Chemical and Biological Characterization of Newly Discovered Iodoacid Drinking Water Disinfection Byproducts

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Iodoacid drinking water disinfection byproducts (DBPs) were recently uncovered in drinking water samples from source water with a high bromide/iodide concentration that was disinfected with chloramines. The purpose of this paper is to report the analytical chemical identification of iodoacetic acid (IA) and other iodoacids in drinking water samples, to address the cytotoxicity and genotoxicity of IA in *Salmonella typhimurium* and mammalian cells, and to report a structure−function analysis of IA with its chlorinated and brominated monohalogenated analogues. The iodoacid DBPs were identified as iodoacetic acid, bromoiodoacetic acid, (*Z*)- and (*E*)-3-bromo-3-iodopropenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid. IA represents a new class (iodoacid DBPs) of highly toxic drinking water contaminants. The cytotoxicity of IA in *S. typhimurium* was 2.9× and 53.5× higher than bromoacetic acid (BA) and chloroacetic acid (CA), respectively. A similar trend was found with cytotoxicity in Chinese hamster ovary (CHO) cells; IA was 3.2× and 287.5× more potent than BA and CA, respectively. This rank order was also expressed in its genotoxicity with IA being 2.6× and 523.3× more mutagenic in *S. typhimurium* strain TA100 than BA and CA, respectively. IA was 2.0× more genotoxic than BA and 47.2× more genotoxic than CA in CHO cells. The rank order of the toxicity of these monohalogenated acetic acids is correlated with the electrophilic reactivity of the DBPs. IA is the most toxic and genotoxic DBP in mammalian cells reported in the literature. These data suggest that chloraminated drinking waters that have high bromide and iodide source waters may contain these iodoacids and most likely other iodo-DBPs. Ultimately, it will be important to know the levels at which these iodoacids occur in drinking water in order to assess the potential for adverse environmental and human health risks.

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Introduction

Each day approximately 250 000 public water purification facilities in the United States provide over 1.3×10^{10} L of high-quality, safe drinking water to 90% of the population (*1*). The production and distribution of disinfected water was a profound public health triumph of the twentieth century that significantly reduced infections by waterborne microbial pathogens. The disinfection of drinking water in public facilities primarily uses chemical disinfectants such as chlorine, chloramines, ozone, and chlorine dioxide (*2*). These disinfectants are strong oxidants that convert naturally occurring and synthetic organic material along with bromide and iodide in the raw water into chemical disinfection byproducts (DBPs). Over 500 DBPs have been identified; however, each disinfection method generates a different suite and distribution of DBPs (*3*).While reducing the public health risk of acute infection by waterborne pathogens, the unintended generation of DBPs poses a chronic health risk. DBPs represent an important class of environmentally hazardous chemicals that carry long-term human health implications (*3*−*5*). Epidemiological studies demonstrate that individuals who consume chlorinated drinking water have an elevated risk of cancer of the bladder, stomach, pancreas, kidney, and rectum as well as Hodgkin's and non-Hodgkin's lymphoma (*6*−*8*). DBPs also have been linked to reproductive and developmental effects, including the induction of spontaneous abortions (*9*, *10*).

Although chlorine has been used for over 100 years as a water disinfectant, the majority of DBPs present in drinking water have yet to be chemically defined (*5*, *11*). Identified DBPs account for less than 50% of the total organic halide (TOX) produced in chlorinated drinking water (*12*−*14*). Percentages of known DBPs are even smaller for drinking water treated with alternative disinfectants, with only 17.1, 8.3, and 28.4% of the TOX accounted for in waters treated with chloramines, ozone, or chlorine dioxide, respectively (*14*). Disinfectants used alone or in combination generate novel DBPs (such as 2,3,5-tribromopyrrole) that express toxic and genotoxic activity (*15*).

The haloacetic acids represent the second largest group of DBPs generated by water disinfection. An estimated 55 000 t of trichloroacetic acid alone are generated annually (*16*). Bromide is naturally present in many source waters that results in an increase in bromine-containing DBPs after water disinfection with chlorine, chloramine, or ozone (*15*, *17*, *18*). The chlorinated and brominated haloacetic acids have been evaluated for their mutagenicity and cytotoxicity in *Salmonella typhimurium* (*19*−*21*). We published an analysis of the induction of chronic and acute cytotoxicity as well as the induction of genomic DNA damage in mammalian cells by specific DBPs (*22*). In general, the brominated haloacetic acids were significantly more cytotoxic and genotoxic than their chlorinated analogues. In a recent U.S. nationwide occurrence study, iodoacid DBPs were uncovered in drinking water samples from source water with a high bromide

