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Occurrence and Comparative Toxicity of Haloacetaldehyde Disinfection Byproducts in Drinking Water

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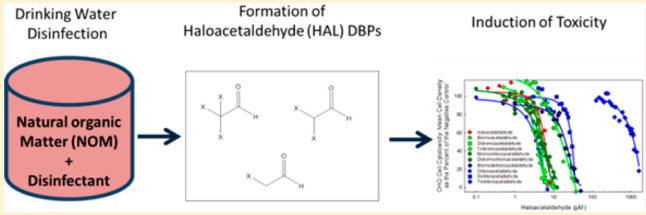
Supporting Information

ABSTRACT: The introduction of drinking water disinfection greatly reduced waterborne diseases. However, the reaction between disinfectants and natural organic matter in the source water leads to an unintended consequence, the formation of drinking water disinfection byproducts (DBPs). The haloacetaldehydes (HALs) are the third largest group by weight of identified DBPs in drinking water. The primary objective of this study was to analyze the occurrence and comparative toxicity of the emerging HAL DBPs. A new HAL DBP, iodoacetaldehyde (IAL) was identified. This study provided the first systematic, quantitative comparison of HAL toxicity in Chinese hamster ovary cells. The rank order of HAL cytotoxicity is tribromoacetaldehyde (TBAL) ≈ chloroacetaldehyde (CAL) > dibromoacetaldehyde (DBAL) ≈ bromochloroacetaldehyde (BCAL) ≈ dibromochloroacetaldehyde (DBCAL) > IAL > bromoacetaldehyde (BAL) ≈ bromodichloroacetaldehyde (BDCAL) > dichloroacetaldehyde (DCAL) > trichloroacetaldehyde (TCAL). The HALs were highly cytotoxic compared to other DBP chemical classes. The rank order of HAL genotoxicity is DBAL > CAL ≈ DBCAL > TBAL ≈ BAL > BDCAL > BCAL ≈ DCAL > IAL. TCAL was not genotoxic. Because of their toxicity and abundance, further research is needed to investigate their mode of action to protect the public health and the environment.



INTRODUCTION

The disinfection of drinking water was an outstanding contribution for the protection of the public health.[1] An unintended consequence of water disinfection is the generation of disinfection byproducts (DBPs). Trihalomethanes (THMs) were discovered as the first chemical class of DBPs in 1974.[2] Since then, research has led to the identification of emerging DBPs[3−7] and determination of their formation kinetics,[8−10] toxicity,[7,11,12] exposure, and risk assessment.[13−17] To date, more than 600 DBPs have been identified, and many are reported to be cytotoxic, genotoxic, teratogenic, or carcinogenic.[7,11,12,18−21] Epidemiologic studies have demonstrated associations between DBPs and increased risk for bladder and colon cancers.[22−26] Furthermore, evidence associating DBPs and adverse pregnancy outcomes, including spontaneous abortion, low birth weight, small-for-gestational-age, still birth, and preterm delivery has also been reported.[14,27−35]

Haloacetaldehydes (HALs) are an important class of emerging (nonregulated) DBPs.[36] HALs were the third largest DBP class by weight in a U.S. Nationwide DBP Occurrence Study, with dichloroacetaldehyde (DCAL) as the most abundant individual HAL reported (maximum concentration: 16 μg/L).[37] Individual HAL concentrations in finished water

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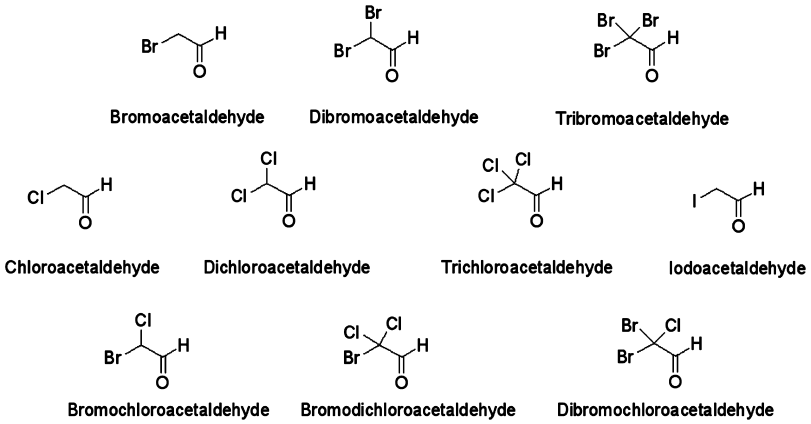

Figure 1. Chemical structures of the ten HALs analyzed in this study.

