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Article

Making Swimming Pools Safer: Does Copper−Silver Ionization with Chlorine Lower the Toxicity and Disinfection Byproduct Formation?

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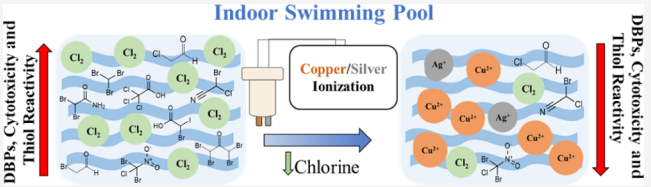
ABSTRACT: Swimming pools are commonly treated with chlorine, which reacts with the natural organic matter and organic matter introduced by swimmers and form disinfection byproducts (DBPs) that are associated with respiratory-related issues, including asthma, in avid swimmers. We investigated a complementary disinfectant to chlorine, copper−silver ionization (CSI), with the aim of lowering the amount of chlorine used in pools and limiting health risks from DBPs. We sampled an indoor and outdoor pool treated with CSI-chlorine during the swimming season in 2017−2018 and measured 71 DBPs, speciated total organic halogen, *in vitro* mammalian cell cytotoxicity, and *N*-acetyl-L-cysteine (NAC) thiol reactivity as a cytotoxicity predictor. Controlled, simulated swimming pools were also investigated. Emerging DBP concentrations decreased by as much as 80% and cytotoxicity decreased as much as 70% in the indoor pool when a lower chlorine residual (1.0 mg/L) and CSI was used. Some DBPs were quantified for the first time in pools, including chloroacetaldehyde (up to 10.6 μg/L), the most cytotoxic haloacetaldehyde studied to date and a major driver of the measured cytotoxicity in this study. Three highly toxic iodinated haloacetic acids (iodoacetic acid, bromoiodoacetic acid, and chloroiodoacetic acid) were also quantified in pools for the first time. We also found that the NAC thiol reactivity was significantly correlated to cytotoxicity, which could be useful for predicting the cytotoxicity of swimming pool waters in future studies.



KEYWORDS: *copper−silver ionization, disinfection byproducts, indoor and outdoor swimming pool, complementary disinfectant, cytotoxicity*

INTRODUCTION

Swimming pools have long provided an effective means of exercise, and swimming is a popular recreational activity throughout the world. In order to inactivate harmful pathogens and minimize other microbial risks, disinfecting swimming pool water is a necessary precaution commonly achieved with the use of chlorine, bromine, ozone, or ultraviolet radiation (UV).[1] In drinking water, DBPs are formed by the reaction of disinfectants with organic matter from the source water.[2] In pools, there are also additional precursors introduced by swimmers, including sweat, urine, sunscreens, and cosmetics.[3−10] DBPs are known to cause adverse health effects,[11−17] and epidemiologic studies have linked DBP exposure to bladder cancer, respiratory issues such as asthma, and adverse pregnancy outcomes.[12,18−24] In addition, there is evidence that some halogenated DPBs are permeable across the skin.[25,26]

In order to limit DBP formation and associated health effects, while also controlling viruses, bacteria, fungi, and algae in pools, alternative disinfection strategies to chlorine are being investigated. One alternative approach is copper−silver ionization (CSI), where copper and silver ions are generated through electrolysis and introduced into the swimming pool water. This is commonly achieved by directly plumbing two copper−silver electrodes into the pool's filling water line and applying a voltage between the electrodes, enabling copper and silver ions to be released into the swimming pool for disinfection. The biocidal and algicidal properties of copper and silver were published, and they have been used in swimming pools, hospitals, and drinking water as residual disinfectants against bacteria such as *Legionella* spp., *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus*.[27−30]

In swimming pools, South Carolina state regulations permit CSI to be used only as a secondary disinfectant in conjunction with chlorine. In many pools, chlorine residuals can be much higher than 1 mg/L, which is excess for appropriate disinfection.[1] Since higher levels of DBPs can form from increased doses of chlorine,[5,31] lowering the amount of chlorine with a secondary CSI treatment could potentially limit DBP formation while being in disinfection compliance. A previous study demonstrated that low levels of free chlorine residual (0.4 mg/L) with CSI controlled total coliform and

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heterotrophic bacteria to the same extent as higher chlorine levels (>1 mg/L).[30] However, DBP formation and toxicity from chlorine plus CSI disinfection have not been previously evaluated. Given that other common pool disinfectants, such as ozone and UV, are known to contribute to DBP formation, the assessment of CSI with chlorine is necessary.[32−37]

Recent comprehensive, broad-screen studies of swimming pools and spas revealed >100 DBPs, including many that were not previously known or not previously identified in swimming pools or drinking water, including new bromoimidazoles, bromoanilines, and bromomethanesulfenic acid esters.[33,38] Gas chromatography−mass spectrometry (GC−MS) has been widely used to quantify DBPs in swimming pools and has focused primarily on trihalomethanes (THMs), haloacetic acids (HAAs), and inorganic chloramines (e.g., trichloramine). As more DBPs were identified in pools, the interest in targeted, quantitative analysis of unregulated DBPs expanded beyond these classes. A recent study from Australia investigated a suite of 64 DBPs from six other emerging DBP classes, including haloacetaldehydes, haloketones, haloacetamides, haloacetonitriles, halonitromethanes, and *N*-nitrosamines.[39] While little is known about the health impacts of these emerging DBPs, which often form at lower concentrations than THMs and HAAs, they are much more cytotoxic and genotoxic, and they control the response of toxic response genes.[40−44] Information on disinfection techniques, as well as occurrence, toxicity, and broader impacts of DBPs in swimming pools can be found in recently published reviews.[3,10] Previous studies have shown swimming pool water to be cytotoxic, genotoxic, and mutagenic in a variety of bioassays.[9,33,38,45−47] Data are not available for pools treated with CSI and chlorine.

The objectives of this research were to (1) conduct the first swimming pool study that integrates quantitative biological cytotoxicity and quantitative chemical analysis of >70 DBPs and speciated total organic halogens and (2) conduct the *N*-acetyl-L-cysteine (NAC) thiol reactivity assay to assess its value as a predictor for mammalian cell cytotoxicity in swimming pool water samples.[48,49] In this assay, the cysteine thiol present in NAC mimics the remediation of soft electrophile toxicity from the toxicants (e.g., DBPs) by the cysteine thiol in the intracellular tripeptide glutathione.[50]

Two chlorinated pools in South Carolina disinfected with sodium hypochlorite (NaOCl) and secondary CSI disinfectant were seasonally sampled. DBP, TOX, cytotoxicity, and NAC thiol reactivity analyses were carried out for the indoor pool during 2018 (May, July, and November), and all other pool and tap water samples were analyzed for TOX, cytotoxicity, and NAC thiol reactivity comparisons. Because it is difficult to make direct comparisons of real swimming pools that have different sources of tap filling water, different bather loads, and different environmental conditions, we also conducted controlled laboratory reactions with body fluid analogue to simulate various chlorinated and CSI swimming pool conditions.

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. Ellman's reagent, 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), and NAC were purchased from Sigma-Aldrich, and 2,5-pyrroledione was purchased from Acros Organics (Geel, Belgium). 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,[51,52] and Diazald, used as the methylating agent for halo-acids,[51] 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.

Sample Collection. The two pools, including a 47,000 L outdoor pool in Murrell's Inlet, SC and a 40,000 L indoor pool in North Myrtle Beach, SC (approximately 40 miles apart), were sampled over the course of the swimming season (March−May, June−July, October−November) in 2017 and 2018. Water samples (52 L) for Chinese hamster ovary (CHO) cell cytotoxicity and thiol reactivity analyses were collected headspace-free in either 2 L Teflon or 10 L and 20 L polytetrafluoroethylene (PTFE)-lined or fluorinated high-density polyethylene (HDPE) carboys. The outdoor pool was first sampled in March 2017 and treated with CSI and free chlorine for each event, excluding the March 2017 sampling when only ionization was used throughout the non-swimming winter months. The indoor pool was first sampled in July 2017, when no CSI was employed (chlorine alone), and subsequent events were sampled when both free chlorine and CSI were used. A Caribbean Clear 50-R Ionization System (Leesville, SC) was used for each pool. The indoor pool was kept at a constant temperature of 29 °C, and the pH values of the outdoor and indoor pools were between 7.2 and 7.5 for all events. All samples were transported on ice and XAD-extracted (details below) within 24 h.

Samples for the quantitative analysis of 71 DBPs and speciated total organic halogen (TOX) were collected headspace-free in 1 L amber glass bottles based on a previously published procedure.[51,53−55] Pool samples were quenched with ammonium chloride to convert free chlorine to chloramines at a target 1.3:1 quencher/chlorine molar ratio for the analysis of 4 THMs, 6 iodinated trihalomethanes (I-THMs), 8 bromo/chloro haloacetic acids (Br/Cl-HAAs), 4 iodinated haloacetic acids (I-HAAs), 10 haloacetonitriles (HANs), 7 halonitromethanes (HNMs), 9 haloketones (HKs), 13 haloacetamides (HAMs), and 4 trihaloacetaldehydes (tri-HALs). Ascorbic acid quencher was used at the same 1.3:1 molar ratio to chlorine residual for TOX analysis and the quantification of six mono- and di-HALs. The incoming tap water in each scenario was treated with chloramines; therefore, a non-quenched and an ascorbic acid-quenched sample were collected. All samples for quantification were acidified between pH 3.5 and 4 with concentrated H_2SO_4 on-site and transported on ice.

Analytical Methods. Nonpurgeable organic carbon (NPOC) was measured along with total nitrogen (TN) (for simulated pool samples) using a Shimadzu total organic carbon analyzer. Samples for copper and silver measurements were collected in plastic 15 mL tubes and measured using a Finnigan ELEMENT XR double focusing magnetic sector field inductively coupled plasma (ICP)-MS instrument (Thermo Electron Corporation). Residual chlorine was measured using a Hach DR/850 Colorimeter.

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Two extraction methods and two derivatization methods (Analytical Methods 1 and 2 in Table S1), modified from previously published works,[51−55] were used to quantify the 71 DBPs in this study. Stock solutions of DBP standards were made by dissolving DBP standards in acetonitrile, methanol (MeOH), or MTBE. Calibration curves were prepared by using individual DBP stocks to make 100 mg/L mixtures of each DBP class in MeOH and then diluting to 10 mg/L in MeOH and spiking into ultrapure (18 MΩ cm) water at various concentrations.

For HANs, HKs, I-THMs, HNMs, tri- HALs, HAMs, and I-HAAs, 100 mL of water (samples quenched with ammonium chloride) was adjusted with H_2SO_4 to pH < 1, followed by multiple liquid−liquid extractions (LLEs) (×3), conducted with 5 mL of MTBE (for each extraction) and 30 g of anhydrous granular sodium sulfate. Organic extracts were dried with sodium sulfate and concentrated to 200 μL under nitrogen. Final extracts were spiked with 1,2-dibromopropane internal standard and analyzed using GC−MS with electron ionization and selected ion monitoring (Agilent 7890 GC, 5977A mass spectrometer, Agilent Technologies, Santa Clara, CA) with a Rtx-200 column (30 m × 0.25 mm × 0.25 μm film thickness; Restek Corporation, Bellefonte, PA). A portion of the extract was removed for I-HAA and Br/Cl-HAA analysis, which required diazomethane derivatization, followed by GC-EI-MS/MS analysis with multiple reaction monitoring (MRM) (TRACE GC Ultra, Quantum GC MS/MS, Thermo Scientific, Waltham, MA). Mono- and di-HALs were analyzed using PFBHA derivatization, followed by LLE and GC-EI-MS analysis (samples quenched with ascorbic acid). Quantifier and qualifier ions, as well as the method reporting limits for each compound, can be found in Table S1. Most compounds had a reporting limit of 0.1 μg/L. Further details outlining GC−MS(/MS) parameters and collection procedures can be found elsewhere.[51,53−55]

TOX Analysis. Analyses for total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI) were performed in duplicate with a sample adsorption and combustion unit (Mitsubishi Chemical Analytech, Chigasaki, Japan; Cosa Xentaur, Yaphank, NY).[53,54,56] Briefly, organic compounds were sorbed onto activated carbon (AC) columns, followed by combustion of the AC, where hydrogen halide gases were bubbled into an aqueous solution, and this solution was analyzed by a Dionex 1600 ion chromatograph (IC) (Dionex, Sunnyvale, CA) to separate and detect the halide ions. The IC limits of quantification for Cl^- and Br^- were 1 μg/L, while I^- was 10 μg/L. For I^- concentrations <10 μg/L, the ICP−MS instrument previously described was used for quantification.[56]

XAD Extraction. For each sample, 52 L of acidified water was extracted over a bed of XAD-2 (Amberlite XAD-2, Sigma Aldrich, MO) and DAX-8 (Supelite DAX-8, Sigma-Aldrich, MO) resin, eluted with ethyl acetate, and concentrated as previously described.[33,57] Portions of the eluate were solvent exchanged into dimethyl sulfoxide (DMSO) or methanol for toxicity assays as described below. Further details on the XAD extraction procedure can be found in Supporting Information (Text S1).

