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Disinfection byproducts in chlorinated or brominated swimming pools and spas: Role of brominated DBPs and association with mutagenicity☆

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ABSTRACT

Although the health benefits of swimming are well-documented, health effects such as asthma and bladder cancer are linked to disinfection by-products (DBPs) in pool water. DBPs are formed from the reaction of disinfectants such as chlorine (Cl) or bromine (Br) with organics in the water. Our previous study (Daiber et al., Environ. Sci. Technol. 50, 6652; 2016) found correlations between the concentrations of classes of DBPs and the mutagenic potencies of waters from chlorinated or brominated swimming pools and spas. We extended this study by identifying significantly different concentrations of 21 individual DBPs in brominated or chlorinated pool and spa waters as well as identifying which DBPs and additional DBP classes were most associated with the mutagenicity of these waters. Using data from our previous study, we found that among 21 DBPs analyzed in 21 pool and spa waters, the concentration of bromoacetic acid was significantly higher in Br-waters versus Cl-waters, whereas the concentration of trichloroacetic acid was significantly higher in Cl-waters. Five Br-DBPs (tribromomethane, dibromochloroacetic acid, dibromoacetonitrile, bromoacetic acid, and tribromoacetic acid) had significantly higher concentrations in Br-spa versus Cl-spa waters. Cl-pools had significantly higher concentrations of Cl-DBPs (trichloroacetaldehyde, trichloromethane, dichloroacetic acid, and chloroacetic acid), whereas Br-pools had significantly higher concentrations of Br-DBPs (tribromomethane, dibromoacetic acid, dibromoacetonitrile, and tribromoacetic acid). The concentrations of the sum of all 4 trihalomethanes, all 11 Br-DBPs, and all 5 nitrogen-containing DBPs were each

☆ In honor of Dr. Michael J. Plewa who, with his collaborator and wife, Dr. Elizabeth D. Wagner, generated a database of the genotoxic potencies of >100 DBPs, providing a foundation for understanding the health effects of drinking water.

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significantly higher in brominated than in chlorinated pools and spas. The 8 Br-DBPs were the only DBPs whose individual concentrations were significantly correlated with the mutagenic potencies of the pool and spa waters. These results, along with those from our earlier study, highlight the importance of Br-DBPs in the mutagenicity of these recreational waters.

Introduction

Swimming is among the most popular sports activities worldwide, being 4th in the United States (US Census Bureau, 2009). The health benefits of swimming are well-documented and include reduced risk of chronic illnesses and approximately half the risk of death compared to inactive people (Chase et al., 2008), improved use of affected joints for people with arthritis (Westby, 2001), and improved bone health in post-menopausal women (Rotstein et al., 2008). Swimming and water-based exercise are also linked to improvements in mood and mental health (Berger and Owen, 1992; Hartmann and Bung, 1999).

Nonetheless, swimming in disinfected pools with elevated concentrations of disinfection by-products (DBPs) has been associated with various health effects, including increased risks for asthma (Zwiener et al., 2007). In addition, increased lung epithelium permeability, urinary mutagenicity, and lymphocyte micronuclei have been found in swimmers after just 40 min of swimming in pool water with a high concentration (28.3 μg/L) (Font-Ribera et al., 2010; Kogevinas et al., 2010) but not a low concentration (9.5 μg/L) (Font-Ribera et al., 2019) of brominated trihalomethanes (Br-THMs).

Epidemiology studies have found an elevated risk for bladder cancer among swimmers exposed to pool water with high concentrations of trihalomethanes (THMs) (Villanueva et al., 2007), especially Br-THMs, and who also possessed two genetic factors (*GSTT1* and a SNP in *GSTZ1*) (Cantor et al., 2010) but not among swimmers with similar exposure levels but missing those genetic factors (Cantor et al., 2010) or exposed to low concentrations of THMs, including low concentrations of Br-THMs (Beane Freeman et al., 2017). Collectively, these studies have associated an increased risk for bladder cancer due to dermal and inhalation exposure to Br-THMs and oral exposure to the haloacetic acids (HAAs) in disinfected waters (DeMarini, 2020; Regli et al., 2015).

DBP formation in pools and spas (hot tubs) results from the reaction of disinfectants such as chlorine or bromine with organic matter, such as natural organic matter from source water, as well as human inputs, such as sweat, urine, pharmaceuticals, and personal-care products (Daiber et al., 2016; Manasfi et al., 2017, 2019). More than 700 DBPs have been identified (Richardson, 2011), nearly all of the >100 that have been studied for genotoxicity are genotoxic, and 20 of 22 studied for rodent carcinogenicity are carcinogenic (DeMarini, 2020; Plewa and Richardson, 2017; Plewa et al., 2011; Richardson et al., 2007; Wagner and Plewa, 2017). In general, Br-DBPs are more cytotoxic, genotoxic, and mutagenic than Cl-DBPs (Kargalioglu et al., 2002; Kundu et al., 2004a; Wagner and Plewa, 2017).

Nearly all organic extracts of chlorinated drinking water tested (1000s of samples) are mutagenic (DeMarini, 2020), and >24 studies have found that pool or spa waters are also genotoxic or mutagenic (Allen et al., 2021; Daiber et al., 2016; Manasfi et al., 2019; Plewa and Richardson, 2017; Plewa et al., 2011; Richardson et al., 2010; Richardson and Ternes, 2018). More than 100 DBPs have been identified in pool and spa waters, some unique to these waters and absent from drinking water (Allen et al., 2021; Carter and Joll, 2017; Carter et al., 2015; 2019a, 2019b; Daiber et al., 2016; Font-Ribera et al., 2019, Manasfi et al., 2019; Richardson et al., 2010; Richardson and Ternes, 2018; Yang et al., 2018; Zwiener et al., 2007), particularly nitrogen-containing DBPs (N-DBPs), which are formed from urea from urine and sweat (Carter et al., 2019a, 2019b; Mustapha et al., 2021; Richardson et al., 2010).