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concentration that was disinfected with chloramines (*23*). Gas chromatography/mass spectrometry (GC/MS) was used to tentatively identify those DBPs as iodoacetic acid, bromoiodoacetic acid, bromoiodopropenoic acid (two isomers), and 2-iodo-3-methylbutenedioic acid. Identifications have since been confirmed for iodoacetic acid and bromoiodoacetic acid. The specific isomers of (*Z*)- and (*E*)-3-bromo-3-iodopropenoic acid and (*E*)-2-iodo-3-methylbutenedioic acid were confirmed (results reported here). The identification of these iodoacids is important toxicologically, as iodoacetic acid (IA) has been shown to induce neural tube closure defects and other developmental abnormalities in mouse embryos (*24*, *25*).

IA is the focus of this paper because it may represent a new class (iodoacid DBPs) of potent drinking water contaminants. The purpose of this paper is (i) to report the analytical chemical identification of IA and other iodoacids in drinking water samples, (ii) to address the cytotoxicity and genotoxicity of IA in *S. typhimurium* and mammalian cells, and (iii) to report a structure−function analysis of IA with its chlorinated and brominated monohalogenated analogues. The health risks of DBPs are poorly understood and are compounded by the lack of a comparative database on the quantitative toxicity of DBPs.

Experimental Section

Drinking Water Analysis. Drinking water samples discussed in this paper were collected from a full-scale drinking water treatment plant in the United States that treated a source water with moderate levels of bromide (0.15 mg/L) and total organic carbon (7.01 mg/L) with chloramines only (chlorine and ammonia were added simultaneously). Samples (39 L each) were collected in November 2001 and December 2003 at the treatment plant in 2-L Teflon bottles, shipped overnight to the U.S. EPA laboratory the following day, and extracted and concentrated using XAD resins. Details regarding the drinking water treatment and concentration can be found in Supporting Information. Methylation derivatizations with BF_3−methanol were used to enable the detection of carboxylic acid DBPs with GC/MS (*15*). GC/MS analyses with electron ionization (EI) were performed on a Micromass Autospec II high-resolution mass spectrometer equipped with an Agilent 6890 gas chromatograph using conditions that can be found in Supporting Information.

Chemicals and Reagents. Bromoacetic acid and chloroacetic acid were purchased from Fluka Chemical Co. (Buchs, Switzerland). IA was purchased from Aldrich Chemical Co. (Milwaukee, WI). Bromoiodoacetic acid was prepared by the reaction of diiodoacetic acid (*26*) with bromine in water. Bromine (2.0 g, 12.5 mmol) was added to a stirred suspension of finely ground diiodoacetic acid (3.1 g, 10 mmol) in water (10 mL) over 10 min. The resultant solution was stirred at room temperature for 2.5 h and then evaporated to give a red oil. Extraction with chloroform, concentration, and cooling of the extract gave bromoiodoacetic acid (1.0 g), mp 82−84 °C. The purity of the product by GC using flame ionization detection was 75% and contained 10% dibromoacetic acid and 15% diiodoacetic acid. A mixture of 85% (*Z*)- and 15% (*E*)-3-bromo-3-iodopropenoic acid was prepared from 3-bromopropiolic acid (*27*) by addition of hydrogen iodide (*28*). The dimethyl ester of (*E*)-2-iodo-3-methylbutenedioic acid was prepared by the sequential addition of methyl copper−magnesium bromide and iodine to dimethylacetylenedicarboxylate (*29*). Hydrolysis of the dimethyl ester by refluxing with 10% hydrochloric acid gave (*E*)-2-iodo-3-methylbutenedioic acid, mp 80−81 °C, after crystallization from hexane. The DBPs were dissolved in dimethyl sulfoxide (DMSO) and stored at −22 °C in sealed, sterile glass vials.

Bacterial and Mammalian Cells. The *S. typhimurium* tester strain, TA100 (*hisG46*, *rfa*; Δ*uvrB-bio*; pKM101, *amp*^{r}) was stored as frozen cultures in Luria−Bonner broth (LB) plus 10% DMSO at −80 °C (*30*). Clone 11-4-8 of the transgenic Chinese hamster ovary (CHO) cell line AS52 was the mammalian cell system used in this research (*31*). The CHO cells were maintained in Ham's F12 medium containing 5% fetal bovine serum (FBS) and grown in 100 mm glass culture plates at 37 °C in an atmosphere of 5% CO_2 in air. For long-term storage, the cells were frozen in FBS:DMSO (9:1, v/v) and kept at −80 °C.