are dependent on the source water quality, including natural organic matter and bromide levels, and disinfection treatment type. The contribution of trichloroacetaldehyde (TCAL), another ubiquitous HAL, that is present in water in its hydrated form (chloral hydrate), to total HALs in water was reported to be highly variable (5−60%), thus, it is important to evaluate other HAL species in order not to underestimate the overall HAL amount present in drinking water.[38] In the U.S. EPA Information Collection Rule, TCAL was found at median and maximum concentrations of 1.7 μg/L and 46 μg/L, respectively, and concentrations observed in finished water did not significantly vary among the investigated disinfection treatments (including chlorine, chloramine, chlorine/chloramine, chlorine dioxide, and ozone).[39] In Canadian drinking water distribution systems, the highest TCAL concentration was 263 μg/L, with the highest HAL concentrations found in waters disinfected with ozone and chlorine.[38] Waters from chloraminated systems had lower levels.[38] In the U.S. Nationwide Occurrence Study, DCAL levels were maximized with chloramines and ozone, but TCAL formation was reduced with this disinfectant combination.[37,40] Brominated HALs, including bromochloroacetaldehyde (BCAL), dibromoacetaldehyde (DBAL), bromodichloroacetaldehyde (BDCAL), dibromochloroacetaldehyde (DBCAL), and tribromoacetaldehyde (TBAL), were formed after chlorination of bromide-containing waters and similarly as for trihalomethanes, bromine incorporation increased with bromide concentration in source waters.[38] Six di- and tri-HALs were measured recently in two microfiltration/reverse osmosis (RO) water recycling plants in Perth, Australia, where HALs were formed by chloramination (used to prevent membrane fouling) but were, for the most part, effectively removed by RO.[41]

The toxicity of a few specific HALs was examined in previous studies.[20] TCAL was mutagenic in *Salmonella typhimurium*[42−45] and induced chromosomal aberrations[42,46] and aneuploidy[47,48] in mammalian cells. TCAL was also reported to induce micronuclei,[49−53] mitotic aberrations,[53−56] and DNA strand-breaks[57−59] in mammalian cells. The toxicity of chloroacetaldehyde (CAL) was studied as a metabolite of the industrial chemical vinyl chloride.[60] CAL was cytotoxic in rat hepatocytes[61] and induced nephrotoxicity in human renal proximal tubule cells.[62] Further, CAL formed DNA adducts, caused mutations,[63−69] and generated mitotic chromosome malsegregation[70] and interstrand cross-links.[71] Similar to TCAL, DCAL induced mitotic aneuploidy.[72] Regarding the toxicity of brominated HALs, BAL irreversibly bound to DNA and proteins in rat liver microsomes,[73] and TBAL induced single- and double-strand DNA breaks.[59] Despite these studies, a systematic investigation of other emerging HAL DBPs has not been conducted, and there is no quantitative, comparative database on the toxicity of the complete set of chloro-bromo HALs or iodo-HALs.

In this context, the objectives of our research were to (i) develop and validate an analytical method to determine 10 chloro-bromo-iodo-HALs in water, (ii) evaluate for the first time the occurrence of iodoacetaldehyde (IAL) in source and drinking waters and to compare its concentrations to those of other target HALs, (iii) analyze the *in vitro* cytotoxicity and genotoxicity of HALs in mammalian cells, (iv) determine the cytotoxicity and genotoxicity index values of HALs and develop a quantitative, comparative toxicity database, and (v) conduct a mechanism-based structure−activity relationship analysis for the observed HAL-mediated cytotoxicity and genotoxicity.

■ MATERIALS AND METHODS

Chemicals and Reagents. General reagents were certified ACS reagent grade and were purchased from Sigma-Aldrich (St. Louis, MO) and Fisher Scientific (Itasca, IL). Media and fetal bovine serum (FBS) were purchased from Fisher Scientific (Itasca, IL). HAL standards were purchased from Sigma-Aldrich, CanSyn Chem. Corp. (Toronto, ON), Aldlab Chemicals (Woburn, MA), and TCI America (Waltham, MA) at the highest level of purity available (chemical properties, purity, and CAS numbers of investigated HALs are provided in Supporting Information (SI), Table S1). 4-Fluorobenzaldehyde and 1,2-dibromopropane, used as the surrogate standard (SS) and internal standard (IS), respectively, and *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA), used as the derivatizing agent, were purchased from Sigma-Aldrich. Oasis HLB cartridges (6 cc, 150 mg, 30 μm particle size) for solid-phase extraction (SPE) were purchased from Waters (Milford, MA). All solvents (acetonitrile, *n*-hexane, methyl *tert*-butyl ether (MTBE), methanol, and ethyl acetate) were of highest purity and were purchased from Fisher Scientific, EMD Millipore (Billerica, MA) or VWR International (Radnor, PA).

Preparation of HAL Solutions. The chemical structures of the investigated HALs are shown in Figure 1. For chemical analyses, individual HAL stock solutions were prepared at a concentration of 100 μg/L by dissolving the appropriate amount of HAL standard in MTBE. Stock solutions were stored in the dark at −20 °C for up to two months. Working

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Table 1. Linearity, Sensitivity, Repeatability, and Recovery Obtained for the Analysis of the Target Analytes in Waters

target analytes		linearity		sensitivity		precision[a]		
		range (μg/L)	R^2	LOD[c] (μg/L)	LOQ[d] (μg/L)	RSD[e] (%) low level	RSD (%) high level	recovery[b] (%)
mono-HALs and di-HALs	CAL	0.25−10	0.99	0.05	0.25	4.6	3.2	N.A.[f]
	BAL	0.50−10	0.99	0.25	0.50	7.1	5.4	N.A.[f]
	IAL	0.50−8	0.99	0.25	0.50	8.3	14.5	N.A.[f]
	DCAL	0.25−10	0.99	0.10	0.25	6.1	4.9	N.A.[f]
	DBAL	0.25−10	0.99	0.05	0.25	3.8	5.4	N.A.[f]
	BCAL	0.10−10	0.99	0.05	0.10	5.6	6.9	N.A.[f]
tri-HALs	TCAL	0.25−25	0.99	0.10	0.25	11.7	4.7	30/23
	TBAL	1−25	0.99	0.50	1	4.4	3.8	82/97
	BDCAL	1−25	0.99	0.50	1	6.4	3.1	68/43
	DBCAL	1−25	0.99	0.50	1	5.8	2.8	72/58