In a previous study (Daiber et al., 2016), we found associations between the concentrations of various chemical classes of DBPs and the mutagenic potencies of pool and spa waters. Brominated pools and spas were almost twice as mutagenic as chlorinated ones, and the mutagenic potencies of both chlorinated and brominated waters were highly correlated with the concentrations of various classes of Br-DBPs. Increased human inputs, i.e., increased use of pools and spas, increased both the concentrations of DBPs and the mutagenicity of the waters.

These observations from Daiber et al. (2016) were based on the combined concentration of the 21 DBPs evaluated and 8 chemical classes of DBPs. However, we did not determine the differences between the concentrations of any of the individual 21 DBPs among the various types of pool and spa waters, nor did we determine associations between the concentrations of these individual DBPs and the mutagenicity of the waters. Here we have performed these additional analyses of the data from Daiber et al. (2016). We identified which individual DBPs and some additional classes of DBPs that were at significantly different concentrations in one type of water versus another in order to determine which DBPs predominated in the Br- or Cl-pools and spas and which were most associated with the mutagenicity of these waters. We also assessed which of the 21 DBPs were at significantly different concentrations in finished versus tap waters, which identified DBPs formed uniquely in pools and spas.

1. Materials and methods

All of the data analyzed in this study were published by Daiber et al. (2016). Table 1 shows the numbers and types of the 28 water samples used in that study. However, for the present analysis, we utilized data for only the 21 waters disinfected by chlorination (hypochlorite or dichloroisocyanuric acid) or bromination (bromochlorodimethylhydantoin [BCDMH] or NaBr in combination with trichloroisocyanuric

Table 1 – Numbers of different types of water samples.[a]

Disinfectant	No.	Raw	Finished	Tap	Public pools	Spas	
						Public	Private
Not disinfected	4	3		1			
Cl	15		3	4	4	3	1
Br	6				2	3	1
Ozone	1						1
Ozone-Cl	2			1	1		
Total	28	3	3	6	7	6	3

[a]Details in Table 1 of Daiber et al. (2016). Only 21 of these 28 water samples were used for the present analysis; the 4 waters not disinfected, 1 ozonated, and 2 ozonated-chlorinated samples were not included.

Table 2 – Abbreviations of DBPs.

DBP	Abbreviation	Class
Chloroacetic acid	CAA	Haloacetic Acids (HAA9)
Dichloroacetic acid	DCAA	
Trichloroacetic acid	TCAA	
Bromoacetic acid	BAA	
Dibromoacetic acid	DBAA	
Bromochloroacetic acid	BCAA	
Bromodichloroacetic acid	BDCAA	
Dibromochloroacetic acid	DBCAA	
Tribromoacetic acid	TBAA	
Trichloromethane (chloroform)	TCM	Trihalomethanes (THM4)
Bromodichloromethane	BDCM	
Dibromochloromethane	DBCM	
Tribromomethane (bromoform)	TBM	
Dichloroacetonitrile	DCAN	Nitrogenous (N-DBPs)
Trichloroacetonitrile	TCAN[a]	
Bromochloroacetonitrile	BCAN	
Dibromoacetonitrile	DBAN	
Trichloronitromethane (chloropicrin)	TCNM[a]	
1,1,1-Trichloropropanone	TCP	Ketones/Aldehydes
1,1-Dichloropropanone	DCP	
Trichloroacetaldehyde (chloral hydrate)	TCAL	

[a] Two DBPs, TCNM and TCAN, were not detected at quantifiable levels in any of the samples.

acid). We did not include the four waters that were not disinfected (three untreated source waters, one well tap water), the one ozonated spa, nor the two ozonated-chlorinated water samples (one tap, one pool). Details of these samples, the sampling procedures, organic extractions, and mutagenicity procedures are described in Daiber et al. (2016).

Briefly, for mutagenicity, 52-L water samples were acidified to pH 1-2, the organics were extracted by XAD/ethyl acetate, and the extracts were solvent-exchanged at 10,000 × into dimethyl sulfoxide (DMSO). These DMSO concentrates were assessed for mutagenicity in the *Salmonella* (Ames) plate-incorporation mutagenicity assay using the base-substitution strain TA100 without metabolic activation (S9); mutagenic potencies were expressed as revertants/L-equivalent (Daiber et al., 2016).

The concentrations of 21 DBPs (listed in Table 2) were quantified by a contract laboratory using EPA Methods 551.1 and 552.2. Briefly, water samples were collected headspace-free in amber glass bottles, and residual disinfectant was quenched with either ammonium chloride or sodium sulfite. Quenched samples were shipped overnight on ice to the contract laboratory where sample preparation, extraction, and analysis were performed according to the respective EPA Methods (Daiber et al. 2016).

Using the data from Daiber et al. (2016), all analyses were done using SAS/STAT v9.4 software. SAS Proc means were used to calculate univariate statistics for water samples and groups of water samples in DBP measurements. SAS Proc Mixed was used to compute linear model analyses to estimate differences in the concentrations of individual DBPs and the sum of the concentration of various classes of DBPs between water sample groups. The model used was essentially a one-way analysis of variance (ANOVA) of the groups in question, with the addition of sample ID as a random effect to account for the two samples of water taken from each water source. SAS Proc Corr was used to calculate Pearson correlation coefficients (r) across the 21 water samples.

