNM	0.1	ND	ND	0.1 (0.6)	0.2 (1.0)	< 0.1
HALs						
TCAL	0.1	ND	ND	0.1 (0.7)	0.2 (1.4)	0.3 (2.0)
BDCAL	0.1	ND	ND	ND	0.7 (3.8)	0.6 (3.1)
DBCAL	0.1	ND	ND	1.1 (4.7)	0.4 (1.6)	0.5 (2.1)
TBAL	0.1	ND	ND	0.2 (0.7)	0.1 (0.3)	0.1 (0.4)
CAL	0.1	ND	ND	ND	ND	ND
DCAL	0.1	ND	ND	ND	0.1 (0.9)	< 0.1
BAL	0.1	ND	ND	ND	ND	ND
BCAL	0.1	ND	ND	0.1 (0.6)	0.1 (0.6)	0.3 (1.9)
IAL	0.1	ND	ND	ND	ND	ND
DBAL	0.1	ND	ND	ND	ND	< 0.1
HANs						
DCAN	0.1	ND	ND	0.7 (6.4)	0.6 (5.5)	0.4 (3.6)
BCAN	0.1	ND	ND	1.3 (8.4)	1.4 (8.8)	1.2 (7.8)
TBAN	0.1	ND	ND	0.5 (1.8)	0.6 (2.2)	0.3 (1.1)
TCAN	0.1	ND	ND	ND	< 0.1	ND
CAN	0.1	ND	ND	ND	0.8 (10.8)	ND
BAN	0.1	ND	ND	ND	ND	ND
DBAN	0.25	ND	ND	1.2 (6.0)	1.1 (5.8)	1.7 (8.5)
IAN	0.5	ND	ND	ND	ND	ND
BDCAN	0.1	ND	ND	NM	NM	ND
DBCAN	0.1	ND	ND	NM	NM	0.1 (0.4)
HKs						
1,1-DCP	0.1	ND	ND	0.3 (2.4)	0.3 (2.7)	0.2 (1.6)
CP	0.1	ND	ND	5.9 (63.8)	0.9 (10.0)	0.7 (7.6)
1,1,1-TCP	0.1	ND	ND	0.2 (1.2)	< 0.1	< 0.1
1,1-DBP	0.1	ND	ND	0.1 (0.5)	< 0.1	< 0.1
1-B-1,1-DCP	0.1	ND	ND	0.2 (1.0)	0.2 (0.7)	ND
1,3-DCP	0.1	ND	ND	0.1 (0.8)	ND	ND

1,1,3-TCP	0.1	ND	ND	ND	ND	ND
1,1,3,3-TeCP	0.1	ND	ND	0.1 (0.5)	< 0.1	< 0.1
1,1,3,3-TeBP	0.1	ND	ND	ND	< 0.1	0.2 (0.5)
I-THMs						
DCIM	0.1	ND	ND	0.2 (0.9)	0.3 (1.6)	0.1 (0.5)
BCIM	0.1	ND	ND	0.3 (1.2)	0.2 (0.9)	0.1 (0.5)
DBIM	0.1	ND	ND	0.1 (0.3)	0.1 (0.3)	< 0.1
CDIM	0.1	ND	ND	ND	ND	ND
BDIM	0.1	ND	ND	ND	ND	ND
TIM	0.1	ND	ND	ND	ND	ND
HAMs						
CAM	1.0	ND	ND	ND	ND	ND
BAM	1.0	ND	ND	ND	ND	ND
IAM	1.0	ND	ND	ND	ND	ND
BCAM	0.3	ND	ND	0.6 (3.5)	0.9 (4.5)	0.6 (3.5)
TCAM	0.2	ND	ND	0.2 (1.2)	< 0.2	ND
DCAM	0.3	ND	ND	ND	ND	0.6 (4.7)
DBAM	0.2	ND	ND	1.6 (7.4)	0.9 (4.3)	1.0 (4.6)
CIAM	0.3	ND	ND	ND	ND	ND
BIAM	0.5	ND	ND	ND	ND	ND
DBCAM	0.1	ND	ND	ND	ND	0.1
TBAM	0.1	ND	ND	ND	0.1 (0.5)	< 0.1
DIAM	0.1	ND	ND	ND	ND	ND
BDCAM	0.1	ND	ND	ND	0.2 (0.9)	< 0.1
IAAs						
IAA	.025	ND	ND	0.032 (0.2)	< 0.010	0.054 (0.3)
CIAA	.050	ND	ND	0.026 (0.1)	0.111 (0.5)	0.153 (0.7)
BIAA	.020	ND	ND	< 0.020	< 0.020	0.058 (0.2)
DIAA	.025	ND	ND	ND	ND	ND

Table S26. Mean THM4 and HAA9 data for Plant 6.