[a]Mono- and di-HALS: $n = 5$ analyses at 0.5 μg/L (low level) and $n = 5$ analyses at 5 μg/L (high level). RSD of the SS peak area was 22% at 0.5 μg/L and 14% at 5 μg/L .Tri-HALs: $n = 6$ analyses at 2 μg/L (low level) and $n = 6$ analyses at 10 μg/L (high level). RSD of the IS peak area was <8% at both spiking levels. [b]Replicate analysis ($n = 6$) of spiked waters at levels of 2 μg/L and 10 μg/L. [c]LOD = limit of detection. [d]LOQ = limit of quantification. [e]RSD = relative standard deviation. [f]N.A. = not available.

solutions were prepared in acetonitrile prior to method validation experiments and sample analyses. Calibration curves were made at concentrations ranging between 0.01 ng/L and 10,000 ng/L by spiking different levels of the calibration standards into purified water and carrying through the complete extraction/derivatization process.

Prior to toxicological analyses, individual HAL stock solutions were prepared in dimethyl sulfoxide (DMSO) from HAL commercial standard solutions, and immediately stored in sterile glass vials under dark conditions at −20 °C.

Chemical Analyses. The methods developed for HAL analysis are based partly on methodologies used to evaluate DBPs in the U.S. Nationwide Occurrence Study.[37,40] Mono- and di-HALs were derivatized with PFBHA, and subsequently liquid−liquid extracted (LLE) with *n*-hexanes, whereas tri-HALs were preconcentrated by means of SPE with Oasis HLB cartridges. Analyte detection was performed by gas chromatography-electron ionization-mass spectrometry (GC/EI-MS) with selected ion monitoring. Further details are provided in the SI (Table S2). These methodologies were evaluated in terms of linearity, sensitivity, repeatability, and recovery. Method performance is discussed later in the Results section. Total organic carbon (TOC), UV absorbance, bromide, and iodide content were also measured in source waters (Table S3, SI).

Water Samples. Source and treated drinking water samples were collected at different water treatment plants (WTPs) in the U.S. from 6 cities in 3 states from geographically diverse regions. Five of the seven investigated plants used chloramines, and two used chlorine, for disinfection. Although treated waters were available for all investigated WTPs the source waters could be collected for five of them only (Table S4, SI). Samples were collected in headspace-free 2-L polytetrafluoroethylene (PTFE) bottles. Ascorbic acid (12.5 mg/L) was used to quench the residual disinfectant, and sulfuric acid was used to lower the sample pH to 3.5 for analyte preservation.[74] Stability of target analytes during sampling, transport, and storage conditions until sample extraction (within 48 h) was evaluated and is discussed in the SI (Table S5). Source waters were passed through 0.45 μm Durapore hydrophilic filters (EMD Millipore) prior to extraction.

Chinese Hamster Ovary Cells. Chinese hamster ovary (CHO) cell line AS52, clone 11-4-8 was used for the toxicity studies.[75−77] The CHO cells were maintained in Ham's F12 medium containing 5% 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.

CHO Cell Chronic Cytotoxicity Assay. This 96-well microplate assay measures the reduction in cell density as a function of the HAL concentration over a period of 72 h (∼3 cell cycles).[11,20] The detailed procedure has been published elsewhere[11,20] and is presented in the SI. In general, for each HAL concentration, 8 replicates were analyzed, and the experiments were repeated 2−4 times. A concentration−response curve was generated for each HAL, and a regression analysis was conducted for each curve. The LC_{50} values were calculated, where the LC_{50} represents the HAL concentration that induced a 50% reduction in cell density as compared to the concurrent negative controls.

Single Cell Gel Electrophoresis Assay. The single cell gel electrophoresis (SCGE) or "comet assay" quantitatively measures genomic DNA damage in individual nuclei.[78−80] The detailed procedure of the microplate methodology used in this study is presented in the SI.[80] The SCGE metric for genomic DNA damage induced by the HALs was the %Tail DNA value, which is the amount of DNA that migrated from the nucleus into the microgel.[81] For each HAL concentration range where the cell viability was >70%, a concentration−response curve was generated. A regression analysis was used to fit the curve, and the concentration inducing a 50% Tail DNA value was calculated.

Statistical Analyses. For the cytotoxicity assay, a one-way analysis of variance (ANOVA) test was conducted to determine if the HAL induced a statistically significant level of cell death. If a significant F value ($P \leq 0.05$) was obtained, a Holm-Sidak multiple comparison versus the control group analysis was performed to identify the lowest cytotoxic concentration. The power of the test statistic ($1-\beta$) was maintained as ≥0.8 at $\alpha = 0.05$.

For the SCGE assay, the %Tail DNA values are not normally distributed, which limits the use of parametric statistics.[82] The mean %Tail DNA value for each microgel was calculated, and these values were averaged among all of the microgels for each HAL concentration. A one-way ANOVA test was conducted on these averaged % Tail DNA values.[83] 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 ≥0.8 at $\alpha = 0.05$.