In order to investigate which DBPs tended to occur together, sample mean concentrations for individual DBPs were used in calculating correlations between pairs of DBP mea-

Table 3 – Significant comparisons of individual DBP concentration between sample types.[a]

Comparison	DBP	Difference	Std Error	*p*-value
Br-Spas vs. Br-Pools	TCAA	-0.2015	0.0815	0.0483
	BAA	0.3839	0.1498	0.0428
Br-Spas vs. Cl-Spas	BAA	0.3225	0.1077	0.0172
	DBCAA	0.0235	0.0099	0.0452
	TBAA	0.3068	0.1044	0.0188
	TBM	0.7685	0.0777	< 0.0001
	DBAN	0.5743	0.1844	0.0144
Br-Pools vs. Cl-Pools	CAA	-0.1345	0.0394	0.0143
	DCAA	-1.2516	0.2931	0.0053
	DBAA	0.5139	0.0356	< 0.0001
	TBAA	0.2417	0.0448	0.0017
	TCM	-0.1642	0.0328	0.0025
	TBM	0.5799	0.0883	0.0006
	DBAN	0.1868	0.0058	< 0.0001
	TCAL	-0.7238	0.2142	0.0149
All Spas vs. All Pools	BAA	0.2138	0.0924	0.0365
Br-Pools+Spas vs.	TCAA	-0.7555	0.2883	0.0202
Cl-Pools+Spas	BAA	0.2186	0.0915	0.0315
	DBAA	2.2473	1.0473	0.0499
	DBCAA	0.0167	0.0068	0.0282
	TBAA	0.2851	0.0598	0.0003
	TCM	-0.2151	0.0948	0.0397
	BDCM	-0.0359	0.0155	0.0359
	TBM	0.7060	0.0628	< 0.0001
	DCAN	-0.0646	0.0291	0.0436
	DBAN	0.4458	0.1220	0.0026
	TCAL	-0.8161	0.2806	0.0114
All Pools vs. All Taps	TCAA	0.7096	0.2441	0.0156

[a] All comparison results provided in Appendix A.

surements. Similarly, sample total DBP group concentrations were correlated with the individual DBP values to show which individual DBPs were most related to the group concentrations. Finally, we determined the correlation between the mutagenic potencies of the waters with the concentrations of the 21 individual and classes of DBPs.

2. Results

Two N-DBPs, TCNM and TCAN, were non-detect or below the limits of quantification in every sample analyzed. Therefore, they were excluded from some analyses reported herein, and result tables will show only 19 individual DBPs.

2.1. *Individual DBPs between sample types*

For each individually quantified DBP, we performed 9 ANOVA comparisons between sample types: (1) chlorinated pools vs. spas, (2) brominated pools vs. spas, (3) brominated vs. chlorinated spas, (4) brominated vs. chlorinated pools, (5) all spas vs. all pools, (6) all brominated recreational waters (pools + spas) vs. all chlorinated recreational waters, (7) all tap waters vs. all finished waters, (8) all spas vs. all tap waters, and (9) all pools vs. all tap waters. The results of all comparisons are shown in Appendix A, and all significant results are shown in Table 3.

2.1.1. *Cl-spas vs. Cl-pools*

In addition to TCNM and TCAN, TBAA was not detected in any of the chlorinated spa or pool waters, and DBAN was detected in only one Cl-spa and not in any Cl-pools (Daiber et al., 2016). There were no significant differences between the concentrations of any of the 21 DBPs between Cl-pools and Cl-spas (Appendix A Table A1).

2.1.2. *Br-spas vs. Br-pools*

In addition to TCNM and TCAN, two of the 21 DBPs (TCP and DCAN) were not detected in any of the brominated spa or pool waters, and 5 DBPs (DCP, BDCM, TCAL, TCM, and CAA) were not detected in the Br-pool waters and were detected in only some Br-spa waters. Only two DBPs (BAA and TCAA) were at significantly different concentrations in the Br-spa waters than in the Br-pool waters (Fig. 1; Appendix A Table A2). BAA concentration was approximately 10-fold greater in the Br-spas than in the Br-pools, whereas TCAA was approximately double the concentration in the pools than in the spas. The remaining DBPs were not at significantly different concentrations between the Br-spa and Br-pool waters.

2.1.3. *Br-spas vs. Cl-spas*

In addition to TCNM and TCAN, DCAN was not detected in Br-spa waters, and TBAA was not detected in Cl-spa waters. Not surprisingly, all 5 DBPs whose concentrations differed significantly between brominated and chlorinated spas were Br-DBPs (TBM, DBCAA, DBAN, BAA, and TBAA); the concentra-

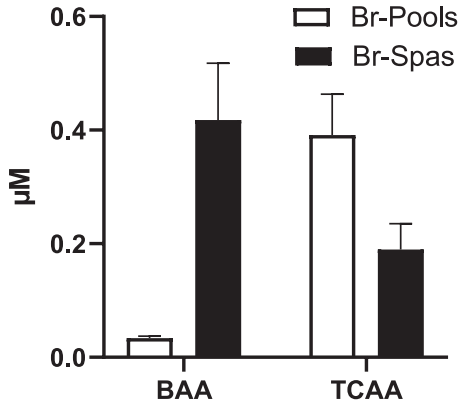

Fig. 1 – Concentrations of DBPs that were significantly different between Br-spas and Br-pools; data from Appendix A Table A2.