Compound	Concentration - µg/L (nM)					
	Dist. Avg. Jan. 2018	Dist. Max. Jan. 2018	Dist. Avg. Aug. 2018	Dist. Max. Aug. 2018	Dist. Avg. Sep. 2019	Dist. Max. Sep. 2019
THMs						
Trichloromethane	3.6 (30.5)	3.8 (31.8)	4.8 (40.2)	3.9 (32.7)	2.8 (23.5)	3.8 (31.8)
Bromodichloromethane	7.6 (46.2)	7.6 (46.4)	8.0 (48.8)	7.0 (42.7)	5.5 (33.6)	6.1 (37.2)
Dibromochloromethane	9.0 (43.4)	9.1 (43.7)	8.7 (41.8)	8.0 (38.4)	8.4 (40.3)	8.6 (41.3)
Tribromomethane	3.7 (14.4)	3.9 (15.4)	2.8 (11.1)	2.6 (10.3)	3.8 (15.0)	3.7 (14.6)
HAAs						
Chloroacetic acid	ND	0.4 (4.2)	ND	ND	ND	ND
Bromoacetic acid	0.6 (4.0)	0.5 (3.6)	ND	ND	ND	ND
Dichloroacetic acid	2.3 (18.1)	2.4 (18.6)	1.9 (14.7)	2.0 (15.5)	1.9 (14.7)	4.0 (31.0)
Bromochloroacetic acid	2.8 (15.9)	2.8 (16.1)	2.3 (13.3)	2.1 (12.1)	3.2 (18.5)	4.3 (24.8)
Dibromoacetic acid	2.2 (10.1)	2.2 (10.1)	1.9 (8.7)	1.7 (7.8)	3.2 (14.7)	4.0 (18.4)
Trichloroacetic acid	0.9 (5.8)	0.9 (5.5)	ND	ND	ND	1.2 (7.3)
Bromodichloroacetic acid	2.0 (9.7)	2.1 (10.1)	1.3 (6.3)	ND	2.0 (9.6)	2.3 (11.1)
Chlorodibromoacetic acid	2.0 (7.9)	2.0 (7.9)	1.0 (4.0)	ND	2.8 (11.1)	2.8 (11.1)
Tribromoacetic acid	0.5 (1.7)	0.5 (1.7)	ND	ND	ND	ND

Table S27. Mean total organic halogen data for Plant 6 - µg/L (µM).

Date	Sample	TOCl (as Cl-)	TOBr (as Br-)	TOI (as I-)
January 2018	Plant 6 A Raw	29.8 (0.84)	7.6 (0.10)	4.0 (0.03)
	Plant 6 B Raw	15.7 (0.44)	7.8 (0.10)	1.0 (0.008)
	Combined Effluent	53.8 (1.52)	60.9 (0.76)	0.8 (0.006)
	Dist. Avg.	49.6 (1.40)	56.8 (0.71)	0.9 (0.007)
	Dist. Max.	89.7 (2.53)	53.9 (0.67)	0.9 (0.007)
August 2018	Plant 6 A Raw	28.7 (0.81)	7.4 (0.09)	7.6 (0.06)
	Plant 6 B Raw	11.2 (0.32)	6.8 (0.08)	1.9 (0.01)
	Combined Effluent	46.0 (1.30)	50.7 (0.63)	0.8 (0.006)
	Dist. Avg.	48.4 (1.36)	44.8 (0.56)	0.9 (0.007)
	Dist. Max.	45.1 (1.27)	43.8 (0.55)	0.9 (0.007)
September 2019	Plant 6 A Raw	38.5 (1.08)	7.7 (0.10)	4.1 (0.03)
	Plant 6 B Raw	31.6 (0.89)	6.1 (0.08)	0.9 (0.007)
	Combined Effluent	95.1 (2.70)	50.3 (0.63)	1.1 (0.008)
	Dist. Avg.	122.1 (3.44)	43.6 (0.55)	1.7 (0.01)
	Dist. Max.	75.1 (2.12)	37.8 (0.47)	1.4 (0.01)

Table S28. Water quality parameters for Plant 6.