CHO Cell Chronic Cytotoxicity Analyses. CHO cell line K1 AS52 (non-neoplastic) was used for the analytical biological assays.[58,59] CHO cells were maintained on cell culture plates in Ham's F12 medium containing 5% fetal bovine serum (FBS), 1% antibiotics (100 U/mL sodium penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B in 0.85% saline), and 1% glutamine at 37 °C in a humidified atmosphere of 5% CO_2. This calibrated mammalian cell cytotoxicity assay detects the reduction in cell density on flat-bottom 96-well microplates as a function of the concentration of the concentrated water sample (CWS) over a period of approximately 72 h (~3 cell cycles).[41,60] Microliters of the sample XAD extract, solvent exchanged in DMSO, were diluted with F12 + FBS medium to analyze a range of concentration factors. A range-finding experiment, plus a minimum of two experimental repetitions, generated between 4 and 24 independent clonal replicate wells and were analyzed for each CWS. The data from these experiments were combined and a concentration−response curve was generated for each pool water sample; an example is shown in Figure S3. A regression analysis was conducted with each curve and an LC_{50} was calculated. This value represents the sample concentration factor that induced a 50% reduction in cell density as compared to the concurrent negative controls.

Thiol Reactivity Analyses. A portion of each XAD ethyl acetate eluate was concentrated to near-dryness and reconstituted in an equal amount of MeOH for NAC thiol reactivity experiments. These experiments were carried out in triplicate on 96-well plates according to a previously published procedure.[48] Briefly, serial dilutions of XAD extract in Tris buffer at pH 8 and 10 μL of 4 mM NAC (50 μL total) were reacted for 20 min on a rocker platform, followed by the addition of 50 μL of 1 mM DTNB, and the resulting absorbance at 412 nm (A_{412}) was measured for each microplate. The 4 mM NAC concentration was empirically determined in the calibration of the assay.[48] For the positive control, 10 μL of 10 mM maleimide was added to 40 μL of Tris buffer (pH 8) and 50 μL of 1 mM DTNB. The negative control contained 40 μL of Tris buffer (pH 8), 10 μL of 4 mM NAC, and 50 μL of 1 mM DTNB. Corresponding blanks (no NAC) contained an equal amount of sample, as well as Tris buffer (pH 8) and 50 μL of 1 mM DTNB, combined to equal 100 μL to correct for background A_{412}. Concentration−response curves, as shown in Figure S4, for each sample were generated using blank-corrected values. EC_{50} values, or effective concentration of the sample that induced a reduction in NAC thiol concentration by 50% compared to negative controls, were then determined by regression analysis.[48]

Statistical Analyses. With the CHO cytotoxicity analyses for each defined water sample, a one-way analysis of variance (ANOVA) test was conducted to determine the lowest summed molar concentration that induced a statistically significant level of cytotoxicity as compared to its concurrent negative control ($P \leq 0.05$). The power of the ANOVA test was maintained at >0.8 at $\alpha = 0.05$.[61] Bootstrap statistics were used to generate a mean and a standard error of the mean for the LC_{50} value for each defined water extract sample.[61,62] A detailed discussion of the statistical methods has been previously published.[41] An LC_{50} value for cytotoxicity and an EC_{50} value for thiol reactivity were determined for each sample using nonlinear regression analyses, followed by the application of a bootstrap statistic to determine the mean cytotoxicity index (CTI) and thiol reactivity index (TRI), respectively, defined as (LC_{50}^{-1}) (10^3) and (EC_{50}^{-1}) (10^3), values (±standard error [SE]).[63,64] See Text S3 for further details.

Simulated Swimming Pool Reactors. Simulated pool reactions were performed to mimic real pool scenarios and isolate variables of interest (e.g., chlorine residual, ionization).

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Each simulated swimming pool reactor contained a total volume of 35 L of tap water buffered at pH 7.5 with Na_2HPO_4 in 10-gallon polypropylene containers. Before use, the containers were thoroughly rinsed with ultrapure water (18 MΩ cm) and NaOCl for 24 h to remove contaminants and/or DBP precursors, followed by tap water and several rinses of purified water, and then allowed to dry. A body fluid analogue (BFA), containing common components of urine (e.g., urea, creatinine), was spiked into tap water based on an average value of 30 L urine contribution in an approximately 416,000 L pool as previously described by Jmaiff Blackstock et al.[65] This BFA formula has been used in previous studies,[66,67] and details regarding its composition can be found in Table S2. For two reactors, NaOCl was added to achieve free chlorine concentrations of 1 and 5 mg/L after 24 h based on chlorine demand testing as described in Text S4. For the target 1 mg/L free chlorine reactor, copper and silver ions were also introduced via a Caribbean Clear Spa Water Treatment System (Leesville, SC) to achieve conditions of a low chlorine plus ionized pool treatment, whereas the 5 mg/L free chlorine reactor represented a mid-range chlorine treatment without secondary ionization disinfection. At a later time, two other reactors, one with only BFA and another with BFA plus CSI, served to investigate whether any DBP formation resulted in the tap water used from (a) BFA alone and (b) CSI combined with BFA. These conditions, along with measured water quality parameters, are summarized in Table S3.

RESULTS AND DISCUSSION

DBPs in Swimming Pools. A description of the samples collected and water quality parameters, including copper, silver, chlorine residual, and NPOC values, is presented in Table 1. While there are no regulatory limits for copper and silver ion concentrations in pools, total copper was measured well below its EPA drinking water regulation limit (1.0 mg/L) at a maximum of 165 μg/L, and total silver was found at a maximum of 53 μg/L, which falls below its secondary regulation limit in drinking water (100 μg/L).[68] In the indoor pool waters, 76% of the DBPs measured were detected at least once, and both μg/L and nM DBP concentrations can be found in the Supporting Information (Tables S4−S7). Haloacetaldehydes (HALs) were among the most prevalent of the emerging DBPs in pools, with summed HAL concentrations ranging from 0.30 to 3.28 μM (56.6−479 μg/L) (Figure 1). Chloroacetaldehyde (CAL) and bromoacetaldehyde (BAL) are quantified here for the first time in swimming pools, and these DBPs were not detected in their corresponding tap waters used to fill the pool. The quantification of CAL is significant because while other HALs have been reported in swimming pool water, CAL is the most cytotoxic studied to date.[41,52] Among HALs that were quantified, trichloroacetaldehyde (TCAL) was consistently found at the highest concentration (up to 439 μg/L; 2.98 μM), although it is the least cytotoxic HAL.[41,52] Haloketones (HKs) were also commonly found in tap and pool waters, and 1,1,3,3-tetrabromopropanone (1,1,3,3-TeBP) was the only HK detected in a pool sample (May 2018 indoor pool at 0.7 μg/L; 1.9 nM) but not in the corresponding tap water.

Figure 1 illustrates the total concentrations (μM) of each DBP class (excluding THMs, as no THMs were quantified in the May 2018 indoor pool sample). Br/Cl-HAAs accounted for the largest overall molar percent of DBPs in swimming pools (35−75%), which is consistent with previous pool studies showing that HAAs accumulate in pools due to their lack of volatility.[3,33,69] Total HAA concentrations were as high as 13.60 μM (1850 μg/L). Furthermore, nitrogen-containing DBPs (N-DBPs) were found at elevated levels in swimming pool waters versus corresponding tap waters, likely due to the presence of more nitrogenous anthropogenic precursors (urine, sweat, etc.) from increased bather load, which is also indicated by higher NPOC.[5,9] Haloacetamide (HAM) formation was the highest during the July sampling (0.41 μM; 70.9 μg/L) when NPOC was the highest (12 mg/L) and was double that of the November sampling (0.20 μM; 36.2 μg/L) and nearly 4 times that of the May sampling (0.12 μM; 27.4 μg/L) when the NPOC values were 4.3 mg/L and 5.2 mg/L, respectively. Although generally found at higher levels in pool waters than tap water, less dramatic differences in concentrations were observed for other classes of N-DBPs (HANs, HNMs), which reached maxima of 0.24 and 0.03 μM (27.5 and 8.2 μg/L) in pools, respectively.

This is the first study that reports iodoacetic acid (IAA), chloroiodoacetic acid (CIAA), and bromoiodoacetic acid (BIAA) in swimming pools. Although IAA and CIAA were also measured in the corresponding tap water for July 2018, BIAA was detected only in the swimming pool water (0.4 nM; 0.1 μg/L). These I-HAAs were only detected in the indoor pool July 2018 sample and only contributed <0.01% of summed molar DBP concentration of individual DBPs quantified. However, while they were present at low concentrations, IAAs are among the most toxic of all DBPs studied to date.[41,70] Of the I-THMs quantified, only bromochloroiodomethane (BCIM) and dibromoiodomethane (DBIM) were detected in the Nov. 2018 indoor pool sample,

Table 1. Water Samples Collected and Water Quality Parameters

sample	date sampled	free Cl_2 (mg/L)	copper (μg/L)	silver (μg/L)	NPOC[a] (mg/L)
Indoor Pool					
July 2017[b]	7/20/2017	4.9	19	0.3	12
Oct. 2017	10/19/2017	4.1	162	16	7.3
May 2018	5/22/2018	4.4	52	4.4	4.3
Tap		<0.2	23	ND	3.9
July 2018	7/24/2018	3.5	58	24	12
Tap		<0.2	1.9	0.1	3.2
Nov. 2018	11/26/2018	1.0	143	17	5.2
tap		<0.2	1.0	0.3	2.9
Outdoor Pool					
March 2017	3/14/2017	0.2	137	32	1.5
June 2017	6/20/2017	5.4	165	53	4.7
Oct. 2017	10/19/2017	1.6	110	33	2.2
May 2018	5/15/2018	12	133	22	1.9
Tap		<0.2	6.3	0.1	3.2
Oct. 2018	10/23/2018	1.0	72	19	5.8
tap		<0.2	1.3	0.3	3.4

[a]Nonpurgeable organic carbon. [b]Collected before CSI system was installed.

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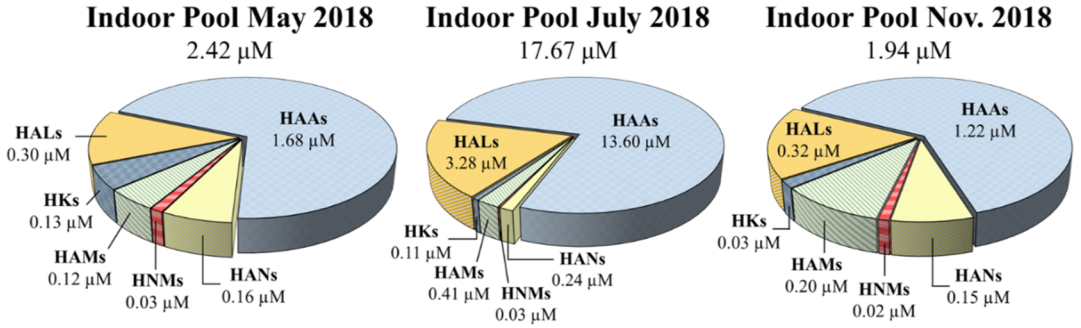

Figure 1. Sum of DBP concentrations (μM) for each class for indoor pool samples treated with chlorine and CSI. THMs were not measured for the indoor pool May 2018 sampling; therefore, THM data are not reflected here. IAAs and I-THMs were not detected or comprised <0.01% molar concentration and thus are not included in charts. Both nM and μg/L DBP concentrations (±standard deviation) are available in Tables S4 and S5.

and BCIM and DBIM were also detected in the tap water at the same concentration (0.1 μg/L; 0.5 and 0.3 nM, respectively), suggesting that they were not formed in the swimming pool. At the same time, the data clearly demonstrate that I-THMs are stable in indoor swimming pools. The presence of I-DBPs is likely a result of iodide in source water reacting with chloramine used to disinfect the tap water, although a recent study reported iodide was below their reporting limit of 25 μg/L in Myrtle Beach source water[71] and helps explain the scarcity of I-DBPs.