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A bootstrap statistical approach was used to generate a series of multiple LC_{50} values or %Tail DNA values for each HAL.[84,85] For each LC_{50} value, a cytotoxicity index (CTI) value was calculated as $(LC_{50})^{-1}(10^3)$. For each %Tail DNA value, a genotoxicity index (GTI) value was calculated as $(50\%\text{Tail DNA})^{-1}(10^3)$. These values (1/M) were then analyzed using an ANOVA test to determine significant differences among the HALs. A Pearson's Product Moment correlation test was conducted to test for correlations among cytotoxicity and genotoxicity data and HAL chemical characteristics.

■ RESULTS AND DISCUSSION

Analysis of HALs in Water. Due to the wide range in volatility and polarity, two separate analytical methods were required to analyze the group of 10 HALs. Most HALs are separated by conventional GC/MS, but the mono- and di-HALs are highly volatile and a few coelute with the extraction solvent. In addition, the mono-HALs are highly polar and are not extracted efficiently by SPE. Therefore, derivatization with PFBHA was advantageous to increase their molecular weight and decrease their volatility so that they elute later in the GC/MS chromatogram, away from the extraction solvent, and so that they are extracted effectively from water. PFBHA derivatization has been used similarly for highly volatile and highly polar nonhalogenated aldehydes.[86−88] Although it would be ideal to measure all 10 HALs using this PFBHA-GC/MS method, the tri-HALs were not derivatized effectively by PFBHA. As a result, they were measured without derivatization using SPE and GC/MS. Table 1 summarizes the performance of the analytical methodologies. Total ion chromatograms obtained for the analysis of investigated HALs are shown in the SI (Figures S1 and S2). Analyte quantification was performed with the internal standard method. Mono- and di-HAL response was normalized to the SS area count, whereas the IS peak area was used to normalize tri-HAL signal. Calibration curves were constructed with the extraction and analysis of fortified Milli-Q water solutions. In general, seven data points were fitted by linear least-squares regression. Coefficients of determination (r^2) above 0.99 were obtained for all analytes (Table 1, Figure S3 in the SI). Linearity was observed from the analyte limit of quantification (LOQ) up to 8 μg/L for IAL, 10 μg/L for the remaining mono-HALs and di-HALs, or 25 μg/L for tri-HALs.

Method sensitivity was estimated from the concentrations observed in analyzed samples and calibration standard solutions. Limits of detection (LODs), i.e., the analyte concentration that provides a signal-to-noise (S/N) ratio of 3, ranged from 0.05 μg/L (CAL, BCAL, and DBAL) to 0.5 μg/L (TBAL, DBCAL, and BDCAL). LOQs, i.e., the analyte concentration that provides a S/N ratio of 10, varied between 0.1 μg/L (BCAL) and 1 μg/L (TBAL, DBCAL, and BDCAL).

Method precision was evaluated with the replicate analysis of fortified Milli-Q water solutions at two different levels (Table 1). Relative standard deviation (RSD) values of the normalized analyte peak areas were <10%, except for IAL (5 μg/L) and TCAL (2 μg/L), which were <15%.

Recovery could not be calculated for mono- and di-HALs because analytical standards of derivatized compounds are not commercially available. Moreover, the smallest ones e.g., CAL and DCAL, are not amenable to GC-MS without derivatization. Haloacetaldehyde conversion during PFBHA derivatization was consistently observed to be 75%.[40] In this respect, any artifact affecting oxime yield during the derivatization step would also affect the SS, and, therefore, it can be corrected. In the case of tri-HALs, SPE recoveries were calculated via IS quantification of the analyte peak areas obtained in the recovery studies using MTBE-based calibration curves. Three different SPE sorbents were tested for tri-HAL extraction, i.e., Oasis HLB, Supelclean LC-18, and StrataC18-E. Best recoveries were achieved with Oasis HLB (SI). The analyte most efficiently extracted with Oasis HLB cartridges was TBAL (>82%), followed by DBCAL (58−72%) and BDCAL (43−68%). In contrast, TCAL had low recovery (23−30%). TCAL is the most polar and soluble compound of the investigated tri-HALs (Table S1, SI), and thus, lower sorption onto the cartridge would be expected. SPE recoveries for IAL and BCAL were also evaluated because these compounds provided good MS signals without derivatization. However, recoveries below 10% were obtained for these polar compounds, and thus, they are determined more reliably with the derivatization approach. Despite this, IAL was kept in the analytical GC/MS analysis of tri-HALs for confirmation purposes.

Occurrence of HALs in Source and Finished Waters. Source waters were similar with regard to TOC concentration (6.4−8.3 mg/L) and specific UV absorbance (SUVA) (2.1−3.1 L/m·mg) (Table S3, SI). The main variation was in bromide content (20−540 μg/L). Iodide levels were below the method LOD (5 μg/L) in all samples.

HALs were barely detected in source waters (Table S6). Only trace levels of DBAL, IAL, TBAL, and DBCAL were observed in the source water of Plant 1, and they could originate from the recirculation of disinfected water within the WTP. All target HALs were detected in all finished waters (Table S7, SI), with the exception of TBAL and IAL, which were detected only in 43% and 57% of the samples, respectively. HAL concentrations in treated waters are summarized in Figure 2.