Date	Sample	Blend	Sucralose (µg/L)	TOC (mg/L)	UV_{254} (abs/cm)	SUVA (L/mg-m)	Total Ammonia (mg/L)	Br- (µg/L)	I- (µg/L)
January 2018	Plant 6 A Raw	36.0 %	12.4	2.6	0.046	1.8	ND	291	22
	Plant 6 A Effluent		2.8	2.1	0.021	1.0	--	--	--
	Plant 6 B Raw	64.0 %	0.2	4.2	0.052	1.2	0.05	59	< 10
	Plant 6 B Effluent		0.2	2.5	0.023	0.9	--	--	--
	Dist. Avg.		1.2	--	--	--	--	--	--
	Dist. Max.		1.0	--	--	--	--	--	--
August 2018	Plant 6 A Raw	31.4%	17.8	2.9	0.058	2.0	0.08	270	< 10
	Plant 6 A Effluent		8.4	2.4	0.031	1.3	--	--	--
	Plant 6 B Raw	68.6 %	0.3	3.6	0.047	1.3	0.09	55	< 10
	Plant 6 B Effluent		0.2	2.1	0.021	1.0	--	--	--
	Dist. Avg.		2.4	--	--	--	--	--	--
	Dist. Max.		2.5	--	--	--	--	--	--
September 2019	Plant 6 A Raw	41.3 %	21	2.8	0.060	2.1	ND	261	< 10
	Plant 6 A Effluent		4.8	1.6	0.021	1.3	--	--	--
	Plant 6 B Raw	58.7 %	0.3	3.0	0.044	1.5	ND	51	< 10
	Plant 6 B Effluent		< 0.2	1.7	0.018	1.1	--	--	--
	Dist. Avg.		1.8	--	--	--	--	--	--
	Dist. Max.		1.9	--	--	--	--	--	--

Table S29. Summary of the CHO cell cytotoxicity statistical analyses of the distribution average water samples.

Water Sample	Lowest Cytotoxic Conc. (CF) [a]	Mean LC_{50} Value (CF ± SE) [b]	r^2 [c]	ANOVA Test Statistic [d]	Mean CTI Value ± SE [e]
Plant 1: 5/7/2018	40.0	149.43 ± 6.71	0.98	$F_{14,98} = 132.1$; $P \leq 0.001$	6.71 ± 0.12
Plant 1: 11/6/2018	50.0	176.75 ± 5.68	0.98	$F_{14,98} = 150.6$; $P \leq 0.001$	5.68 ± 0.13
Plant 1: 3/5/2018	25.0	137.41 ± 7.38	0.98	$F_{14,95} = 206.4$; $P \leq 0.001$	7.38 ± 0.27
Plant 2: 12/14/2017	25.0	98.48 ± 10.21	0.99	$F_{12,92} = 85.7$; $P \leq 0.001$	10.21 ± 0.26
Plant 2: 2/20/2019	100.0	131.68 ± 7.64	0.99	$F_{10,63} = 85.7$; $P \leq 0.001$	7.64 ± 0.18
Plant 2: 9/17/2019	40.0	109.90 ± 9.12	0.97	$F_{11,99} = 145.2$; $P \leq 0.001$	9.12 ± 0.11
Plant 3: 6/12/2018	40.0	116.33 ± 8.81	0.99	$F_{11,98} = 132.7$; $P \leq 0.001$	8.81 ± 0.43
Plant 3: 1/28/2019	50.0	128.48 ± 8.01	0.96	$F_{10,78} = 69.9$; $P \leq 0.001$	8.01± 0.39
Plant 4: 2/26/2018	40.0	63.79 ± 15.72	0.99	$F_{11,99} = 454.1$; $P \leq 0.001$	15.72 ± 0.25
Plant 4: 12/10/2018	50.0	72.35 ± 14.28	0.99	$F_{11,91} = 58.9$; $P \leq 0.001$	14.28 ± 0.75
Plant 4: 7/15/2019	40.0	123.67 ± 8.32	0.99	$F_{10,102} = 96.7$; $P \leq 0.001$	8.32 ± 0.42
Plant 5: 10/10/2017	25.0	148.01 ± 6.79	0.99	$F_{11,97} = 221.6$; $P \leq 0.001$	6.79 ± 0.15
Plant 5: 7/10/2018	20.0	101.29 ± 9.93	0.99	$F_{10,101} = 339.4$; $P \leq 0.001$	9.93 ± 0.24
Plant 6: 1/9/2018	80.0	152.97 ± 6.57	0.99	$F_{12,96} = 190.3$; $P \leq 0.001$	6.57 ± 0.15
Plant 6: 8/6/2018	10.0	105.25 ± 9.53	0.98	$F_{14,97} = 136.8$; $P \leq 0.001$	9.53 ± 0.17
Plant 6: 9/23/2019	40.0	128.41 ± 8.34	0.99	$F_{10,98} = 76.6$; $P \leq 0.001$	8.34 ± 0.64

[a] Lowest cytotoxic concentration was the lowest concentration factor of the sample that induced a statistically significant reduction in cell density as compared to the negative control. [b] The LC_{50} value is the concentration factor of the water sample, determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative controls. The mean and the standard error (SE) of each LC_{50} value were derived from multiple regression analyses using bootstrap statistics. [c] The r^2 is the coefficient of determination for the regression analysis of the concentration-response data upon which the LC_{50} value was calculated. [d] The degrees of freedom for the between-groups and residual associated with the calculated *F*-test result and the resulting probability value.
[e] The Cytotoxicity Index Value is the $(LC_{50}^{-1})(10^3)$. The mean and the standard error (SE) of each CTI value were derived from multiple regression analyses using bootstrap statistics.