Higher levels of TOX are an indicator of higher DBP levels (including both known target and unknown DBPs). TOX values for water samples are shown in Table S9. TOCl dominated the overall DBP formation (Figure 2), which was

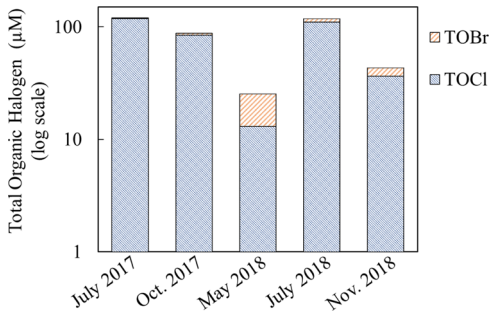

Figure 2. TOCl and TOBr (as X^-) data for indoor pool samples. TOCl and TOBr values (±standard deviation) are available in Table S9.

not surprising since both pools are chlorinated. TOBr formation was highest in the May 2018 sample (12.4 μM; 990 μg/L) and 1.5× the July 2018 sample (8.2 μM; 652 μg/L) and 6.5× higher than the July 2017 sample (1.9 μM; 149 μg/L) (Figure 2). The higher TOBr corresponded to the highest levels of emerging Br-containing DBPs (Figure 3A), with particularly high concentrations of dibromoacetonitrile (DBAN), tribromoacetamide (TBAM), and tribromoacetaldehyde (TBAL), which were found up to 86.9, 17.8, and 28.2 nM (17.3, 5.3, and 7.9 μg/L), respectively (Tables S4 and S5). These Br-DBPs are significantly more toxic than their chlorinated analogues.[41] Total organic iodine (TOI) levels were extremely low (never more than 5 μg/L) in tap filling water and pool waters, which was consistent with low levels of I-DBPs in pools and tap water.

Swimming Pool Toxicity. Figure 4 shows that all indoor pool samples were more cytotoxic than the corresponding tap water and the outdoor pool samples, indicated by higher CTI values. When the lowest amount of free chlorine was used for disinfection (1.0 mg/L) with secondary CSI treatment (November 2018), DBP formation and the resulting toxicity were the lowest among indoor pool samples. While it should be noted that November is not during the peak swimming season, the indoor pool is in operation and open for swimming year-round. The sampling occurred after Thanksgiving holidays when the pool was frequently occupied by swimmers, indicated by the increase in NPOC from the tap filling water (2.9 mg/L) to pool water (5.2 mg/L). Additionally, the equivalent seasonal sampling a year prior (indoor pool Oct. 2017) that was higher in free chlorine also had higher TOX (Figure 2) and cytotoxicity (Figure 4). It is worth noting that the NPOC data suggest a slightly higher bather load in the Oct. 2017 sampling (7.3 mg/L) versus the Nov. 2018 sampling (5.2 mg/L), although the NPOC of the tap filling water for the indoor pool Oct. 2017 was not measured, so its contribution cannot be accounted for.

The considerably lower toxicity observed for the outdoor pool samples is likely due to volatilization and photodegradation of DBPs over time,[72,73] as the indoor pool is less susceptible to these environmental factors. Cytotoxicity and thiol reactivity were actually higher after CSI was introduced (July 2018) compared to before ionization during the same month the previous year (July 2017) (Figure 4). Although CSI was being used, a considerable amount of free chlorine (3.5 mg/L) was still measured in the July 2018 sampling, leading to similar TOX formation (Figure 2) and likely comparable amounts of DBPs to those observed the previous year. The amount of chlorine, combined with increased bather load and DBP precursors present during peak swimming season (July), appears to drive the overall DBP formation and resulting toxicity (Figures 1 and 4).

The toxicity of the May 2018 indoor pool was most similar to the July sampling events, although the overall quantified DBP formation (Figure 1) and TOX (Figure 2) was closer to the November 2018 event. Figure 3B expresses the calculated toxicity associated with each indoor pool sample. Calculated toxicity, called "TIC-Tox",[74] has been used to predict cytotoxicity in previous studies based on individual DBP concentrations and known toxicity values of these DBPs.[39,53,74,75] This is calculated by multiplying each individual molar DBP concentration by the CTI value (inverse of its molar LC_{50} [10^3]) and then multiplying by a factor of 10^6. A singular assumption of the calculated toxicity approach is that the toxicity of the individual DBPs is additive in a

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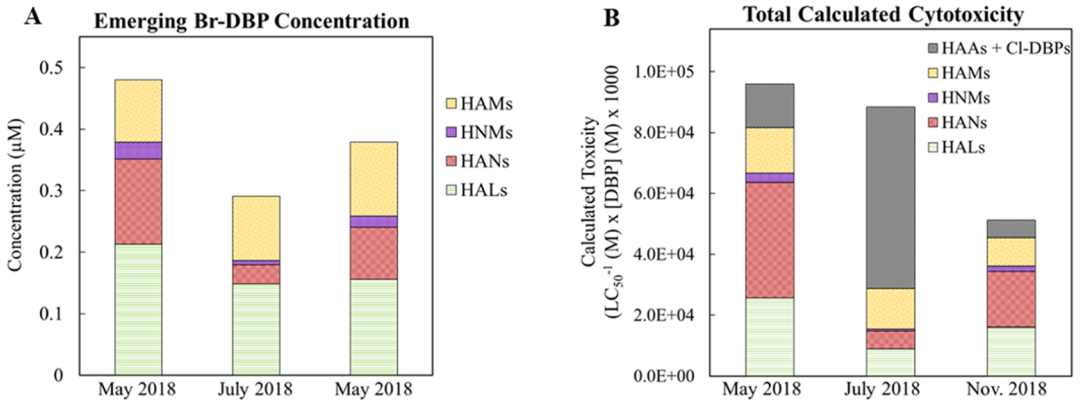

Figure 3. (A) Indoor pool sample stack plots of summed emerging Br-containing haloacetamide (HAM), halonitromethane (HNM), haloacetonitrile (HAN), and haloacetaldehyde (HAL) concentrations (μM). Haloketones (HKs) are not included because no cytotoxicity data are available for these compounds. I-DBPs were not included because most were not detected. (B) Total calculated toxicity from quantified DBPs in indoor pool samples.

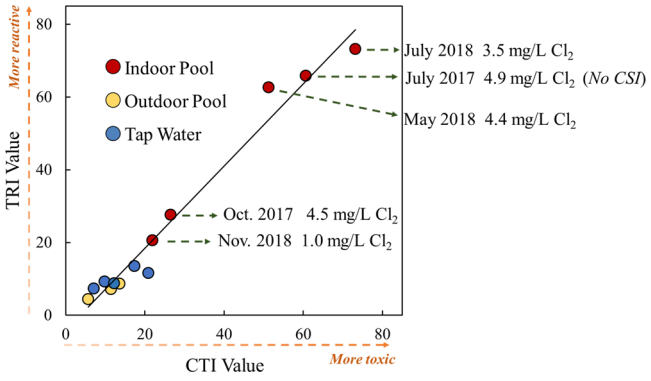

Figure 4. Thiol reactivity index (TRI) values vs cytotoxicity index (CTI) values (generated from LC_{50} and EC_{50} values [± standard error] in Table S8) for pool and tap water samples. March 2017 and Oct. 2017 Outdoor Pool samples are excluded due to no induction of NAC thiol concentration reduction. TRI and CTI were statistically and significantly correlated ($r = 0.99$, $P \leq 0.001$).

mixture. Recently, the additivity assumption for CHO cell cytotoxicity was verified.[76] Despite having lower overall DBP formation and TOX, the May 2018 indoor pool sample had higher calculated toxicity due to the predominance of Br-DBPs compared to the other indoor pool samples (Figure 3B). Thus, while total quantified DBPs were lower for May 2018 compared to that for the July 2018 event (Figure 1), the higher levels of more toxic Br-DBPs and increased TOBr, in which there are likely more unknown toxic Br-DBPs, could account for more similar toxicity to the July events than November and October. The calculated cytotoxicity in the indoor pool July 2018 sample had a significant contribution from Br/Cl-HAAs and other DBPs containing only chlorine (Cl-DBPs) (Figure 3B). In fact, chloroacetaldehyde contributed 44% of the total calculated toxicity, suggesting that this compound is a major source of cytotoxicity that has not been previously accounted for in pools and was a major driver of the measured cytotoxicity in this study.

NAC Thiol and Cytotoxicity Correlation. The Pearson Product Moment Correlation between the CHO cell CTI values and the TRI values is highly and statistically significantly correlated ($r = 0.99$; $P < 0.001$; Figure 4). These data show that thiol reactivity can be a reliable predictor of CHO cell cytotoxicity in swimming pool samples. The CTI and TRI values were generated from the mean LC_{50} and EC_{50} (±SE) found for each sample in Table S8. Two samples that were among the lowest in cytotoxicity (outdoor pool Oct. 2017, outdoor pool March 2017) did not generate a reduction in thiol reactivity concentration compared to the negative control, labeled as "NR" in Table S8.

N-DBP and TOX Cytotoxicity Correlations. Several N-DBPs were detected in indoor pool samples and associated tap waters, and there was generally an increase seen in the pool water. N-DBPs are particularly concerning because they are generally more genotoxic and cytotoxic than DBPs without nitrogen (e.g., THMs, HAAs).[40,77] Likely nitrogen-sources for N-DBPs are sweat and urine released by swimmers, which contain urea and other N-containing precursors.[5,9,78] Linear regressions of CTI values versus N-DBP molar concentrations are shown in Figure S2. There was a significant correlation between total N-DBPs and CTI values, as well as each individual N-DBP class, in all indoor pool samples. The best correlation was seen for HNMs ($r = 0.99$, $P < 0.05$), followed by HANs ($r = 0.91$, $P < 0.05$). A previous study showed an average correlation between toxicity (mutagenicity in Salmonella) and total N-DBPs ($r = 0.73$) in chlorinated pools and tap waters, but only four HANs and a single HNM (trichloronitromethane) were measured.[33] Our study has uncovered a strong correlation ($r = 0.89$, $P < 0.05$) to toxic potency with a much broader suite of N-DBPs that included 10 HANs, 13 HAMs, and 7 HNMs.

While TOX increased from tap to pool in each scenario, there was no significant correlation between cytotoxicity and TOX between all samples and was mainly due to differences in TOCl. The outdoor pool samples were much higher in TOCl compared to tap waters, and similar to indoor pool samples, which is expected because this was a chlorinated pool. Yet, outdoor pools were among the least toxic samples overall. As mentioned previously, this is likely due to compounds volatilizing or UV-mediated photodegradation.[72]

TOBr and cytotoxicity were highest in the indoor pool samples, and across all samples, a moderate correlation was observed ($r = 0.70$, $P < 0.05$). Since brominated DBPs are much more cytotoxic than their chlorinated analogues, it is logical that higher measurements of TOBr typically resulted in higher cytotoxicity. This was especially true in indoor pool samples, particularly for the May 2018 sample discussed

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previously, where TOBr and cytotoxicity were the highest (Figures 2 and 4).

Simulated Pools. Because real pools can have substantial variability due to differences in tap filling water, bather loads,[79,80] DBP precursors added by the bathers, temperature, pH, levels of chlorine, air exchange, sunlight, and other conditions,[6,80,81] simulated pools using a BFA[66,67,82] (Table S2) were also investigated so that the parameters could be carefully controlled and results compared directly. Treatments included a low chlorine target residual (1 mg/L) with CSI, and a higher chlorine residual (5 mg/L), representing a mid-range chlorine dose used for real pools, along with BFA/CSI and BFA controls and two tap water controls. Figure 5A summarizes the quantitative DBP results from these simulated pools.