IAL was only detected in chloraminated waters. This is consistent with previous research in which other iodinated DBPs (iodo-THMs, iodo-acids, and an iodo-amide) maximized with chloramination[12,89−93] because unlike chlorine, which

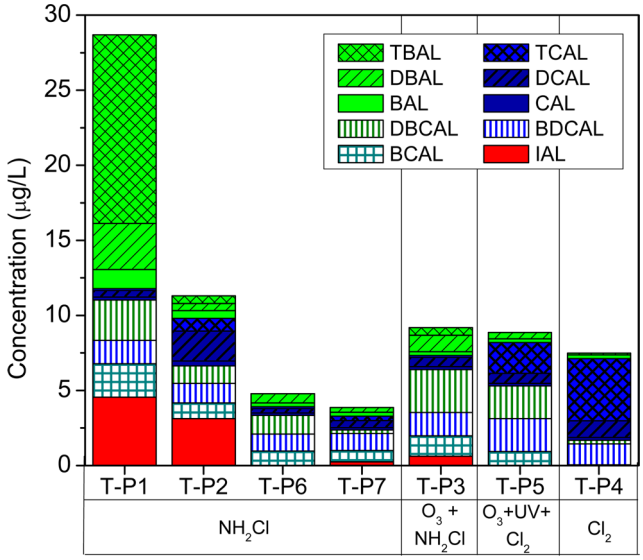

Figure 2. Levels of HALs in the investigated finished waters. Samples are classified according to the disinfection process applied at the water treatment plant. <LOQ levels were included as half of the analyte LOQ value.

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Table 2. Summary of the CHO Cell Chronic Cytotoxicity of the Target HALs

		concn range (μM)	lowest cytotoxic concn (μM)[a]	LC_{50} (μM)[b]	R^{2c}	ANOVA test statistic[d]
mono-HALs and di-HALs	CAL	0.1−7	0.5	3.51	0.99	$F_{11,\ 176} = 241$; $P \leq 0.001$
	BAL	1−42	8	17.28	0.98	$F_{23,\ 248} = 76.1$; $P \leq 0.001$
	IAL	0.2−10	5	6.00	0.96	$F_{12,\ 163} = 79.6$; $P \leq 0.001$
	DCAL	1−15	8	29.25	0.91	$F_{20,\ 335} = 37.5$; $P \leq 0.001$
	DBAL	1−6	2	4.7	0.99	$F_{10,\ 177} = 165$; $P \leq 0.001$
	BCAL	0.1−10	2.5	5.34	0.97	$F_{14,\ 169} = 31.5$; $P \leq 0.001$
tri-HALs	TCAL	125−1600	375	1163	0.94	$F_{24,\ 333} = 34.0$; $P \leq 0.001$
	TBAL	0.5−10	2	3.58	0.99	$F_{15,\ 316} = 256$; $P \leq 0.001$
	BDCAL	1−50	10	20.35	0.89	$F_{14,\ 209} = 23.4$; $P \leq 0.001$
	DBCAL	0.5−10	4	5.15	0.95	$F_{16,\ 167} = 36.1$; $P \leq 0.001$

[a]Lowest cytotoxic concentration was the lowest concentration of the HAL in the concentration−response curve that induced a statistically significant reduction in cell density as compared to the concurrent negative controls. [b]The LC_{50} value is the concentration of the HAL, determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative controls. [c]R^2 is the coefficient of determination for the regression analysis upon which the LC_{50} value was calculated. [d]The degrees of freedom for the between-groups and residual associated with the calculated F-test result and the resulting probability value.

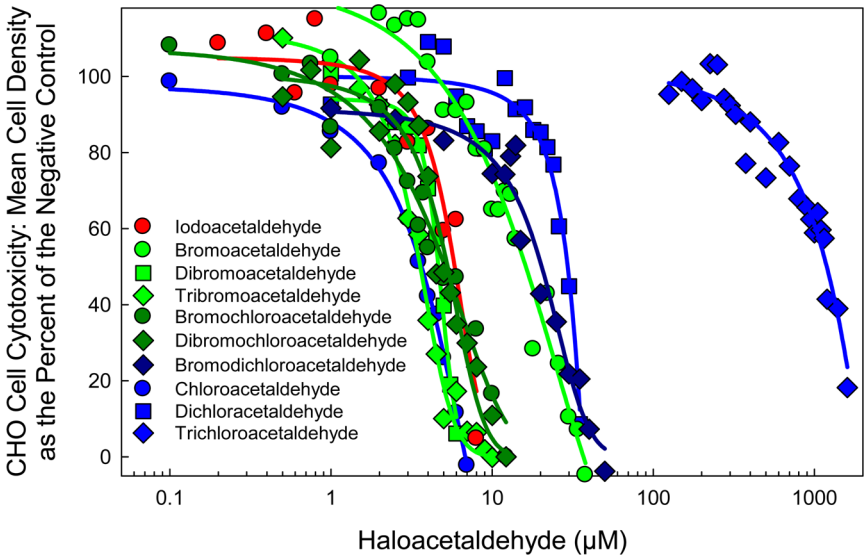

Figure 3. Comparison of the CHO cell chronic cytotoxicity concentration−response curves of the target HALs.