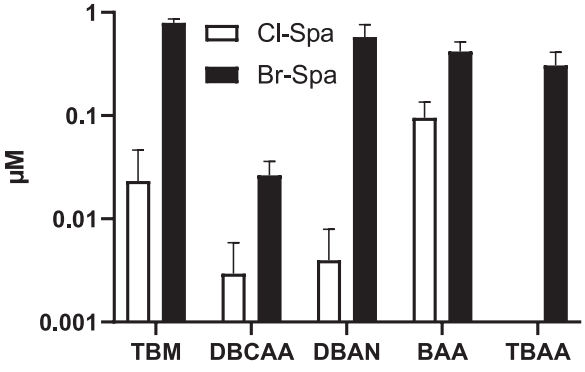

Fig. 2 – Concentrations of DBPs that were significantly different between Cl-spas and Br-spas; data from Appendix A Table A3 (note logarithmic scale).

tions of these DBPs were orders-of-magnitude higher in Br-spas than in Cl-spas (Fig. 2; Appendix A Table A3).

2.1.4. *Br-pools vs. Cl-pools*

In addition to TCNM and TCAN, 7 DBPs (TCP, DCP, BDCM, TCAL, TCM, DCAN, and CAA) were not detected in the Br-pool waters, and 1 DBP (TBAA) was not detected in the Cl-pool waters. Eight DBPs (TBM, TCAL, TCM, DBAA, DBAN, DCAA, CAA, and TBAA) were at concentrations that were significantly different between the two types of pool waters (Fig. 3; Appendix A Table A4). Four were Br-DBPs that were at least an order-of-magnitude higher concentration in Br-pools, and the other 4 were Cl-DBPs that were at least an order-of-magnitude higher concentration in Cl-pools. As with the spas, these results show that for pool waters, bromination promotes Br-DBP formation, whereas chlorination promotes Cl-DBP formation.

2.1.5. *Spas vs. Pools*

In comparing all spas versus all pools, only one DBP (BAA, $p = 0.03645$) was present at a higher concentration in spa (0.26 µM) than in pool (0.04 µM) waters, and the remaining DBPs were present at concentrations that were not signifi-

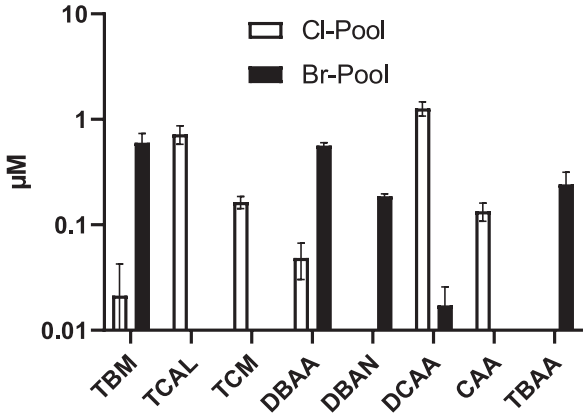

Fig. 3 – Concentrations of DBPs that were significantly different between Cl-pools and Br-pools; data from Appendix A Table A4 (note logarithmic scale).

cantly different between the spa and pool waters (Appendix A Table A5). DBP formation is accelerated at higher temperatures (Richardson et al., 2007); thus, it is likely that most DBPs in this study were formed at higher concentrations in the spa waters, but due to volatility and high temperatures, they lost significant concentrations due to evaporation to the air. HAAs are more hydrophilic and less volatile (Loos and Barceló, 2001) than other DBPs reported by Daiber et al. (2016), so it is not surprising that the one DBP at a significantly higher concentration in spas is an HAA. Additionally, the average concentrations of many other HAAs appeared higher (at least 2-fold) in spas vs. pools, but variability across the different pool/spa sites, disinfection types, and usage levels prevented significance in these differences.

2.1.6. *Br-recreational waters vs. Cl-recreational waters*

In this comparison, the disinfection method applied to recreational waters was evaluated, comparing Br-pools + Br-spas to Cl-pools + Cl-spas. In addition to TCNM and TCAN, 2 DBPs (TCP and DCAN) were not detected in Br-pools + spas, and 1 DBP (TBAA) was not detected in Cl-pools + spas. Eleven DBPs (BDCM, TBM, TCAL, DBCAA, TCM, DBAA, DBAN, DCAN, BAA, TBAA, and TCAA) were at significantly different concentrations between Br-pools + spas and Cl-pools + spas (Fig. 4; Appendix A Table A6). Among those 11 DBPs, 6 were Br-DBPs whose concentrations were significantly higher in the brominated waters; 4 Cl-DBPs, and BDCM, which is ubiquitous in chlorinated waters (Richardson et al., 2007), were at significantly higher concentrations in the chlorinated waters (Fig. 4; Appendix ATable A6).