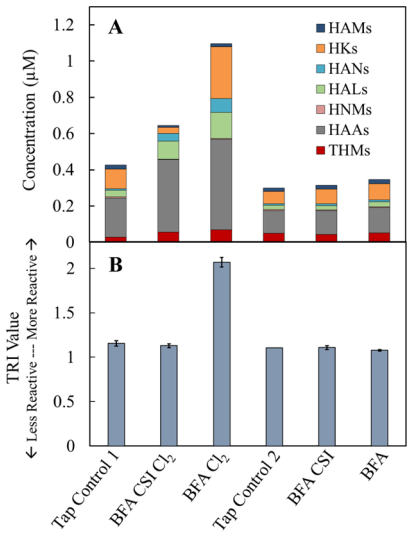

Figure 5. Simulated pool reactions: (A) Stack plots of summed DBP concentrations (μM) for all classes of DBPs and (B) TRI values. All I-DBPs were below detection and thus not included. Note: Tap Control 2 represents only a single measurement. Both nM and μg/L DBP concentrations (±standard deviation) are available in Tables S6 and S7.

Summed DBP formation increased from the tap water control (0.42 μM; 55.3 μg/L) in the low Cl_2/CSI reactor (0.64 μM; 88.2 μg/L) and the high Cl_2 reactor (1.09 μM; 139 μg/L), which was mainly driven by increases in Cl-DBPs (Tables S6 and S7). HANs, particularly dichloroacetonitrile (DCAN), increased by the highest percent of all classes in both the low Cl_2/CSI reactor (341%) and the high Cl_2 reactor (681%) (Figure 5A), likely due to the presence of N-DBP precursors in the BFA reacting with the additional chlorine. This is consistent with previous studies where L-histidine was shown to be a precursor to DCAN.[5,82] However, the largest % increases in real pool samples during the peak swimming seasons were seen in HAAs and HALs, likely because inputs such as sunscreens and lotions act as major precursors to these classes and were not present in the simulated reactors.[3,4] Chlorinated HAAs, including chloroacetic acid, dichloroacetic acid, and trichloroacetic acid, progressively increased from the tap water control to the low Cl_2/CSI reactor to the high Cl_2 reactor, likely from the reaction of citric acid in the BFA and chlorine.[67]

HAL formation also increased in Cl_2 plus BFA reactors. Notably, CAL was not detected in the tap water control but was found at 10.1 nM (0.8 μg/L) in the low Cl_2/CSI reactor and 23.2 nM (1.8 μg/L) in the high Cl_2 reactor. Similarly, CAL was also only detected in real pool samples and not in associated tap filling waters. The high concentrations of HALs in real pool samples compared to tap filling waters in this study are consistent with previous studies,[3,33,39,83] and their increased formation in simulated pool reactors is further evidence that components of human inputs, such as urine, act as precursors for this DBP class. Minimal differences in DBP concentrations between the second tap water control, BFA only, and BFA/CSI reactors indicated that DBP formation is not linked to copper and silver ions produced from ionization.

Figure 5B compares the NAC thiol reactivity results between simulated swimming pool samples. The high Cl_2 reactor, which nearly tripled in total DBP concentration from the tap water control, exhibited the highest reactivity and was double that of the tap water control. Although DBP formation slightly increased in the low Cl_2/CSI reactor, increased thiol reactivity was not observed, likely because the increase in DBPs was not enough to induce more reactivity or consisted of relatively low-cytotoxic (with low thiol reactivity) DBPs. Based on the strong correlation observed between TRI and CTI values, it is highly probable that the high Cl_2 reactor is also more cytotoxic than the low Cl_2/CSI sample and tap water control. This result is consistent with results from our real pool samples, as the low Cl_2/CSI pool sample exhibited the lowest DBP formation and cytotoxicity. The second tap water control, BFA only, and BFA/CSI reactors all had the same TRI value (1.1), demonstrating that the reactivity was not affected by CSI or BFA.

Broader Implications. The work presented here combines extensive analytical chemistry and analytical biology analyses to characterize swimming pools disinfected with copper and silver ions and chlorine for the first time. Our data from controlled experiments suggest that a lower chlorine residual, combined with a secondary CSI treatment, can reduce the formation of DBPs, TOX, NAC thiol reactivity, and resulting mammalian cell cytotoxicity. We recognize that swimming pools are dynamic, complex matrices with many variables that can contribute to DBP formation (e.g., seasonal bather load, varying precursors introduced, pH, etc.).[3,4,9,79,80] There are also emergency situations when it is necessary to add large amounts of chlorine (i.e., shock treatment) in response to fecal accidents. However, we believe that when proper care is taken in a normal day-to-day pool operation, it could be possible to limit the formation of DBPs and associated health risks.

Our data also show that NAC thiol reactivity can be used to predict mammalian cell cytotoxicity in swimming pool waters. Although not a replacement for analytical biology, it is a high-throughput assay that can be used effectively as a screening for cytotoxicity by chemistry labs without access to specialized facilities needed to carry out biology assays.

This study builds upon previous work of cytotoxicity measurements in pools[9,47] with extensive analytical chemistry measurements to bridge the gap between observed cytotoxicity and the associated toxic agents responsible. We observed a clear increase in DBP formation, TOX, and cytotoxicity from tap to pool water in the indoor pool. Although TOX in the outdoor pool increased, enhanced cytotoxicity did not correlate. Thus, TOX may not be a reliable metric for predicting toxicity in pool samples. The presence of N-DBPs,

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likely augmented by precursors introduced by bathers, strongly correlated with cytotoxicity and could be a useful indicator for pool health. Encouraging swimmers to refrain from urinating in pools and taking further precautions like showering before entering pools could also limit the introduction of DBP precursors. While the formation of DBPs and cytotoxic potency raises concern and should not be ignored, our goal is not to discourage swimming, as this is a well-established healthy form of exercise; rather, our aim is to make the swimming environment safer by seeking ways to lower the byproduct formation. The use of CSI with lower amounts of chlorine appears to be a promising way to accomplish this.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06287.

XAD resin extraction; CHO cell experimental procedure; statistical analyses; chlorine demand for simulated swimming pools; chlorine residual versus chlorine dose linear regression; cytotoxicity index versus N-DBP concentration linear regressions; GC-MS quantifier and qualifier ions, vendor information, and minimum reporting limits (MRLs) for DBPs quantified; BFA composition; simulated pool water quality parameters; concentrations (μg/L and nM) of DBPs for indoor pool 2018 samples; concentrations (μg/L and nM) of DBPs for simulated pool samples; CHO LC50 and NAC thiol reactivity EC50 values for water samples; example CHO concentration−response curve; example NAC thiol concentration−response curve; and total organic halogen data for water samples (PDF)

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Notes

The authors declare no competing financial interest.

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SUPPORTING INFORMATION FOR:

MAKING SWIMMING POOLS SAFER: DOES COPPER-SILVER IONIZATION WITH CHLORINE LOWER TOXICITY AND DBP FORMATION?

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Text S1. XAD Resin Extraction

Before each extraction, resins were cleaned and conditioned according to a previously published Standard Operating Procedure and packed into glass chromatography columns.[1] Samples were first acidified to pH < 1 with concentrated H_2SO_4 and passed over the resins, and the organics were eluted with ethyl acetate funnel once all sample passed through. The ethyl acetate extract was collected in a separatory funnel, and this extract was then passed through a column of anhydrous sodium sulfate to remove any water. The eluate was subsequently concentrated under N_2 in a Turbovap (Turbovap®II, Biotage) to 3 mL (17,333-fold concentration) and stored at -20°C.

Text S2. Chinese hamster ovary (CHO) cell experimental procedure.

Each concentrated water sample (CWS) was solvent exchanged from ethyl acetate into dimethyl sulfoxide (DMSO) at a concentration factor of 1×10^5. The samples were stored in the dark at −20°C. Measuring the reduction in cell viability compared to untreated controls, cytotoxicity captures a wide array of toxic insults. This assay measures cytotoxicity as the reduction in cell density after exposure of the cells to a CWS for 72 h (i.e., a chronic exposure for these cells) compared to untreated control.[2] For each experiment, a dilution series (generally 10 concentrations) was constructed by diluting the CWS into cell culture medium just prior to the treatment. These CWS treatment dilutions were exposed to CHO cells in 96-well microplates covered with AlumnaSeal to prevent volatilization during the 72 h exposure period. After exposure, the cell density per microplate was determined by histological staining using crystal violet and absorbance at 595 nm using a SpectraMax microplate reader (Molecular Devices, CA). The dilution series constructed from the CWS represents a range of concentration factors for the organics in the original water. The range in concentration factors was selected to span the

range between no significant reduction in growth and increasing reduction in cell density per microplate well.

Text S3. Statistical analyses.

Precision statistical analyses were conducted on each analytical biology dataset. The process followed the generation of a concentration-response curve from combined replicate experiments with a test for significance using a one-way analysis of variance (ANOVA) test. If a significant *F* value of $P \leq 0.05$ was obtained, a Holm-Sidak multiple comparison versus the control group analysis was conducted with the power $(1-\beta)$ 0.8 at $\alpha = 0.05$ to identify the lowest concentration that induced an adverse biological impact.[3] After non-linear regression analyses, a LC_{50} value was determined for the CHO cell cytotoxicity assay. A bootstrap statistic was conducted and the CHO cell cytotoxicity data were calculated.[4,5] The same bootstrap method was used to determine thiol reactivity index (TRI), defined as $(EC_{50}^{-1})(10^3)$, values (±SE) for the *N*-acetyl-L-cysteine (NAC) thiol reactivity assay . Using these index values, an ANOVA test could be conducted to identify significant differences among specific CWS groups.

Text S4. Chlorine demand for simulated pools.

Chlorine demand for the body fluid analogue (BFA) and laboratory tap water was measured to achieve both 1 and 5 mg/L free chlorine residual after 24-h reaction time in simulated swimming pool reactors. NaOCl was first standardized at $\lambda_{max} = 243$ nm using a SpectraMax spectrophotometer. Demand tests were carried out in 250 mL polypropylene beakers with 100 mL of tap water. Each reactor containing tap water as its base was buffered to pH 7.5 with Na_2HPO_4 and spiked with BFA and various concentrations of NaOCl diluted from the standardized stock solution. Free chlorine was measured after 24 h using a Hach DR/850 colorimeter, and the chlorine residual vs. chlorine dose curve (Figure S1) was generated.

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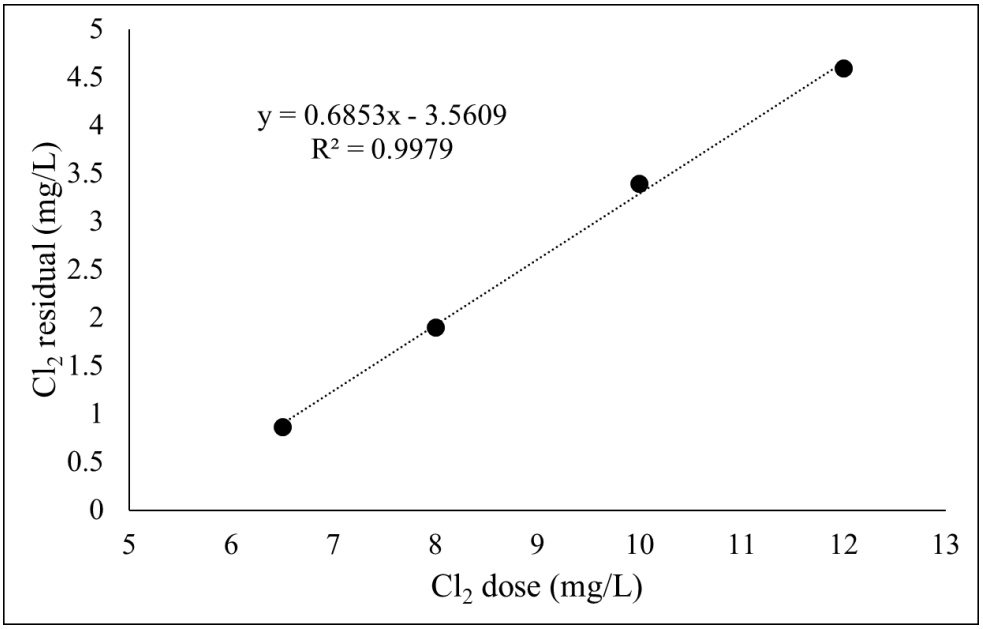

Figure S1. Chlorine residual vs. chlorine dose linear regression generate from chlorine demand experiment.