oxidizes iodide rapidly to iodate, monochloramine preferentially forms iodo-DBPs.[90,91] Lower IAL concentrations were observed in the plant where preozonation was also applied. However, more research is needed to understand the effect of preozonation on IAL levels because it could also be related to a low iodide concentration and the type of NOM present in the source water.[94] Previous research has shown that the application of ozone before chlorination can significantly increase HAL formation, likely due to initial formation of aldehydes by ozone and subsequent halogenation.[38,95]

IAL was detected (0.62−4.5 μg/L) in chloraminated drinking water even with iodide below the detection limit (5 μg/L) in the source waters. In this respect, other iodide sources, e.g., X-ray contrast media present in the water, could also contribute to IAL formation.[96] IAL together with TBAL were the HALs detected at highest levels in treated waters. Their maximum levels (4.5 μg/L for IAL and 12.6 μg/L for TBAL) were observed in finished water from Plant 1, which originated from a source water with the highest content of bromide (540 μg/L). Moreover, these two DBPs are the main contributors (16% and 44% in the case of IAL and TBAL, respectively) to the total load of HALs in Plant 1 (29 μg/L), which presents the highest HAL load of all the drinking water samples. Chromatograms for Plant 1 are shown in the SI (Figures S4, S5). DBCAL ($\leq$2.85 μg/L) and/or BDCAL ($\leq$2.20 μg/L) were the predominant brominated acetaldehydes in treated waters originating from source waters with low bromide ($\leq$120 μg/L). This is in agreement with data published previously on HAL occurrence in treated waters.[38] These results highlight the importance of monitoring bromine- and iodine-containing HALs in drinking water.

High levels of chlorinated HALs, particularly TCAL (4 μg/L), were found in finished waters from Plant 4, which is the only investigated plant applying chlorine exclusively for water disinfection. However, this level is below the maximum TCAL concentrations found in treated waters in Canada[38] and Spain.[97] Overall, despite being ubiquitous, CAL was found at the lowest concentrations in the treated water samples (below 1 μg/L). Concentrations of DCAL varied between 0.3 and 1.99 μg/L, and its formation appeared independent of the disinfection treatment applied.

CHO Cell Chronic Cytotoxicity. CHO cell chronic cytotoxicity analyses (72 h exposures) of each HAL are summarized in Table 2. Figure 3 illustrates the concentration−response curves for the HALs. The individual concentration−

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Table 3. Summary of the CHO Cell Acute Genotoxicity of the Target HALs

		concn range (μM)	lowest %TDNA genotoxic concn (μM)[a]	50% TDNA (μM)[b]	R^{2c}	ANOVA test statistic[d]
mono-HALs and di-HALs	CAL	50−500	100	142.8	0.99	$F_{10, 59} = 62.6$; $P \leq 0.001$
	BAL	100−550	200	381.2	0.98	$F_{10, 68} = 57.2$; $P \leq 0.001$
	IAL	100−1000	900	1009	0.98	$F_{13, 103} = 22.5$; $P \leq 0.001$
	DCAL	50−2000	800	795	0.98	$F_{19, 60} = 64.0$; $P \leq 0.001$
	DBAL	50−300	50	111.3	0.98	$F_{9, 44} = 41.5$; $P \leq 0.001$
	BCAL	100−700	500	621.4	0.92	$F_{10, 51} = 22.0$; $P \leq 0.001$
tri-HALs	TCAL	50−5000	NS[e]	NS[e]	NS[e]	$F_{20, 37} = 0.556$; $P = 0.918$
	TBAL	25−500	100	340.3	0.99	$F_{11, 64} = 168$; $P \leq 0.001$
	BDCAL	60−600	300	470.4	0.91	$F_{17, 106} = 16.4$; $P \leq 0.001$
	DBCAL	60−220	100	143.7	0.99	$F_{5, 29} = 34.4$; $P \leq 0.001$

[a]The lowest genotoxic concentration was the lowest concentration of the haloacetaldehyde in the concentration−response curve that induced a statistically significant amount of genomic DNA damage as compared to the negative control. [b]The SCGE 50% Tail DNA value is the haloacetaldehyde concentration determined from a regression analyses of the data that was calculated to induce, on average, 50% of the genomic DNA of the nucleoids to migrate into the gel. [c]R^2 is the coefficient of determination for the regression analysis upon which the SCGE % Tail DNA value was calculated. [d]The degrees of freedom for the between-groups and residual associated with the calculated F-test result and the resulting probability value. [e]NS = not significantly different from the negative control.