***Salmonella* Microplate Cytotoxicity Assay.** The detailed procedures for the *Salmonella* microplate cytotoxicity assay were published (*21*), and an expanded description of the assay can be found in the Supporting Information. This assay measured the cytotoxicity of the DBPs during a growth period of ∼3 cell divisions. A concurrent negative control of bacteria without DBP exposure was included with each microplate, and the blank-corrected data for the negative control was set at 100% growth. The blank-corrected data for each DBP concentration was converted into a percentage of the negative control. In general, each DBP concentration was replicated 4−8 times per microplate, and each experiment was repeated a minimum of 2 times.

***Salmonella* Preincubation Mutagenicity Assay.** The detailed procedures for the *Salmonella* mutagenicity assay were published (*21*), and an expanded description of the assay can be found in the Supporting Information. The induction of histidine revertants per 5×10^8 cells treated for each DBP concentration was used to generate the concentration−response data and to calculate the mutagenic potency. The mutagenicity experiments were repeated 3 times.

Mammalian Cell Microplate Cytotoxicity Assay. The detailed procedures for the CHO cell microplate cytotoxicity assay were published (*32*) and can be found in the Supporting Information. This assay measures the reduction in cell density as a function of DBP concentration over a period of ∼3 cell divisions (72 h). The blank-corrected absorbency value of the negative control (cells with medium only) was set at 100%. The absorbency for each treatment group well was converted into a percentage of the negative control. For each DBP concentration 8 replicate wells were analyzed per experiment, and the experiments were repeated twice.

Single Cell Gel Electrophoresis (SCGE) Assay. The procedures for the CHO cell microplate SCGE assay were published (*22*, *32*), and they can be found in the Supporting Information. SCGE is a sensitive assay that quantitatively determines genomic DNA damage and predictive carcinogenic potency. After the CHO cells were treated with the DBP for 4 h, acute cytotoxicity with a vital dye was measured (*33*). A computerized image analysis system (Komet 3.1, Kinetic Imaging Ltd., Liverpool, UK) was used to measure various SCGE parameters (i.e., % tail DNA and tail moment) of 25 randomly chosen nuclei per slide. The tail moment (integrated value of migrated DNA density multiplied by the migration distance) was used as the primary measure of DNA damage. Each experiment was repeated 3 times.

Safety and Data Analysis. Manipulations of toxic and mutagenic chemicals were conducted using disposable papers and gloves in a certified biological/chemical safety hood. Data from the experiments were transferred to Excel spreadsheets (Microsoft Corp., Redmond, WA) and analyzed using the statistical and graphical functions of SigmaPlot 8, SigmaStat 3, and Table Curve 4.03 (SPSS Inc., Chicago, IL). The tail moment values in the SCGE assay were not normally distributed and violated the requirements for analysis by parametric statistics. The median tail moment value for each slide was determined, and the data were averaged. Averaged median values express a normal distribution according to