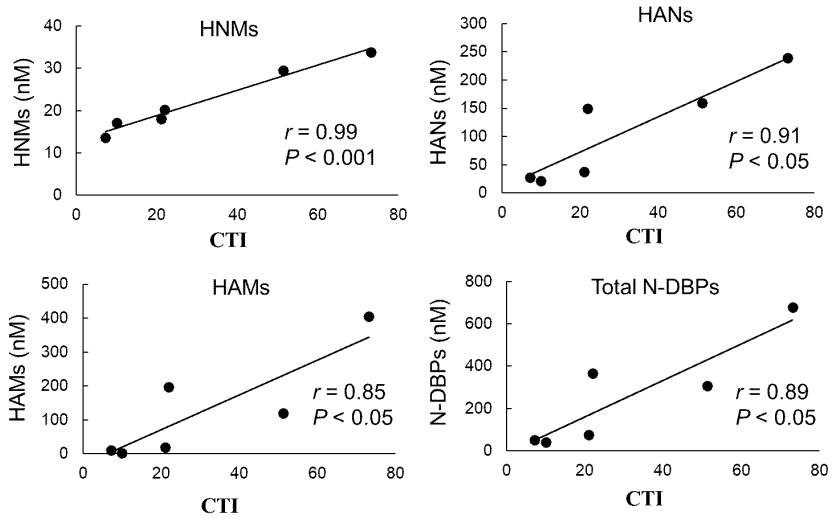

Figure S2. Linear regressions of cytotoxicity index (CTI) values vs. concentration (nM) of halonitromethanes (HNMs), haloacetonitriles (HANs), haloacetamides (HAMs), and total N-DBPs (HANs + HNMs + HAMs) for indoor pool 2018 samples and corresponding tap water samples. All had Pearson's *r* values of ≥ 0.85.

Table S1. Gas chromatography-mass spectrometry (GC-MS) quantifier and qualifier ions, vendor information, and minimum reporting limits (MRLs) for DBPs quantified in this study.[a]

DBP Name	Abbreviation	Quantitative Ion (m/z)	Qualifier Ion (m/z)	MRL (µg/L)
Analytical Method 1				
Trichloromethane[b]	TCM	83.0	85.0	0.1
Tribromomethane[b]	TBM	173.0	252.0	0.1
Dibromochloromethane[b]	DBCM	129.0	126.9	0.1
Bromodichloromethane[b]	BDCM	83.0	129.0	0.1
Trichloroacetaldehyde[b]	TCAL	82.0	110.9	0.1
Bromodichloroacetaldehyde[c]	BDCAL	111.0	83.0, 163.8	0.1
Dibromochloroacetaldehyde[c]	DBCAL	128.9	127.9	0.1
Tribromoacetaldehyde[b]	TBAL	172.8	171.8	0.1
Trichloroacetonitrile[b]	TCAN	108.0	110.0	0.1
Dichloroacetonitrile[b]	DCAN	74.0	82.0	0.1
Chloroacetonitrile[b]	CAN	75.0	48.0	0.5
Bromochloroacetonitrile[b]	BCAN	155.0	74.0	0.1
Bromoacetonitrile[b]	BAN	118.9	120.9	0.1
Dibromoacetonitrile[b]	DBAN	117.9	199.0	0.1
Iodoacetonitrile[b]	IAN	167.0	126.9	0.1

Bromodichloroacetonitrile[c]	BDCAN	154.0	108.0	0.1
Dibromochloroacetonitrile[c]	DBCAN	154	152	0.1
Tribromoacetonitrile[c]	TBAN	197.8	195.8	0.1
1,1-Dichloropropanone[b]	11DCP	83.0	43.0	0.1
Chloropropanone[b]	CP	92.0	43.0	0.1
1,1,1-Trichloropropanone[b]	111TCP	43.0	125.0	0.1
1,1-Dibromopropanone[c]	11DBP	215.9	43.0	0.1
1-Bromo-1,1-dichloropropanone[c]	1B11DCP	125.0	43.0	0.1
1,3-Dichloropropanone[c]	13DCP	77.0	49.0	0.1
1,1,3-Trichloropropanone[b]	113TCP	77.0	83.0	0.1
1,1,3,3-Tetrachloropropanone[c]	1133TeCP	83.0	85.0	0.1
1,1,3,3-Tetrabromopropanone[d]	1133TeBP	200.8	119.9	0.1
Trichloronitromethane[b]	TCNM	116.9	119.0	0.1
Dichloronitromethane[c]	DCNM	83.0	85.0	0.1
Bromochloronitromethane[c]	BCNM	129.0	127.0	0.1
Dibromonitromethane[c]	DBNM	172.8	171.0	0.1
Bromodichloronitromethane[c]	BDCNM	163.0	161.0	0.1
Dibromochloronitromethane[c]	DBCNM	206.8	209.0	0.1
Tribromonitromethane[c]	TBNM	251.0	253.0	0.5
Dichloroiodomethane[c]	DCIM	83.0	126.9	0.1
Bromochloroiodomethane[c]	BCIM	128.9	126.9	0.1
Dibromoiodomethane[c]	DBIM	172.8	299.7	0.1
Chlorodiiodomethane[c]	CDIM	174.9	126.9	0.1
Bromodiiodomethane[c]	BDIM	218.8	220.8	0.1
Iodoform[b]	TIM	266.8	393.7	0.1
Chloroacetamide[b]	CAM	93.0	44.0	1.0
Bromoacetamide[b]	BAM	139.0	137.0, 44.0	1.0
Dichloroacetamide[e]	DCAM	44.0	127.0	0.25
Bromochloroacetamide[c]	BCAM	44.0	173.0	0.1
Trichloroacetamide[b]	TCAM	44.0	82.0	0.1
Iodoacetamide[b]	IAM	185.0	58.0	1.0
Dibromoacetamide[c]	DBAM	44.0	217.0	0.1
Chloroiodoacetamide[c]	CIAM	92.0	219.0	0.1
Bromodichloroacetamide[c]	BDCAM	44.0	128.0	0.1
Bromoiodoacetamide[c]	BIAM	136.0	138.0	0.5
Dibromochloroacetamide[c]	DBCAM	44.0	128.0	0.1
Tribromoacetamide[c]	TBAM	44.0	295.0	0.1
Diiodoacetamide[c]	DIAM	184.0	311.0	0.1

Chloroacetic acid[b]	CAA	108>76	77>49	0.1
Dichloroacetic acid[b]	DCAA	83>48	76>48	0.1
Trichloroacetic acid[b]	TCAA	116.9>81.9	141>113	0.1
Bromoacetic acid[b]	BAA	121>93	72>42	0.1
Dibromoacetic acid[b]	DBAA	172.9>91.9	119.9>91.9	0.1
Bromochloroacetic acid[b]	BCAA	157>129	128.9>48	0.1
Dibromochloroacetic acid[b]	DBCAA	186.9>158.9	206.8>127.9	0.1
Bromodichloroacetic acid[b]	BDCAA	141>113	162.9>81.9	0.1
Iodoacetic acid[b]	IAA	200>73	200>45	0.025
Chloroiodoacetic acid[c]	CIAA	234>79	234>107	0.025
Bromoiodoacetic acid[c]	BIAA	278>123	278>151, 280>125	0.025
Diiodoacetic acid[c]	DIAA	326>171	326>199	0.025
Analytical Method 2				
Chloroacetaldehyde[b]	CAL	238.0	181.0/182.0	0.1
Bromoacetaldehyde[d]	BAL	287.0	238.0	0.1
Iodoacetaldehyde[d]	IAL	293.0	335.0	0.5
Dichloroacetaldehyde[e]	DCAL	272.0	181.0/182.0	0.1
Bromochloroacetaldehyde[c]	BCAL	272.0	238.0	0.1
Dibromoacetaldehyde[c]	DBAL	137.0	135.0	0.1

[a] DBPs are classified by their corresponding analytical method and DBP class. [b] Sigma Aldrich. [c] CanSyn Chem. Corp. [d] Aldlab Chemicals. [e] TCI America.

Table S2. Body fluid analogue concentrations.

Ingredient	mg/L
NH_4Cl	2000
Urea	14800
L-Histidine	1210
Hippuric acid	1710
Uric acid	490
Citric acid	640
Na_2HPO_4	4300
Creatinine	1800

Table S3. Simulated pool water quality parameters.

Reactor	Free Cl_2 (mg/L)	Copper (µg/L)	Silver (µg/L)	NPOC[a] (mg/L)	Total Nitrogen (mg/L)
Tap Control 1	< 0.2	20	0.03	1.8	1.2
BFA Cl_2	5.1	15	ND	2.1	0.7
BFA CSI Cl_2	1.4	183	3.68	2.3	0.9
Tap Control 2	< 0.2	12	0.20	1.3	1.1
BFA	< 0.2	11	0.01	1.9	1.7
BFA CSI	< 0.2	211	6.76	1.8	1.7

[a] Non-purgeable organic carbon

Table S4. Concentrations (µg/L) of 2018 indoor pool DBPs.[a]

		Indoor May 2018		Indoor July 2018		Indoor Nov. 2018	
Class	**Compound**	Tap	Pool	Tap	Pool	Tap	Pool
HNMs	DCNM	ND	ND	ND	0.4 ± 0.007	ND	ND
	BCNM	< 0.1	< 0.1	ND	0.1 ± 0.001	ND	0.2 ± 0.01
	DBNM	< 0.1	0.3 ± 0.02	ND	ND	< 0.1	0.3 ± 0.01
	TCNM	1.2 ± 0.1	0.4 ± 0.1	1.1 ± 0.2	3.9 ± 0.3	0.6±0.04	0.3 ± 0.1
	BDCNM	0.7 ± 0.02	0.3 ± 0.01	0.5 ± 0.05	0.5 ± 0.02	0.8±0.02	0.6 ± 0.02
	DBCNM	1.0 ± 0.001	1.1 ± 0.05	0.6 ± 0.04	0.6 ± 0.01	1.2±0.03	1.2 ± 0.1
	TBNM	1.0 ± 0.04	6.1 ± 0.2	0.7 ± 0.04	0.6 ± 0.04	1.6±0.04	2.5 ± 0.4
HALs	CAL	NM	NM	ND	10.6 ± 0.06	ND	0.3 ± 0.1
	BAL	NM	NM	ND	1.7 ± 0.04	ND	0.6 ± 0.01
	IAL	NM	NM	ND	ND	ND	ND
	DCAL	NM	NM	0.3 ± 0.01	1.4 ± 0.05	0.1 ± 0.001	0.2 ± 0.01
	BCAL	NM	NM	< 0.1	0.3 ± 0.05	0.1 ± 0.001	0.3 ± 0.02
	DBAL	NM	NM	ND	1.3 ± 0.07	< 0.1	0.6 ± 0.04
	TCAL	4.3 ± 0.2	12.9 ± 1.2	3.2 ± 0.6	439 ± 4	2.0 ± 0.1	24.1 ± 1.8
	BDCAL	1.2 ± 0.2	24.0 ± 1.9	1.5 ± 0.3	23.9 ± 0.7	0.9 ± 0.02	18.3 ± 0.7
	DBCAL	0.3 ± 0.03	14.2 ± 2.0	0.2 ± 0.04	0.4 ± 0.02	0.2 ± 0.001	11.5 ± 0.8
	TBAL	< 0.1	7.9 ± 0.910	0.2 ± 0.004	0.2 ± 0.001	< 0.1	0.7 ± 0.03
HANs	CAN	< 0.1	0.3 ± 0.02	0.7 ± 0.02	3.8 ± 0.03	0.6 ± 0.07	0.8 ± 0.04
	BAN	< 0.1	1.2 ± 0.01	0.3 ± 0.001	1.0 ± 0.01	ND	ND
	IAN	ND	ND	ND	ND	ND	ND
	DCAN	2.9 ± 0.1	1.9 ± 0.04	1.1 ± 0.2	16.9 ± 0.1	1.0 ± 0.1	5.8 ± 0.3
	BCAN	1.5 ± 0.1	5.8 ± 0.5	0.7 ± 0.08	3.1 ± 0.002	0.5 ± 0.02	5.8 ± 0.4
	DBAN	0.3 ± 0.01	17.3 ± 2.2	0.2 ± 0.01	0.4 ± 0.007	0.2 ± 0.01	7.8 ± 0.5
	TCAN	< 0.1	< 0.1	ND	0.6 ± 0.006	ND	0.2 ± 0.001
	BDCAN	NM	NM	NM	NM	ND	0.2 ± 0.01