2.1.7. *Tap vs. finished*

In addition to TCNM and TCAN, 6 of the 21 DBPs (BCAN, TBM, DBCAA, DBAN, CAA, and TBAA) were not detected in any of the tap or finished waters, DCP was not detected in tap waters, and DBAA was not detected in finished waters. The 6 DBPs not detected in finished or tap were typically present in the pool and spa waters, indicating that they were likely formed by the chemistry of those recreational waters, possibly involving human inputs as indicated by Daiber et al. (2016). The remaining

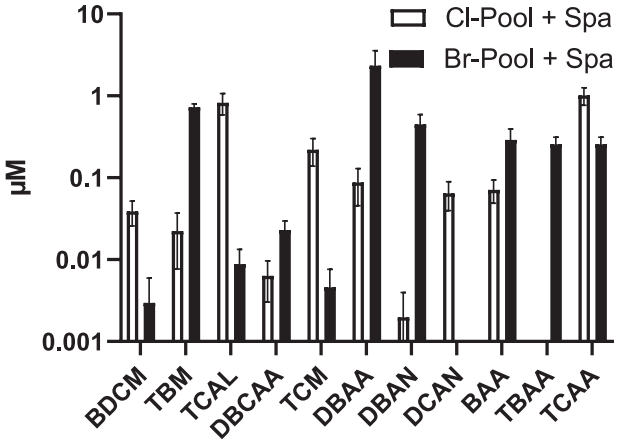

Fig. 4 – Concentrations of DBPs that were significantly different between brominated pools + spas and chlorinated pools + spas; data from Appendix A, Table A6 (note logarithmic scale).

13 DBPs were present at concentrations that were not significantly different between tap and finished waters (Appendix A Table A7).

2.1.8. *Recreational vs. tap waters*

Seven DBPs (DCP, BCAN, TBM, DBCAA, DBAN, CAA, and TBAA) were present in both pool and spa waters that were not detected in the tap waters (Appendix A Tables A8 and A9), suggesting that they were likely formed by the reaction of pool/spa disinfectants with organic compounds introduced by humans (e.g., personal care products, sweat, urine) (Daiber et al., 2016). The concentrations of the remaining DBPs were not significantly different between the tap waters and pool or spa waters except for TCAA, which was higher in pool (0.77 µM) than in tap (0.06 µM) waters.

2.2. *DBP classes between sample types*

All comparisons for DBP groups across sample types are shown in Appendix B, and significant differences are shown in Table 4.

2.2.1. *All DBPs*

Among the comparisons in Table 4 and Appendix B (Table B1) for the total concentration of all 21 DBPs, the concentrations were significantly higher in the chlorinated pools than in the brominated pools (Fig. 5A). The concentration of all 21 DBPs was 6-fold greater in the pool waters compared to the tap waters (Fig. 5B) and ~13-fold greater in the spa waters compared to the tap waters (Fig. 5C). These results illustrate the extensive DBP formation in pools and spas relative to the water used to fill them, likely due to the reaction of human inputs with the disinfectants (Daiber et al., 2016).

2.2.2. *THMs*

Among the comparisons in Appendix B (Table B2) for the total concentration of the 4 THMs, the concentrations of the 4 THMs were significantly higher (>2-fold) in brominated pools (Fig. 6A), spas (Fig. 6B), and pools + spas (Fig. 6C) than in chlorinated. These results indicate that bromination promotes THM formation more than does chlorination in these recreational waters, and 3 of 4 THMs were Br-THMs. This may also be due to the likelihood of greater formation of TCM in a Cl-pool followed by the more rapid volatilization of TCM due its elevated Henry's Law constant.

2.2.3. *HAAs*

Although many individual HAAs were at significantly different concentrations between sample types, the total concentration of all 9 HAAs as a group was not significantly different between Cl/Br spas or Cl/Br spas + pools, and there was a significance of $0.05 < p < 0.06$ for Cl/Br pools (Table 4; Appendix

Table 4 – Significant comparisons ($p < 0.06$) of DBP groups between sample types.[a,b]

Comparison	DBP class	Difference	Std Error	*p*-value
Br-Spas vs. Cl-Spas	THM4	0.4618	0.1836	0.0361
	N-DBPs	0.4860	0.1977	0.0394
Br-Pools vs. Cl-Pools	21DBPs	-1.6079	0.4712	0.0143
	THM4	0.3503	0.1080	0.0176
	HAA9	-1.3558	0.5855	0.0598*
	Br-DBPs	1.2911	0.1491	0.0001
	N-DBPs	0.1298	0.0362	0.0116
Br-Pool+Spas vs	THM4	0.4433	0.1165	0.0019
Cl-Pool+Spas	Br-DBPs	4.0064	1.5833	0.0240
	N-DBPs	0.3742	0.1295	0.0119
All Spas vs. All Taps	21DBPs	6.1276	2.8371	0.0517*
	THM4	0.3795	0.1783	0.0547*
	HAA9	4.9995	2.3923	0.0586*
All Pools vs. All Taps	21DBPs	2.6589	0.4992	0.0003
	HAA9	1.9846	0.4749	0.0019
	N-DBPs	0.0859	0.0398	0.0564*

[a] All comparison results provided in Appendix B.
[b] $0.05 < p < 0.06$ marked with asterisk (*)

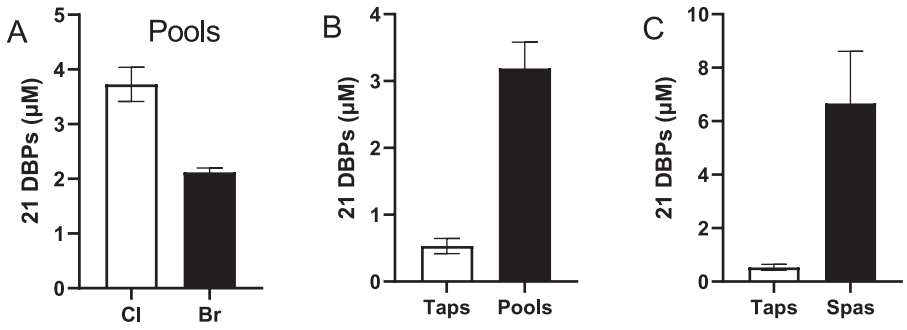

Fig. 5 – Total concentrations of 21 DBPs that were significantly different between chlorinated and brominated pools (A), tap and pool waters (B), and tap and spa waters (C); data from Appendix B, Table B1.