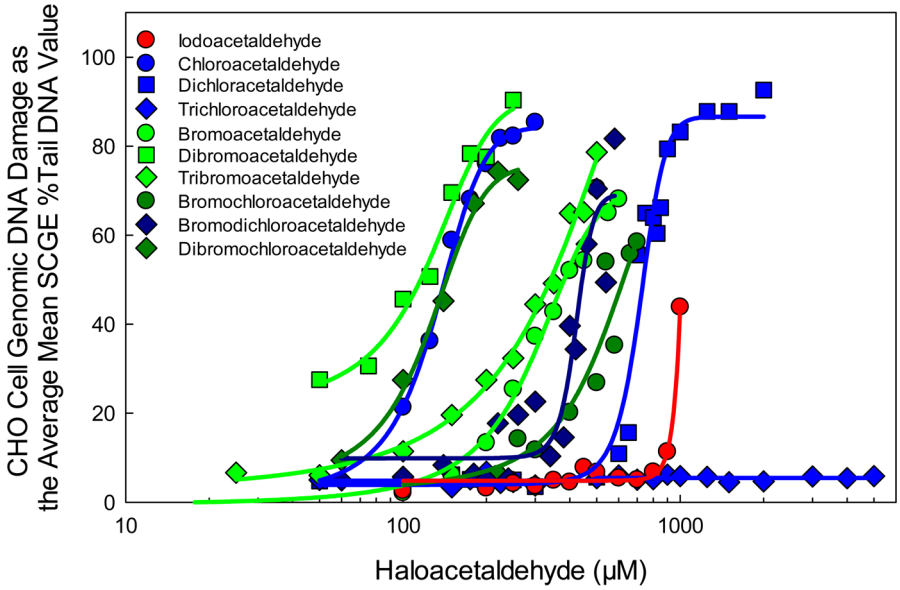

Figure 4. Comparison of the CHO cell acute genotoxicity concentration−response curves of the target HALs.

response curves of each HAL are presented in the SI (Figures S6−S15).

CHO Cell Acute Genotoxicity. CHO cell acute genotoxicity analyses (4 h exposures) of each HAL are summarized in Table 3. Figure 4 illustrates the concentration−response curves for the HALs. The individual concentration−response curves of each HAL with the cell viability data are presented in the SI (Figures S17−S26).

Structure−Activity Relationships of Haloacetaldehyde Toxicity. This study presents the first systematic, quantitative comparison of HAL cytotoxicity and genotoxicity. An all pairwise ANOVA test of the CTI values generated a descending rank order of chronic cytotoxicity as TBAL ≈ CAL > DBAL ≈ BCAL ≈ DBCAL > IAL > BAL ≈ BDCAL > DCAL > TCAL. The mean bootstrap CTI (±SE) values are presented in Table 4 and Figure S16. An all pairwise ANOVA test of the GTI values generated a descending rank order of genotoxicity of the ten HALs as DBAL > CAL ≈ DBCAL > TBAL ≈ BAL > BDCAL > BCAL ≈ DCAL > IAL. TCAL was not genotoxic. The mean bootstrap GTI (±SE) values are presented in Table 4 and Figure S27.

The cytotoxicity and genotoxicity of these ten HALs were not significantly correlated ($r = 0.36$; $P = 0.308$). The HALs did not follow the pattern in which the halogen affected toxicity (iodinated > brominated > chlorinated DBPs) in contrast to other DBP classes including the haloacetic acids (HAAs),[98] THMs,[20] or haloacetamides (HAcAms).[93]

The toxicity of HALs is complex in that these compounds possess two potential sites to react with nucleophiles in cells. One is the halogen α-carbon bond, which is associated with S_N2 type reactions. The halogen substituent, through bond dissociation energy and other factors, determines the relative bimolecular nucleophilic substitution (S_N2) reactivity of the compound. With monohaloacetic acids (mono-HAAs) and monohaloacetamides, the rank order of toxicity followed I > Br ≫ Cl, which corresponds to the leaving tendency of the halogens of alkyl halide.[93,98,99] We found that the mono-HAAs irreversibly inhibited glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity with a high correlation with the dissociation energy of the halogen α-carbon bond and with the alkylation potential of the HAA.[98,99] The rate of GAPDH inhibition and the toxic potency of the mono-HAAs showed the

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Table 4. Comparison of Calculated $K_{hydration}$ Values, Cytotoxicity Index (CTI) Values, and Genotoxicity Index (GTI) Values of HALs

		SPARC[a] $K_{hydration}$	CTI (±SE)[b]	GTI (±SE)[c]
mono-HALs and di-HALs	CAL	17.8	279.0 ± 7.0	7.20 ± 0.42
	BAL	11.0	64.6 ± 3.5	2.68 ± 0.11
	IAL	4.37	170.4 ± 7.3	0.96 ± 0.03
	DCAL	1.95×10^3	35.7 ± 0.8	1.26 ± 0.03
	DBAL	1.58×10^3	207.5 ± 2.1	9.11 ± 0.60
	BCAL	1.70×10^3	207.4 ± 11.0	1.61 ± 0.21
tri-HALs	TCAL	3.24×10^4	0.94 ± 0.03	NS[d]
	TBAL	1.15×10^4	279.8 ± 4.8	3.00 ± 0.03
	BDCAL	4.37×10^4	51.1 ± 4.3	2.24 ± 0.05
	DBCAL	2.00×10^4	200.2 ± 1.4	6.99 ± 0.28

[a]SPARC (SPARC Performs Automated Reasoning in Chemistry) models are mechanistic perturbation models developed by the U.S. EPA to calculate chemical reactivity and physical processes for compounds from molecular structure.[100,101] [b]The Cytotoxicity index (CTI) value was calculated from the individual LC_{50} values generated from the bootstrap analyses. The mean CTI was calculated as the $(LC_{50})^{-1}(10^3)$. [c]The Genotoxicity index (GTI) value was calculated from the individual 50%TDNA values generated from the bootstrap analyses. The mean GTI was calculated as the $(50\%TDNA)^{-1}(10^3)$. A Pearson correlation analysis demonstrated that no significant correlation exists among the hydration constants and the CTI or the GTI. [d]NS = not significantly different from the negative control.

same rank order (I > Br ≫ Cl) in a concentration-dependent manner. With the combined data of tri-HALs and di-HALs from the present study (Table 4), a strong significant correlation was found between the number of Br atoms and the CTI ($r = 0.90$; $P \leq 0.006$), while a good but not significant correlation was found with the GTI values ($r = 0.63$; $P = 0.13$). However, the impact of the halogen was not observed in the mono-HALs.