	DBCAN	NM	NM	NM	NM	ND	0.6 ± 0.03
	TBAN	ND	1.0 ± 0.02	ND	ND	< 0.1	1.0 ± 0.1
HKs	CP	8.0 ± 0.1	10.3 ± 1.3	2.2 ± 0.2	4.4 ± 0.2	4.6 ± 1.1	0.4 ± 0.03
	11DCP	2.3 ± 0.08	ND	3.5 ± 0.4	ND	ND	ND
	13DCP	< 0.1	ND	0.3 ± 0.05	1.3 ± 0.1	ND	ND
	1B11DCP	1.0 ± 0.08	0.9 ± 0.02	0.3 ± 0.004	0.4 ± 0.004	0.2 ± 0.003	0.7 ± 0.04
	11DBP	0.2 ± 0.004	0.8 ± 0.03	0.4 ± 0.05	ND	0.3 ± 0.01	0.4 ± 0.02
	111TCP	2.1 ± 0.1	0.9 ± 0.01	1.1 ± 0.2	7.9 ± 0.1	1.2 ± 0.03	3.8 ± 0.2
	113TCP	0.1 ± 0.005	ND	0.2 ± 0.03	ND	ND	ND
	1133TeCP	0.3 ± 0.02	ND	0.3 ± 0.02	0.6 ± 0.05	0.3 ± 0.001	ND
	1133TeBP	ND	0.7 ± 0.06	ND	ND	ND	ND
I-THMs	DCIM	ND	ND	0.6 ± 0.03	ND	ND	ND
	BCIM	0.1 ± 0.01	ND	< 0.1	< 0.1	0.1 ± 0.01	0.1 ± 0.001
	DBIM	< 0.1	ND	0.2 ± 0.001	0.2 ± 0.001	0.1 ± 0.001	0.1 ± 0.001
	CDIM	ND	ND	ND	ND	ND	ND
	BDIM	< 0.1	ND	ND	ND	ND	ND
	TIM	ND	ND	0.1 ± 0.001	0.1 ± 0.002	ND	ND
HAMs	CAM	ND	ND	ND	ND	ND	ND
	BAM	ND	ND	ND	ND	ND	ND
	IAM	ND	ND	ND	ND	ND	ND
	DCAM	3.2 ± 0.06	1.5 ± 0.002	ND	ND	ND	ND
	BCAM	2.2 ± 0.06	3.6 ± 0.02	1.2 ± 0.4	4.9 ± 0.08	0.5 ± 0.1	9.0 ± 0.7
	DBAM	0.5 ± 0.04	6.2 ± 0.9	ND	0.9 ± 0.01	< 0.1	13.6 ± 0.4
	CIAM	ND	ND	ND	ND	ND	ND
	BIAM	ND	ND	ND	ND	ND	ND
	DIAM	ND	ND	ND	ND	ND	ND
	TCAM	0.6 ± 0.01	2.9 ± 0.01	0.5 ± 0.1	48.8 ± 0.9	ND	12.4 ± 0.3
	BDCAM	0.2 ± 0.003	3.0 ± 0.2	ND	10.4 ± 0.4	< 0.1	ND
	DBCAM	< 0.1	4.9 ± 0.8	ND	3.5 ± 0.07	ND	0.8 ± 0.05
	TBAM	ND	5.3 ± 1.5	ND	2.4 ± 0.04	ND	0.4 ± 0.09
I-HAAs	IAA	ND	ND	0.0 ± 0.007	0.1 ± 0.02	ND	ND
	CIAA	0.1 ± 0.01	ND	0.1 ± 0.007	0.1 ± 0.007	ND	ND
	BIAA	ND	ND	ND	0.1 ± 0.001	ND	ND
	DIAA	ND	ND	ND	ND	ND	ND
THMs	TCM	NM	NM	3.0 ± 0.6	23.3 ± 2.8	6.0 ± 0.04	104 ± 3
	BDCM	NM	NM	2.1 ± 0.4	21.6 ± 0.1	3.5 ± 0.2	50.4 ± 2.7
	DBCM	NM	NM	0.7 ± 0.1	3.9 ± 0.03	0.9 ± 0.04	42.9 ± 2.5
	TBM	NM	NM	< 0.5	< 0.5	0.2 ± 0.002	30.2 ± 1.9
Br/Cl-HAAs	CAA	0.5 ± 0.001	16.6	1.4 ± 0.2	91.3 ± 19.4	< 0.25	1.9 ± 0.3
	BAA	0.1 ± 0.001	16.3	0.4 ± 0.07	12.5 ± 2.8	0.2 ± 0.01	3.4 ± 0.6
	DCAA	1.3 ± 0.07	10.5	4.6 ± 0.7	1230 ± 11	1.4 ± 0.02	45.8 ± 9.4
	TCAA	0.6 ± 0.07	19.6	1.3 ± 0.2	275 ± 9	0.4 ± 0.04	99 ± 11
	BCAA	0.4 ± 0.001	53.0	1.5 ± 0.2	171 ± 55	0.6 ± 0.04	14.4 ± 2.9
	BDCAA	0.3 ± 0.07	14.0	0.9 ± 0.07	28.1 ± 13.2	0.4 ± 0.01	11.2 ± 1.6
	DBAA	0.2 ± 0.07	87.0	0.4 ± 0.001	40.0 ± 21.2	0.3 ± 0.02	14.3 ± 3.0
	DBCAA	ND	9.0	ND	ND	0.2 ± 0.003	4.8 ± 1.3

[a] Values reported as avg ± SD of duplicate measurements; ND: not detected; "<": Detected below MRL; NM: not measured; Br/Cl-HAAs for May 2018 sample reported as concentration determined by single measurement.

Table S5. Concentrations (nM) of 2018 indoor pool DBPs.[a]

		Indoor May 2018		Indoor July 2018		Indoor Nov. 2018	
Class	**Compound**	Tap	Pool	Tap	Pool	Tap	Pool
HNMs	DCNM	ND	ND	ND	2.9 ± 0.05	ND	ND
	BCNM	< 0.6	< 0.6	ND	0.4 ± 0.001	ND	1.0 ± 0.03
	DBNM	< 0.5	1.3 ± 0.07	ND	ND	< 0.5	1.3 ± 0.06
	TCNM	7.3 ± 0.6	2.3 ± 0.6	6.6 ± 1.1	23.7 ± 1.5	3.5 ± 0.3	1.9 ± 0.6
	BDCNM	3.2 ± 0.1	1.3 ± 0.03	2.3 ± 0.2	2.4 ± 0.08	3.7 ± 0.1	2.8 ± 0.1
	DBCNM	4.1 ± 0.01	4.2 ± 0.2	2.5 ± 0.2	2.5 ± 0.05	4.7 ± 0.1	4.9 ± 0.3
	TBNM	3.4 ± 0.1	20.3 ± 0.7	2.2 ± 0.1	1.9 ± 0.1	5.2 ± 0.1	8.4 ± 1.2
HALs	CAL	NM	NM	ND	135.6 ± 0.7	ND	3.8 ± 0.6
	BAL	NM	NM	ND	14.0 ± 0.4	ND	3.4 ± 0.06
	IAL	NM	NM	ND	ND	ND	ND
	DCAL	NM	NM	2.3 ± 0.1	12.3 ± 0.4	0.9 ± 0.003	1.3 ± 0.06
	BCAL	NM	NM	< 0.6	2.0 ± 0.3	1.2 ± 0.01	2.8 ± 0.2
	DBAL	NM	NM	ND	6.7 ± 0.3	< 0.5	3.1 ± 0.2
	TCAL	29.2 ± 1.3	87.6 ± 8.4	21.5 ± 4.1	2981 ± 27	13.8 ± 1.0	164 ± 12
	BDCAL	6.1 ± 1.0	125.0 ± 9.9	8.1 ± 1.7	124.4 ± 3.8	4.7 ± 0.1	95.5 ± 3.9
	DBCAL	1.3 ± 0.1	60.0 ± 8.4	0.8 ± 0.2	1.8 ± 0.1	1.0 ± 0.004	48.9 ± 3.3
	TBAL	< 0.4	28.2 ± 3.2	0.6 ± 0.01	0.6 ± 0.002	< 0.4	2.3 ± 0.1
HANs	CAN	< 1.3	4.1 ± 0.3	8.9 ± 0.3	49.7 ± 0.3	8.1 ± 0.9	10.7 ± 0.5
	BAN	< 0.8	9.7 ± 0.05	2.8 ± 0.001	8.6 ± 0.1	ND	ND
	IAN	ND	ND	ND	ND	ND	ND
	DCAN	26.8 ± 1.3	17.2 ± 0.3	10.4 ± 1.5	154 ± 1	9.1 ± 0.9	52.8 ± 2.4
	BCAN	9.4 ± 0.7	37.8 ± 3.3	4.8 ± 0.5	20.2 ± 0.01	3.3 ± 0.1	37.7 ± 2.5
	DBAN	1.5 ± 0.1	86.9 ± 11.0	1.0 ± 0.07	2.2 ± 0.04	1.0 ± 0.06	39.2 ± 2.4
	TCAN	< 0.7	< 0.7	ND	4.1 ± 0.04	ND	1.5 ± 0.001
	BDCAN	NM	NM	NM	NM	ND	1.0 ± 0.06
	DBCAN	NM	NM	NM	NM	ND	2.6 ± 0.1
	TBAN	ND	3.8 ± 0.08	ND	ND	< 0.4	3.8 ± 0.4
HKs	CP	86.2 ± 1.1	111.3 ± 13.8	23.6 ± 2.2	48.0 ± 1.7	49.5 ± 11.8	4.6 ± 0.3
	11DCP	17.9 ± 0.7	ND	27.3 ± 3.5	ND	ND	ND
	13DCP	ND	ND	2.5 ± 0.4	10.5 ± 0.8	ND	ND
	1B11DCP	4.8 ± 0.4	4.5 ± 0.09	1.4 ± 0.02	1.7 ± 0.02	0.8 ± 0.01	3.3 ± 0.2
	11DBP	0.9 ± 0.02	3.8 ± 0.1	1.8 ± 0.2	ND	1.3 ± 0.05	1.8 ± 0.09
	111TCP	13.0 ± 0.8	5.3 ± 0.05	6.8 ± 1.0	49.0 ± 0.8	7.7 ± 0.2	23.3 ± 1.3
	113TCP	0.8 ± 0.03	ND	1.5 ± 0.2	ND	ND	ND
	1133TeCP	1.3 ± 0.08	ND	1.6 ± 0.1	3.2 ± 0.2	1.3 ± 0.001	ND
	1133TeBP	ND	1.9 ± 0.2	ND	ND	ND	ND
I-THMs	DCIM	ND	ND	2.6 ± 0.1	ND	ND	ND
	BCIM	0.5 ± 0.05	ND	< 0.4	< 0.4	0.5 ± 0.02	0.5 ± 0.004
	DBIM	< 0.3	ND	0.6 ± 0.002	0.6 ± 0.004	0.3 ± 0.001	0.3 ± 0.001
	CDIM	ND	ND	ND	ND	ND	ND
	BDIM	< 0.3	ND	ND	ND	ND	ND
	TIM	ND	ND	0.1 ± 0.001	0.2 ± 0.004	ND	ND
HAMs	CAM	ND	ND	ND	ND	ND	ND
	BAM	ND	ND	ND	ND	ND	ND
	IAM	ND	ND	ND	ND	ND	ND
	DCAM	24.8 ± 0.5	11.9 ± 0.01	ND	ND	ND	ND