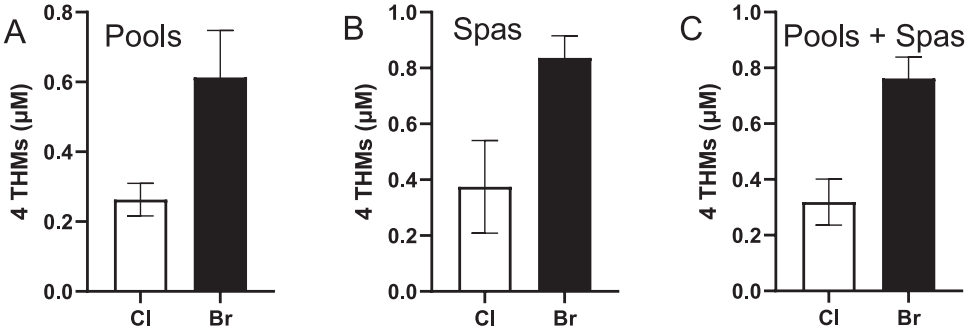

Fig. 6 – Total concentrations of 4 THMs that were significantly different between chlorinated and brominated (A) pools, (B) spas, and (C) pools + spas; data from Appendix B, Table B2.

BTable B3). This illustrates the value of extending the analysis to individual DBPs because evaluating differences between disinfection type only by DBP class can be misleading, especially with the variability that comes with combining multiple sites.

2.2.4. *Br-DBPs*

Among the comparisons in Appendix B (Table B4) for the total concentration of the 11 Br-DBPs, the concentrations were significantly higher (4-7-fold) in brominated pools (Fig. 7A) and pools + spas relative to chlorinated waters (Fig. 7B). These results confirm that bromination promotes Br-DBP formation.

2.2.5. *N-DBPs*

Among the comparisons in Appendix B (Table B5) for the total concentration of the 5 N-DBPs, the concentrations were significantly higher (3-5-fold) in brominated pools (Fig. 8A), spas (Fig. 8B), and pools + spas (Fig. 8C) relative to chlorinated waters, indicating that bromination promotes N-DBP formation. Similar to the THMs, 2 of the 3 N-DBPs detected contained bromine, which formed to a higher extent with a bromine-based oxidant, and bromination with HOBr occurs at higher reaction rates than chlorination with HOCl (Westerhoff et al., 2004). It is also possible that nitrogen contributed by the organic-based disinfectants (cyanuric acids and hydantoin) used in bromine-treatment of recreational waters serve as another precursor to N-DBPs.

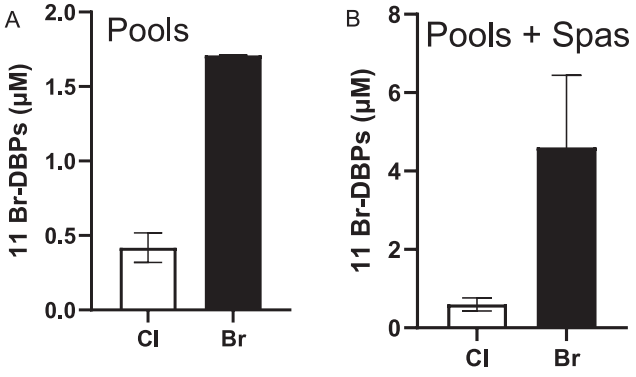

Fig. 7 – Total concentrations of 11 Br-DBPs that were significantly different between chlorinated and brominated (A) pools and (B) pools + spas; data from Appendix B, Table B4.

2.3. *DBPs and mutagenicity*

The concentrations of 8 individual DBPs correlated with the mutagenic potencies of the water samples, and all 8 were brominated DBPs (BAA, DBAA, BCAA, BDCAA, DBCAA, TBAA, TBM, and DBAN) (Table 5). There were also significant correlations between the mutagenicity of these waters and the sum of the concentrations of the 21 DBPs, the 4 THMs, the 9 HAAs, the Br-DBPs, and the N-DBPs (Table 5), indicating that all of

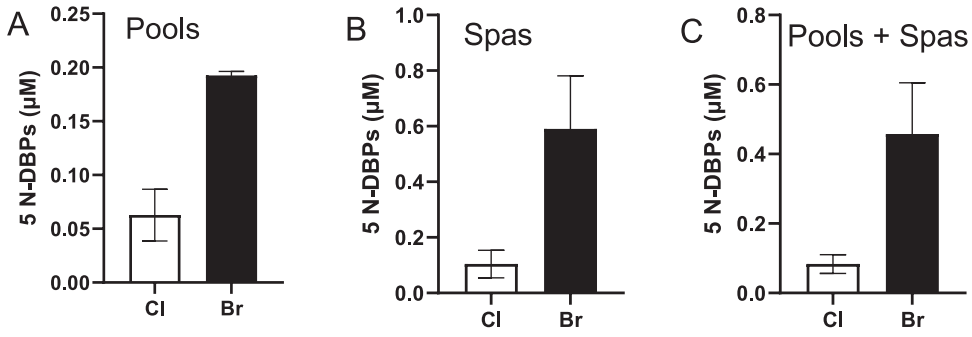

Fig. 8 – Total concentrations of 5 N-DBPs that were significantly different between chlorinated and brominated (A) pools, (B) spas, and (C) pools + spas; data from Appendix B, Table B5.