The other reactive site of the HALs is the carbonyl C═O bond of the aldehyde group. Aliphatic aldehydes are able to undergo Schiff base formation (Figure S28, SI). The Schiff base formation is a mechanism used by enzymes to catalyze reactions between an amine group with either an aldehyde or ketone. It proceeds through the carbinolamine intermediate resulting in an imine as a final product. HALs may induce genotoxic effects, such as DNA adducts, DNA−DNA cross-links, or DNA−protein cross-links by reacting with DNA chains through Schiff base formation.[63,68,69] Therefore, the overall toxic potency may differ by individual compound depending on the combinative reactivity of S_N2 type reaction and Schiff base formation in a biological system.

In an aqueous phase, HALs exist in equilibrium between an aldehyde and a hydrate form (Figure S29). This hydration equilibrium constant is defined as $K_{hydration}$ = [hydrate]/[aldehyde]. As $K_{hydration}$ increases, the hydrate species is dominant in the aqueous system. Theoretical $K_{hydration}$ values were calculated for each HAL from a predictive modeling system, SPARC (SPARC Performs Automated Reasoning in Chemistry), that was developed by the U.S. EPA[100,101] (Table 4). Based on these values, $K_{hydration}$ increases as the number of halogens increases. As the number of halogens increases, the electron withdrawing capacity of the $C(X)_n$ group is greater and the carbonyl carbon becomes more partial positive, enabling attack of a water molecule and hydration. The $K_{hydration}$ values for mono-HALs were 2−4 orders of magnitude lower than those for di-HALs and tri-HALs. The halogen-induced toxicity pattern seen with other DBP classes was not expressed in the mono-HALs. Mono-HALs have distinct $K_{hydration}$ values where the distribution of reactive aldehyde species will differ by halogen type. It is interesting that there was no correlation between the $K_{hydration}$ values and cytotoxicity or genotoxicity. Therefore, mono-HALs may induce overall toxicity outcomes through more than one mode of action. For the di- and tri-HALs the halogen-mediated S_N2 reaction may perform the predominant role in the induction of toxicity.

Comparison of the Toxicity of Haloacetaldehydes to Other DBP Classes. We compared the CHO cell toxic potencies of the HALs to those for other DBP chemical classes using calculated cytotoxicity and genotoxicity indices (Figure 5). The cytotoxicity index was determined by calculating the mean LC_{50} value of all of the individual compounds of a single class of DBPs. The genotoxicity index was determined by calculating the mean SCGE genotoxic potency value, which is defined by the SCGE tail moment from the individual compounds within a single class of DBPs.[20] Six DBP chemical classes were compared, including THMs, HAAs, HALs, halonitromethanes, haloacetonitriles, and HAcAms. HALs constitute the second most cytotoxic DBP class, whereas they rank as the second least genotoxic DBP class.

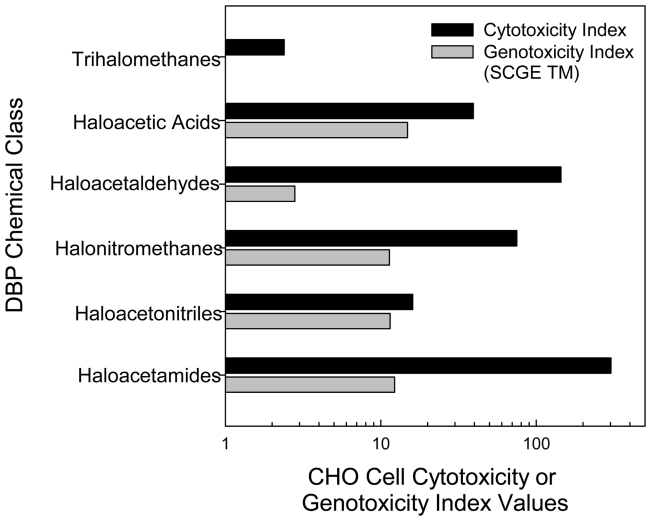

Figure 5. Comparison of the CHO cell chronic cytotoxicity index values and acute genotoxicity index values of various DBP chemical classes. Of the THMs analyzed, none were genotoxic in the CHO cell assay.

Research Implications. This study presented a precise analytical chemical method for the most comprehensive HAL identification and quantification to date and reports for the first time the formation of IAL during water disinfection. We conducted systematic quantitative comparative analyses of the cytotoxicity and genotoxicity in mammalian cells of the HALs, performed structure−activity relationships analyses on their toxicity, and compared the HALs with other DBP classes. Considering that HALs constitute the third largest group by weight of identified DBPs, attention should be given to determine their possible health risks and to their control by engineering practices.

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■ ASSOCIATED CONTENT

Supporting Information

Additional information on experimental methods, including additional figures and tables. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/es506358x.

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