	BCAM	12.9 ± 0.3	21.1 ± 0.1	7.2 ± 2.2	28.3 ± 0.5	2.9 ± 0.8	52.3 ± 4.2
	DBAM	2.3 ± 0.2	28.4 ± 4.6	ND	4.0 ± 0.05	< 0.5	62.9 ± 2.1
	CIAM	ND	ND	ND	ND	ND	ND
	BIAM	ND	ND	ND	ND	ND	ND
	DIAM	ND	ND	ND	ND	ND	ND
	TCAM	3.7 ± 0.05	17.9 ± 0.03	3.2 ± 0.8	301 ± 6	ND	76.5 ± 1.6
	BDCAM	0.7 ± 0.01	14.4 ± 1.1	ND	50.2 ± 1.7	< 0.5	ND
	DBCAM	< 0.4	19.4 ± 2.9	ND	13.8 ± 0.3	ND	3.0 ± 0.2
	TBAM	ND	17.8 ± 5.0	ND	8.1 ± 0.1	ND	1.5 ± 0.3
I-HAAs	IAA	0.2 ± 0.001	ND	0.2 ± 0.04	0.3 ± 0.1	ND	ND
	CIAA	0.4 ± 0.03	ND	0.5 ± 0.03	0.5 ± 0.03	ND	ND
	BIAA	ND	ND	ND	0.4 ± 0.001	ND	ND
	DIAA	ND	ND	ND	ND	ND	ND
THMs	TCM	NM	NM	25.3 ± 4.9	195 ± 23	50.5 ± 0.3	874 ± 22
	BDCM	NM	NM	13.0 ± 2.6	131.8 ± 0.6	21.2 ± 1.4	308 ± 16
	DBCM	NM	NM	3.2 ± 0.6	18.6 ± 0.1	4.3 ± 0.2	206 ± 12
	TBM	NM	NM	< 16.2	< 16.2	< 16.2	120 ± 7
Br/Cl-HAAs	CAA	5.3 ± 0.001	176	14.3 ± 2.2	966 ± 206	< 2.6	20.1 ± 3.0
	BAA	0.7 ± 0.001	117	2.5 ± 0.5	90.0 ± 20.3	1.4 ± 0.06	24.5 ± 4.1
	DCAA	9.7 ± 0.5	81.4	35.7 ± 5.5	9555 ± 88	10.8 ± 0.1	355 ± 73
	TCAA	3.4 ± 0.4	120	7.7 ± 1.3	1680 ± 56	2.4 ± 0.2	603 ± 67
	BCAA	2.3 ± 0.001	306	8.4 ± 1.2	988 ± 316	3.5 ± 0.2	82.8 ± 16.7
	BDCAA	1.2 ± 0.3	67.4	4.1 ± 0.3	135 ± 64	1.7 ± 0.05	53.6 ± 7.8
	DBAA	0.7 ± 0.3	399	1.8 ± 0.001	184 ± 97	1.3 ± 0.07	65.6 ± 13.6
	DBCAA	ND	35.7	ND	ND	0.8 ± 0.01	19.0 ± 5.0

[a] Values reported as avg ± SD of duplicate measurements; ND: not detected; "<": Detected below MRL; NM: not measured; Br/Cl-HAAs for May 2018 sample reported as concentration determined by single measurement.

Table S6. Concentration (µg/L) of simulated swimming pool DBPs.[a]

Class	Compound	Tap Control A	BFA Cl2 CSI	BFA Cl2	Tap Control B	BFA CSI	BFA
HNMs	DCNM	ND	ND	ND	ND	ND	ND
	BCNM	ND	ND	ND	ND	ND	ND
	DBNM	ND	ND	ND	ND	ND	ND
	TCNM	0.5 ± 0.04	0.4 ± 0.01	0.6 ± 0.01	0.5 ± 0.02	0.4 ± 0.01	0.5 ± 0.03
	BDCNM	0.2 ± 0.01	0.2 ± 0.002	0.2 ± 0.003	0.2 ± 0.001	0.2 ± 0.002	0.2 ± 0.002
	DBCNM	0.3 ± 0.01	ND	ND	0.2 ± 0.01	0.2 ± 0.001	0.2 ± 0.002
	TBNM	ND	ND	ND	ND	ND	ND
HALs	CAL	ND	0.8 ± 0.001	1.8 ± 0.001	ND	ND	ND
	BAL	ND	ND	ND	ND	ND	ND
	IAL	ND	ND	ND	ND	ND	ND
	DCAL	0.1 ± 0.001	0.1 ± 0.001	0.2 ± 0.001	ND	ND	ND
	BCAL	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
	DBAL	ND	ND	ND	ND	ND	ND
	TCAL	4.1 ± 0.6	11.5 ± 0.8	15.9 ± 0.2	2.6 ± 0.1	2.7 ± 0.05	2.9 ± 0.1
	BDCAL	1.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.05	1.0 ± 0.03	1.1 ± 0.02	1.2 ± 0.05

	DBCAL	0.3 ± 0.01	0.3 ± 0.004	0.3 ± 0.001	0.2 ± 0.001	0.2 ± 0.003	0.2 ± 0.004
	TBAL	ND	ND	ND	ND	ND	ND
HANs	CAN	ND	ND	ND	ND	ND	ND
	BAN	ND	ND	ND	ND	ND	ND
	IAN	ND	ND	ND	ND	ND	ND
	DCAN	1.0 ± 0.04	4.0 ± 0.1	7.4 ± 0.1	0.8 ± 0.03	0.8 ± 0.03	0.9 ± 0.04
	BCAN	ND	0.7 ± 0.04	0.9 ± 0.02	0.3 ± 0.01	0.3 ± 0.01	0.4 ± 0.02
	DBAN	< 0.1	< 0.1	< 0.1	ND	ND	ND
	TCAN	< 0.1	0.2 ± 0.01	0.3 ± 0.1	ND	ND	ND
	BDCAN	ND	0.1 ± 0.001	0.1 ± 0.01	ND	ND	ND
	DBCAN	ND	< 0.1	< 0.1	ND	ND	ND
	TBAN	ND	ND	ND	ND	ND	ND
HKs	CP	8.8 ± 0.4	0.7 ± 0.01	21.8 ± 1.1	5.8 ± 0.7	7.0 ± 1.7	7.3 ± 0.4
	11DCP	ND	ND	ND	ND	ND	ND
	13DCP	0.7 ± 0.07	ND	2.6 ± 0.08	ND	ND	ND
	1B11DCP	ND	ND	ND	ND	ND	ND
	11DBP	ND	ND	ND	ND	ND	ND
	111TCP	0.6 ± 0.1	3.5 ± 0.07	3.9 ± 0.1	0.3 ± 0.002	0.3 ± 0.001	0.4 ± 0.01
	113TCP	0.1 ± 0.002	0.2 ± 0.01	0.3 ± 0.01	ND	ND	ND
	1133TeCP	0.5 ± 0.05	0.5 ± 0.03	0.9 ± 0.05	0.6 ± 0.03	0.6 ± 0.08	0.6 ± 0.03
	1133TeBP	ND	ND	ND	ND	ND	ND
I-THMs	DCIM	ND	ND	ND	ND	ND	ND
	BCIM	ND	ND	ND	ND	ND	ND
	DBIM	ND	ND	ND	ND	ND	ND
	CDIM	ND	ND	ND	ND	ND	ND
	BDIM	ND	ND	ND	ND	ND	ND
	TIM	ND	ND	ND	ND	ND	ND
HAMs	CAM	ND	ND	ND	ND	ND	ND
	BAM	ND	ND	ND	ND	ND	ND
	IAM	ND	ND	ND	ND	ND	ND
	DCAM	2.5 ± 0.3	0.7 ± 0.1	1.0 ± 0.02	1.9 ± 0.3	2.1 ± 0.06	2.3 ± 0.1
	BCAM	0.6 ± 0.01	ND	ND	0.4 ± 0.04	0.5 ± 0.02	0.5 ± 0.02
	DBAM	ND	ND	ND	ND	ND	ND
	CIAM	ND	ND	ND	ND	ND	ND
	BIAM	ND	ND	ND	ND	ND	ND
	DIAM	ND	ND	ND	ND	ND	ND
	TCAM	0.2 ± 0.02	0.5 ± 0.001	1.1 ± 0.05	0.2 ± 0.05	0.2 ± 0.02	0.3 ± 0.01
	BDCAM	ND	0.2 ± 0.01	0.2 ± 0.002	0.2 ± 0.003	0.2 ± 0.001	0.2 ± 0.001
	DBCAM	ND	ND	ND	ND	ND	ND
	TBAM	ND	ND	ND	ND	ND	ND
THMs	TCM	3.0 ± 0.4	6.2 ± 3.5	7.7 ± 0.9	4.2 ± 0.02	3.7 ± 0.02	4.4 ± 0.012
	BDCM	0.6 ± 0.07	0.7 ± 0.02	0.7 ± 0.04	2.1 ± 0.04	1.6 ± 0.04	1.9 ± 0.02
	DBCM	0.1 ± 0.002	0.1 ± 0.001	0.1 ± 0.002	0.5 ± 0.01	0.4 ± 0.01	0.5 ± 0.02
	TBM	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Br/Cl-HAAs	CAA	1.8 ± 0.01	3.3 ± 0.4	4.7 ± 1.4	1.0 ± 0.1	1.1 ± 0.07	1.1 ± 0.1
	BAA	ND	ND	ND	ND	ND	ND
	DCAA	17.6 ± 0.5	33.1 ± 3.6	38.1 ± 5.9	9.0 ± 1.1	9.4 ± 0.3	10.0 ± 0.2
	TCAA	5.7 ± 0.2	11.4 ± 1.1	18.1 ± 0.9	3.7 ± 0.3	4.0 ± 0.2	4.2 ± 0.001
	BCAA	2.4 ± 0.1	4.0 ± 0.4	4.3 ± 0.7	2.0 ± 0.3	2.0 ± 0.06	2.1 ± 0.03

	BDCAA	1.8 ± 0.06	2.2 ± 0.2	2.9 ± 0.4	1.6 ± 0.1	1.6 ± 0.03	1.8 ± 0.06
	DBAA	0.3 ± 0.01	0.4 ± 0.03	0.4 ± 0.06	0.3 ± 0.04	0.3 ± 0.01	0.4 ± 0.01
	DBCAA	0.4 ± 0.01	0.4 ± 0.03	0.4 ± 0.07	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02

[a] Values reported as avg ± SD of duplicate measurements; ND: not detected; "<": Detected below MRL.

Table S7. Concentration (nM) of simulated swimming pool DBPs.[a]

Class	Compound	Tap Control A	BFA Cl2 CSI	BFA Cl2	Tap Control B	BFA CSI	BFA
HNMs	DCNM	ND	ND	ND	ND	ND	ND
	BCNM	ND	ND	ND	ND	ND	ND
	DBNM	ND	ND	ND	ND	ND	ND
	TCNM	3.3 ± 0.2	2.3 ± 0.05	3.8 ± 0.04	3.3 ± 0.1	2.3 ± 0.07	3.0 ± 0.2
	BDCNM	0.9 ± 0.03	0.7 ± 0.01	0.8 ± 0.01	0.8 ± 0.005	0.8 ± 0.01	0.8 ± 0.008
	DBCNM	1.1 ± 0.05	ND	ND	0.7 ± 0.04	0.7 ± 0.002	0.7 ± 0.009
	TBNM	ND	ND	ND	ND	ND	ND
HALs	CAL	ND	10.1 ± 0.2	23.2 ± 0.3	ND	ND	ND
	BAL	ND	ND	ND	ND	ND	ND
	IAL	ND	ND	ND	ND	ND	ND
	DCAL	1.0 ± 0.02	1.1 ± 0.06	2.2 ± 0.06	ND	ND	ND
	BCAL	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
	DBAL	ND	ND	ND	ND	ND	ND
	TCAL	28.1 ± 3.7	78.0 ± 5.2	108 ± 2	17.4 ± 0.7	18.0 ± 0.3	19.9 ± 0.8
	BDCAL	5.2 ± 0.3	9.3 ± 0.6	9.8 ± 0.2	5.3 ± 0.1	5.6 ± 0.1	6.4 ± 0.3
	DBCAL	1.1 ± 0.02	1.3 ± 0.02	1.5 ± 0.003	0.8 ± 0.006	0.8 ± 0.01	0.9 ± 0.02
	TBAL	ND	ND	ND	ND	ND	ND
HANs	CAN	ND	ND	ND	ND	ND	ND
	BAN	ND	ND	ND	ND	ND	ND
	IAN	ND	ND	ND	ND	ND	ND
	DCAN	9.3 ± 0.4	36.0 ± 1.2	67.4 ± 1.2	7.2 ± 0.3	7.1 ± 0.3	8.5 ± 0.3
	BCAN	ND	4.8 ± 0.2	5.5 ± 0.1	2.0 ± 0.09	2.0 ± 0.05	2.3 ± 0.1
	DBAN	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	TCAN	0.5 ± 0.02	1.4 ± 0.03	2.3 ± 0.7	ND	ND	ND
	BDCAN	ND	0.6 ± 0.008	0.7 ± 0.05	ND	ND	ND
	DBCAN	ND	< 0.4	< 0.4	ND	ND	ND
	TBAN	ND	ND	ND	ND	ND	ND
HKs	CP	95.2 ± 3.8	8.0 ± 0.1	236 ± 12	62.3 ± 7.3	75.7 ± 17.9	78.5 ± 4.8
	11DCP	ND	ND	ND	ND	ND	ND
	13DCP	5.4 ± 0.6	ND	20.4 ± 0.6	ND	ND	ND
	1B11DCP	ND	ND	ND	ND	ND	ND
	11DBP	ND	ND	ND	ND	ND	ND
	111TCP	3.5 ± 0.4	21.6 ± 0.5	24.2 ± 0.9	2.1 ± 0.01	2.1 ± 0.008	2.4 ± 0.09
	113TCP	0.9 ± 0.01	1.0 ± 0.08	1.8 ± 0.04	ND	ND	ND
	1133TeCP	2.3 ± 0.3	2.5 ± 0.1	4.6 ± 0.3	3.0 ± 0.1	3.0 ± 0.4	3.0 ± 0.2
	1133TeBP	ND	ND	ND	ND	ND	ND
I-THMs	DCIM	ND	ND	ND	ND	ND	ND
	BCIM	ND	ND	ND	ND	ND	ND
	DBIM	ND	ND	ND	ND	ND	ND