Table 5 – Correlations between concentrations of DBPs with mutagenic potencies of waters.[a,b]

DBPs	Pearson *r*	*p*-value
CAA	0.3982	0.0738
DCAA	0.3829	0.0867
TCAA	0.2623	0.2508
BAA*	0.7237	0.0002
DBAA*	0.6063	0.0036
BCAA*	0.6065	0.0036
BDCAA*	0.5898	0.0049
DBCAA*	0.7331	0.0002
TBAA*	0.6767	0.0008
TCM	0.1938	0.3999
BDCM	-0.4294	0.0521
DBCM	-0.0079	0.9728
TBM*	0.6557	0.0013
DCAN	0.2120	0.3563
BCAN	0.2324	0.3107
DBAN*	0.6625	0.0011
TCP	-0.0278	0.9047
DCP	0.2805	0.2181
TCAL	0.2528	0.2689
Sum of 21 DBPs*	0.7482	< 0.0001
Sum of 4 THMs*	0.8281	< 0.0001
Sum of 9 HAAs*	0.7339	0.0002
Sum of Br-DBPs*	0.6869	0.0006
Sum of N-DBPs*	0.7303	0.0002

[a] DBP concentration (μM) and mutagenic potency (rev/L-eq) data from Daiber et al. (2016) Supporting Information Tables S6-S9 and S19, respectively. Asterisk (*) denotes significant correlation at 95% confidence.
[b] Class sum correlations with mutagenicity from Daiber et al. (2016) Table 3.

these classes of DBPs likely played a role in the mutagenicity of the waters.

3. Discussion

3.1. *Spa vs. pool*

Five DBPs (DCP, BDCM, TCAL, TCM, and CAA) were present uniquely in Br-spas relative to Br-pools, indicating that spa conditions, such as higher temperatures and less water exchange, might account for the formation of these DBPs. Five Br-DBPs (TBM, DBCAA, DBAN, BAA, and TBAA) were at higher concentrations in Br-spas than in Cl-spas. Thus, bromination likely promoted their formation; it is also known that brominated DBPs form at higher rates than do chlorinated (Westerhoff et al., 2004). Seven DBPs (TCP, DCP, BDCM, TCAL, TCM, DCAN, and CAA) were not detected in Br-pools, suggesting that bromination preferentially promoted the formation of many Br-DBPs at the expense of the formation of some Cl-DBPs. This would be consistent with the finding that 4 Br-DBPs were at higher concentrations in Br-pools, and 4 Cl-DBPs were at higher concentrations in Cl-pools. Considering pools + spas, 6 Br-DBPs were at higher concentrations in brominated waters, whereas 4 Cl-DBPs were at higher concentrations in chlorinated waters.

3.2. *Tap vs. recreational waters*

Several DBPs not present in tap water were present in pools and spas, and the total concentration of 21 DBPs was 6-fold higher in pools and ~13-fold higher in spas relative to the tap water used to fill the pools and spas, indicating that the chemistry of these recreational waters promoted the formation of unique and higher concentrations of DBPs relative to DBPs in tap water. For example, 6 DBPs (BCAN, TBM, DBCAA, DBAN, CAA, and TBAA) were unique to recreational waters and absent from tap or finished water. Several of these were N-DBPs, which would be expected in pool and spa waters due to the presence of urea to provide nitrogen for their formation (Daiber et al., 2016).

3.3. *Br- vs. Cl-recreational waters*

Bromination preferentially promoted the formation of THMs, Br-DBPs, and N-DBPs relative to chlorination; however, chlorinated pools had higher total concentrations of the 21 DBPs than did brominated pools. The greater formation of N-DBPs in brominated waters may be due to the fact that to the disinfectant used in brominated pools is bromochlorodimethylhydantoin ($C_5H_6BrClN_2O_2$), which contains nitrogen, chlorine, and bromine. The proportion of total THM and Br-THMs concentrations has been shown to influence the induction of (a) genotoxic endpoints in swimmers and (b) bladder cancer among people with a specific genotype exposed to disinfected

waters primarily via dermal/inhalation. Thus, swimmers in pools with high concentrations (28.3 μg/L) of Br-THMs have higher frequencies of urinary mutagenicity, lymphocyte micronuclei, and lung epithelium permeability relative to swimmers in pools with low concentrations (9.5 μg/L) of Br-THMs, even though the total concentrations of all 4 THMs were similar (45.4-48.5 μg/L) (DeMarini, 2020).

Bladder cancer risk is higher among those with a specific genotype who are exposed dermally or via inhalation to high concentrations of Br-THMs in disinfected waters relative to low concentrations (DeMarini, 2020). Although total HAA concentration is also associated with bladder cancer risk via oral exposure to disinfected waters (Cantor et al., 2010), the data do not distinguish whether Br- vs. Cl-HAAs preferentially are the cause of this elevated risk (DeMarini, 2020; Regli et al., 2015). Bromination is used less frequently than chlorination to disinfect pools but more frequently than chlorination to disinfect spas (Daiber et al., 2016), making the high concentrations of Br-DBPs in spas (Fig. 7) potentially important in the induction of genotoxicity and bladder cancer.