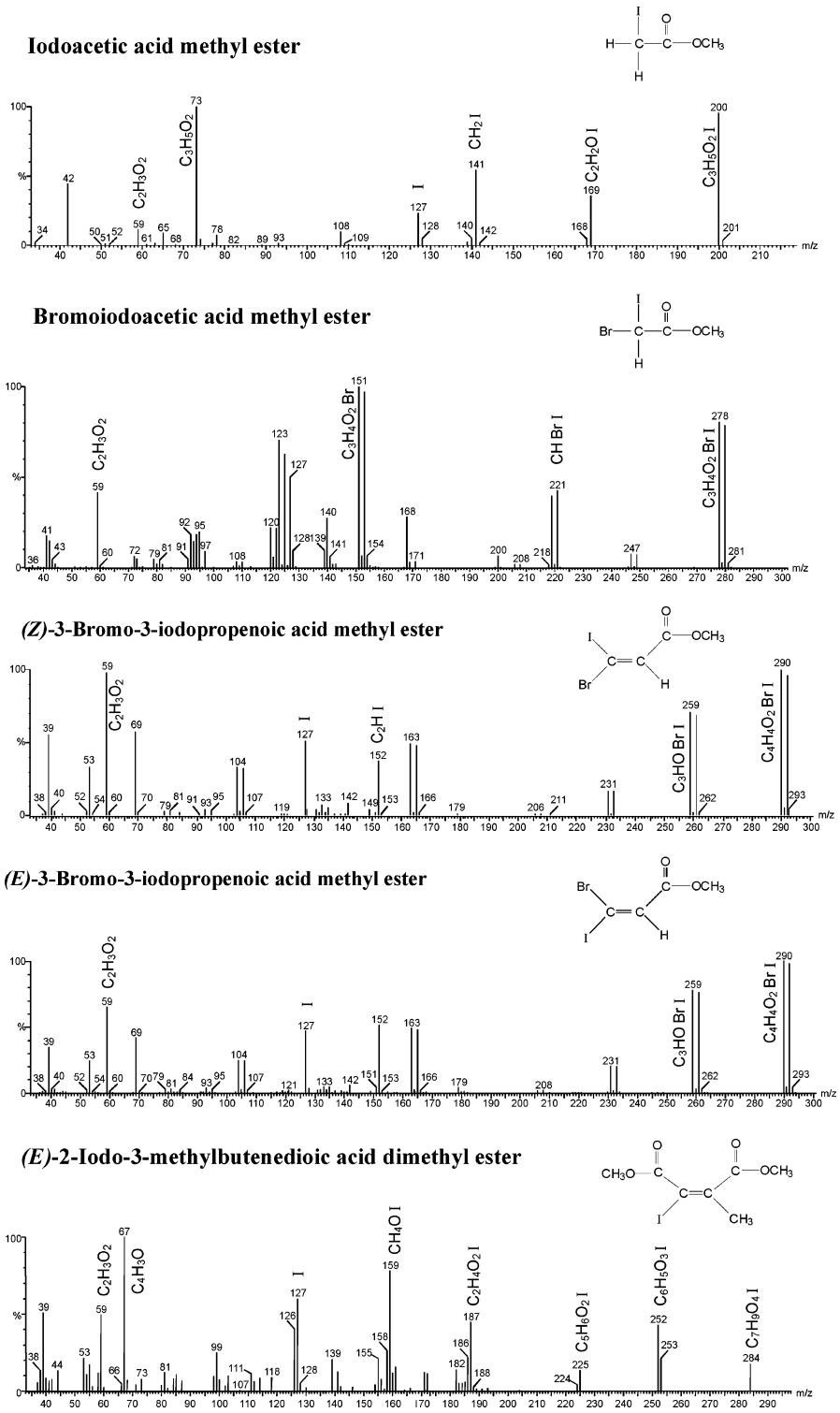

FIGURE 1. Mass spectra for iodoacids (identified in their methyl ester forms).

the central limit theorem (*34*). The averaged median tail moment values obtained from repeated experiments were used with a one-way analysis of variance test (*35*). If a significant F value of $P \leq 0.05$ was obtained, a Holm−Sidak multiple comparison versus the control group analysis was conducted. The power of the test statistic (β) was ≥ 0.8 at $\alpha = 0.05$.

Results and Discussion

The chemistry and toxicity of DBPs have been investigated for more than a quarter of a century. However, it is important to evaluate the toxicity of important new classes of DBPs, such as these iodoacids, which have just been identified for the first time in drinking water.

Analytical Chemistry. Five iodoacids—IA, bromoiodoacetic acid, (*Z*)- and (*E*)-3-bromo-3-iodopropenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid—were identified in their methyl ester forms using GC/MS. Figure 1 shows their EI mass spectra; empirical formulas shown above the molecular ions and fragments were obtained using high-resolution MS (10000 resolution). These iodoacids were found in the finished drinking waters treated by chloramination (Figure 2) and