	CDIM	ND	ND	ND	ND	ND	ND
	BDIM	ND	ND	ND	ND	ND	ND
	TIM	ND	ND	ND	ND	ND	ND
HAMs	CAM	ND	ND	ND	ND	ND	ND
	BAM	ND	ND	ND	ND	ND	ND
	IAM	ND	ND	ND	ND	ND	ND
	DCAM	19.2 ± 2.0	5.8 ± 1.0	7.7 ± 0.1	15.1 ± 2.4	16.3 ± 0.4	17.6 ± 0.5
	BCAM	3.4 ± 0.06	ND	ND	2.6 ± 0.2	2.6 ± 0.1	2.8 ± 0.09
	DBAM	ND	ND	ND	ND	ND	ND
	CIAM	ND	ND	ND	ND	ND	ND
	BIAM	ND	ND	ND	ND	ND	ND
	DIAM	ND	ND	ND	ND	ND	ND
	TCAM	1.3 ± 0.1	3.2 ± 0.001	6.7 ± 0.3	1.4 ± 0.3	1.4 ± 0.1	1.6 ± 0.05
	BDCAM	ND	0.8 ± 0.03	1.0 ± 0.008	0.8 ± 0.02	0.8 ± 0.001	0.8 ± 0.005
	DBCAM	ND	ND	ND	ND	ND	ND
	TBAM	ND	ND	ND	ND	ND	ND
THMs	TCM	25.0 ± 3.5	51.9 ± 28.9	64.5 ± 7.7	35.3 ± 0.2	31.3 ± 0.1	37.1 ± 0.2
	BDCM	3.7 ± 0.4	4.2 ± 0.1	4.2 ± 0.2	12.7 ± 0.2	9.8 ± 0.3	11.8 ± 0.1
	DBCM	0.5 ± 0.01	0.5 ± 0.006	0.5 ± 0.008	2.5 ± 0.07	1.8 ± 0.05	2.2 ± 0.09
	TBM	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
Br/Cl-HAAs	CAA	18.7 ± 0.1	34.9 ± 3.9	50.0 ± 14.3	10.1 ± 1.1	11.3 ± 0.8	12.2 ± 1.1
	BAA	ND	ND	ND	ND	ND	ND
	DCAA	137 ± 4	257 ± 28	296 ± 46	69.5 ± 8.7	73.2 ± 2.2	77.7 ± 1.6
	TCAA	35.0 ± 1.1	69.8 ± 6.7	111 ± 5	22.7 ± 2.0	24.2 ± 1.3	25.6 ± 0.005
	BCAA	14.1 ± 0.5	23.1 ± 2.3	24.5 ± 4.0	11.4 ± 1.5	11.4 ± 0.3	12.3 ± 0.2
	BDCAA	8.7 ± 0.3	10.5 ± 1.1	14.0 ± 1.9	7.8 ± 0.6	7.9 ± 0.1	8.7 ± 0.3
	DBAA	1.4 ± 0.03	1.9 ± 0.1	2.0 ± 0.3	1.6 ± 0.2	1.6 ± 0.04	1.6 ± 0.02
	DBCAA	1.6 ± 0.04	1.7 ± 0.1	1.8 ± 0.3	1.9 ± 0.08	1.8 ± 0.1	2.0 ± 0.06

[a] Values reported as avg ± SD of duplicate measurements; ND: not detected; "<": Detected below MRL.

Table S8. CHO LC_{50} and NAC thiol reactivity EC_{50} values for water samples.

Sample	LC_{50}[a]	EC_{50}[b]
Outdoor Pool March 2017	247.6 ± 20.2	NR
Outdoor Pool June 2017	80.5 ± 4.6	1499.0 ± 0.7
Outdoor Pool Oct. 2017	116.8 ± 11.8	NR
Outdoor Pool May 2018	73.2 ± 1.9	1136.0 ± 0.9
Outdoor Pool Oct. 2018	172.6 ± 5.5	2593.7 ± 0.4
Indoor Pool July 2017	17.2 ± 1.2	151.8 ± 6.6
Indoor Pool Oct. 2017	37.8 ± 0.9	388.3 ± 2.6
Indoor Pool May 2018	19.5 ± 0.2	159.5 ± 6.3
Indoor Pool July 2018	13.7 ± 0.2	130.1 ± 7.7
Indoor Pool Nov. 2018	52.2 ± 4.3	495.1 ± 2.0
Indoor Tap May 2018	47.6 ± 0.7	862.4 ± 1.2
Outdoor Tap May 2018	81.7 ± 0.9	1166.7 ± 0.9
Indoor Tap July 2018	139.5 ± 2.9	1377.5 ± 0.7
Outdoor Tap Oct. 2018	57.1 ± 0.9	729.6 ± 1.4
Indoor Tap Nov. 2018	102.9 ± 7.3	1047.8 ± 1.0
Tap Control 1	--	868.9 ± 1.2
BFA CSI 1.4 ppm Cl2	--	887.3 ± 1.1
BFA 5.1 ppm Cl2	--	485.8 ± 2.1
BFA CSI	--	903.5 ± 1.1
BFA	--	927.1 ± 1.1
Tap Control 2[*]	--	903.4

[a] The mean LC_{50} ± 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] The mean EC_{50} value is the concentration of the water sample, determined from bootstrap multiple regression analysis of the data, that induced a reduction in the NAC thiol concentration by 50% as compared to the concurrent negative controls. [*] No replicate measurements.

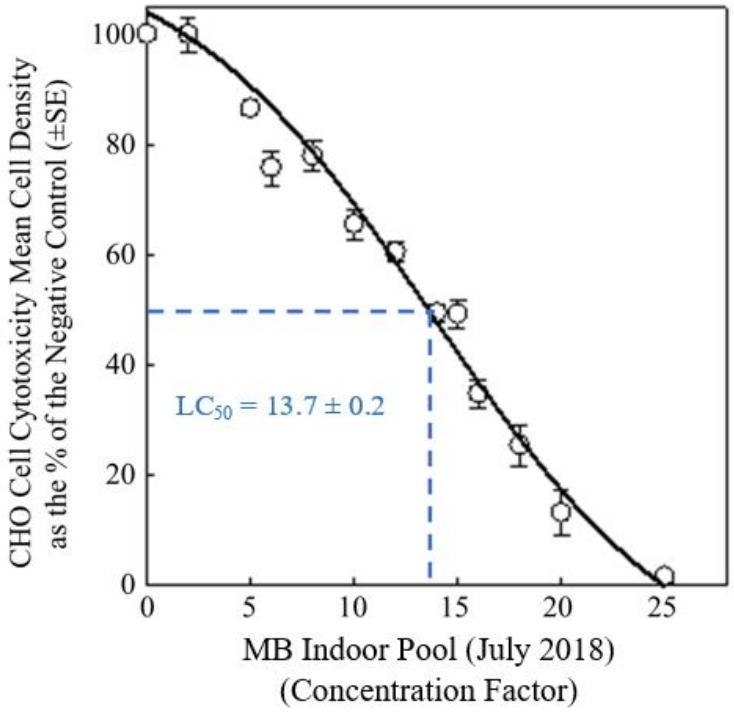

Figure S.3. Example CHO concentration-response curve.

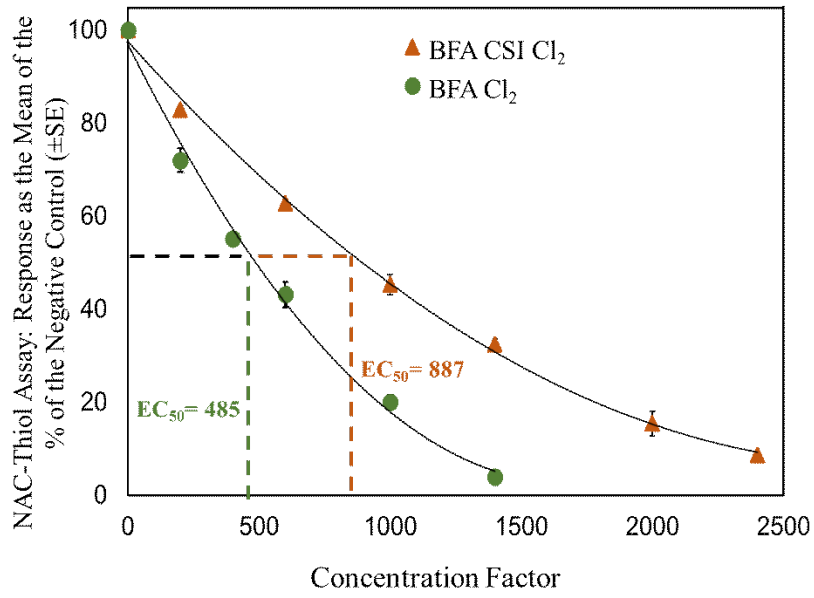

Figure S.4. Example NAC-thiol concentration-response curve.

Table S9. Total organic halogen (TOX) data for water samples.[a]

Sample	TOCl (µg/L as Cl^-)	TOBr (µg/L as Br-)	TOI (µg/L as I^-)	ΣTOX (as Cl-)
Outdoor Pool June 2017	4599 ± 11	18.4 ± 0.1	2.9 ± 0.2	4608
Outdoor Pool Oct. 2017	5044 ± 18	78.8 ± 1.9	1.5 ± 0.1	5079
Outdoor Pool May 2018	3453 ± 22	35.4 ± 0.2	3.0 ± 0.7	3470
Outdoor Pool Oct. 2018	2539 ± 151	64.2 ± 2.1	2.5 ± 0.0	2568
Indoor Pool July 2017	4218 ± 38	149.4 ± 6.0	1.9 ± 0.1	4285
Indoor Pool Oct. 2017	3006 ± 67	255.6 ± 0.1	2.1 ± 0.0	3120
Indoor Pool May 2018	463.4 ± 3.4	986.7 ± 12.3	2.4 ± 0.9	902.5
Indoor Pool July 2018	3907 ± 89	651.9 ± 38.1	4.9 ± 0.1	4198
Indoor Pool Nov. 2018	1288 ± 192	549.3 ± 62.2	4.4 ± 0.7	1534
Indoor Tap May 2018	148.6 ± 0.5	38.7 ± 0.2	3.2 ± 0.1	166.7
Outdoor Tap May 2018	148.9 ± 4.8	12.7 ± 0.1	3.8 ± 0.1	155.6
Indoor Tap July 2018	125.6 ± 4.5	27.9 ± 1.2	2.0 ± 0.6	138.6
Outdoor Tap Oct. 2018	151.1 ± 13.6	9.1 ± 0.2	3.4 ± 0.6	156.1
Indoor Tap Nov. 2018	86.6 ± 10.7	19.2 ± 1.6	0.9 ± 0.1	95.4
Tap Control 1	120.3 ± 2.4	16.2 ± 0.8	0.9 ± 0.1	127.7
BFA CSI 1.4 ppm Cl_2	231.7 ± 8.4	11.8 ± 0.1	3.0 ± 0.1	237.8
BFA 5.1 ppm Cl_2	235.7 ± 0.5	12.9 ± 0.3	0.6 ± 0.0	241.6
BFA CSI	79.5 ± 2.0	15.8 ± 0.4	1.9 ± 0.2	87.1
BFA	87.5 ± 4.0	16.1 ± 0.1	1.1 ± 0.3	95.0
Tap Control 2	77.4 ± 0.7	16.5 ± 0.1	0.8 ± 0.1	85.0

[a] Values reported as avg ± SD of duplicate measurements.