As shown by Cantor et al. (2010), exposure alone to Br-THMs via the dermal/inhalation route or to HAAs via the oral route is insufficient to increase bladder cancer risk. Instead, a genotype having two genes that occur together in 24% of the population, along with sufficient exposure, is required for increased bladder cancer risk from DBPs. The *GSTT1* gene metabolizes Br-THMs to mutagens (DeMarini et al., 1997; Pegram et al., 1997), and a single-nucleotide polymorphism (SNP) in *GSTZ1* (*GSTZ1 rs1046428 CT/TT*) results in less inactivation (less detoxification) of HAAs. Thus, in the absence of this combined genotype, exposures to Br-THMs and HAAs alone do not increase the risk for bladder cancer (Cantor et al., 2010). Details of this paradigm have been reviewed (DeMarini, 2020; Regli et al., 2015).

3.4. *Br-DBPs and mutagenicity*

As we showed previously (Daiber et al., 2016), (a) increasing human inputs generally increased the DBP concentrations and mutagenic potencies of pool and spa waters, (b) spa waters had higher DBP concentrations and were more mutagenic than pool waters, and (c) Br-waters generally had lower DBPs concentrations but were more mutagenic than Cl-waters. The present study showed that the concentrations of individual Br-DBPs were the DBPs that correlated most with the mutagenic potencies of the waters. This observation extends our previous finding (Daiber et al., 2016) that the concentrations of various classes of DBPs, such as N-DBPs, Br-DBPs, and Br-HAAs correlated highly ($r > 0.90$) with the mutagenic potencies of Cl-waters, and a bit less so ($r > 0.82$) with the mutagenicity of Br-waters. Although we did not analyze for the presence of Br-N-DBPs as a group in the present study, our earlier study showed that the concentration of this class of DBPs correlated poorly ($r = 0.04$) with the mutagenicity of Cl-waters but highly ($r = 0.78$) with the mutagenicity of Br-waters (Daiber et al., 2016).

Our previous analysis also showed that the concentration of Br-THMs correlated poorly ($r = 0.29$) with the mutagenicity of Cl-waters but much more so ($r = 0.63$) with the mutagenicity of Br-waters (Daiber et al., 2016). Further indication of the important role of Br-THMs, which a mechanistic role in the risk for bladder cancer from disinfected waters (Cantor et al., 2010), was confirmed by our previous study showing that the mutagenic potencies of these waters were significantly greater in a GSTT1-containing strain of *Salmonella*, which converts Br-THMs but not iodinated THMs (DeMarini et al., 2021) to mutagens. High (Cantor et al., 2010) but not low (Bean Freeman et al., 2017) concentrations of Br-THMs in drinking water have been associated with increased risk for bladder cancer. Likewise, high (Kogevinas et al., 2010) but not low (Font-Ribera et al., 2019) concentrations of Br-THMs have been associated with increased genotoxicity in swimmers exposed to pool water even though the total concentration of THMs is similar (DeMarini, 2020).

Our data provide additional support for the observation that (a) the Br-THMs and the HAAs are clearly linked to increased risk for bladder cancer among specific genotypic populations exposed to disinfected water (Cantor et al., 2010) and (b) elevated concentrations of Br-THMs in pool water are linked to genotoxicity in swimmers (Kogevinas et al.). However, our data have not permitted us to explore the role of other potentially important classes of DBPs in pools and spas that are not yet linked to health effects. For example, the halonitromethanes (HNMs) are more cytotoxic and genotoxic in mammalian cells in vitro (Wagner and Plewa, 2017) and more cytotoxic and mutagenic in *Salmonella* (Kundu et al., 2004a,b) than their parent halomethanes. However, we did not analyze for their presence in the recreational waters studied here, and no studies have evaluated their potential role in genotoxicity or cancer risk in humans.

Although we have shown that the concentrations of N-DBPs were associated with the mutagenic potencies of pool and spa waters, and Wagner and Plewa (2017) have shown that the N-DBPs are among the most genotoxic DBPs in mammalian cells in vitro, the N-DBPs have not been evaluated for their association with genotoxicity or carcinogenicity in humans. Other highly cytotoxic or genotoxic DBPs such as haloacetonitriles and iodoacetic acids should also be examined for their possible linkage to human health effects (Allen et al., 2022). In addition, alternative disinfection methods for pools and spas should be explored further based on the recent findings by Allen et al. (2021) that copper-silver ionization with chlorine reduced the concentrations of DBPs and the cytotoxicity of pool water relative to chlorination alone.

4. Conclusions

The results reported here show that among 21 DBPs analyzed in various pool and spa waters, BAA and TCAA were at significantly higher concentrations in Br-waters, and 5 Br-DBPs (TBM, DBCAA, DBAN, BAA, and TBAA) were at significantly higher concentrations in Br-spa versus Cl-spa waters. Not surprisingly, Cl-pools had significantly higher concentrations of Cl-DBPs (TCAL, TCM, DCAA, and CAA), whereas Br-pools had significantly higher concentrations of Br-DBPs (TBM, DBAA, DBAN, and TBAA). The total concentrations of all 4 THMs, all 11 Br-DBPs, and all 5 N-DBPs were significantly higher in brominated pools and spas than in chlorinated pools and spas. The eight individual DBPs that had concentrations that were

significantly correlated with the mutagenic potencies of the pool and spa water were all Br-DBPs. These results, along with those from our earlier study (Daiber et al., 2016) identify the importance of Br-DBPs in the mutagenicity of these recreational waters.

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Appendix Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.04.049.

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