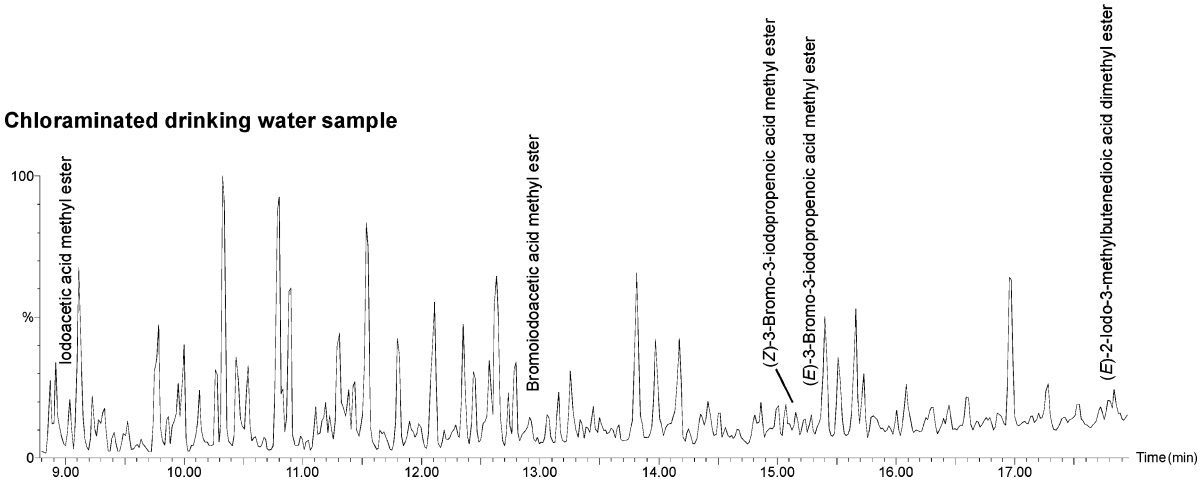

FIGURE 2. GC/MS chromatogram of chloraminated drinking water sample.

were not in the corresponding raw water blanks. As a result, they were determined to be DBPs of the treatment process. These iodinated DBPs were likely formed by the reaction of monochloramine with naturally occurring iodide to form hypoiodous acid (HOI), which in turn reacts with natural organic matter (*36*, *37*) to form the iodoacids. Because none of the iodoacid methyl esters were present in the NIST or Wiley Library Databases, extensive interpretation of their mass spectra was performed to generate possible structures for the unknown compounds. Unlike other halogenated compounds, such as brominated and chlorinated compounds that are easily recognizable from their distinctive isotopic patterns, the presence of iodine is not easy to detect in mass spectra since iodine has only one naturally occurring isotope: 127 Da. A compound with one bromine in its structure will give a doublet isotopic pattern, which is due to the overlap of the two naturally occurring bromine isotopes (79 and 81 Da). For example, the presence of one bromine atom is indicated from the mass spectrum of bromoiodoacetic acid methyl ester (Figure 1). The molecular ion doublet at m/z 278/280 is due to the overlap of the two bromine isotopes. On the other hand, the presence of iodine is not immediately obvious in this spectrum nor in the spectra of the other iodoacids (Figure 1). However, it is fortunate that the molecular ions of these methyl esters were present. It was evident that the highest m/z ions in these mass spectra were the molecular ions (and not fragment ions), due to the loss of 31 Da (for the monoacid esters), and in some cases 59 Da, which is common for carboxylic methyl esters. The presence of the m/z 59 ion in each of these mass spectra also supports the assignment of these as carboxylic acid methyl esters. The diacid ester ((*E*)-2-iodo-3-methylbutenedioic acid dimethyl ester) showed the characteristic loss of 59 Da and the presence of m/z 59 but underwent a rearrangement to lose 32 Da (CH_3OH) instead of the more common 31 Da (OCH_3). Once the molecular ions were known, and it was likely that these were carboxylic acid methyl esters, then the presence of iodine could be uncovered from careful inspection of the mass spectra. For example, in most cases, there was a small ion at m/z 127, representing the I^+ ion, and often the loss of iodine $(M - I)^+$ was often present at substantial relative abundance.

From the low resolution EI-MS data, tentative structures were proposed. For the first two iodoacid methyl esters (iodoacetic acid methyl ester and bromoiodoacetic acid methyl ester), only a single isomer was possible. However, several structural isomers were possible for the remaining iodoacids (six different isomers were possible for the two bromoiodopropenoic acid methyl ester isomers observed, and two isomers were possible for the single 2-iodo-3-methylbutenedioic acid dimethyl ester isomer observed). These structures are illustrated in Figure 3. High-resolution MS data supported the empirical formulas for these tentative structural assignments and the presence of iodine in their structures, with exact mass data of 199.939 (observed) and 199.933 (theoretical) for iodoacetic acid methyl ester, 277.849 (observed) and 277.844 (theoretical) for bromoiodoacetic acid methyl ester, 289.850 (observed) and 289.844 (theoretical) for (*Z*)-3-bromo-3-iodopropenoic acid methyl ester, and 283.957 (observed) and 283.955 (theoretical) for (*E*)-2-iodo-3-methylbutenedioic acid dimethyl ester. The mass spectral signal of (*E*)-3-bromo-3-iodopropenoic acid methyl ester was too weak to obtain high resolution data. After we tentatively identified these iodoacids, we purchased the IA standard and synthesized the remaining standards and methylated these standards to check their mass spectra and GC retention times against those in our drinking water samples. Because

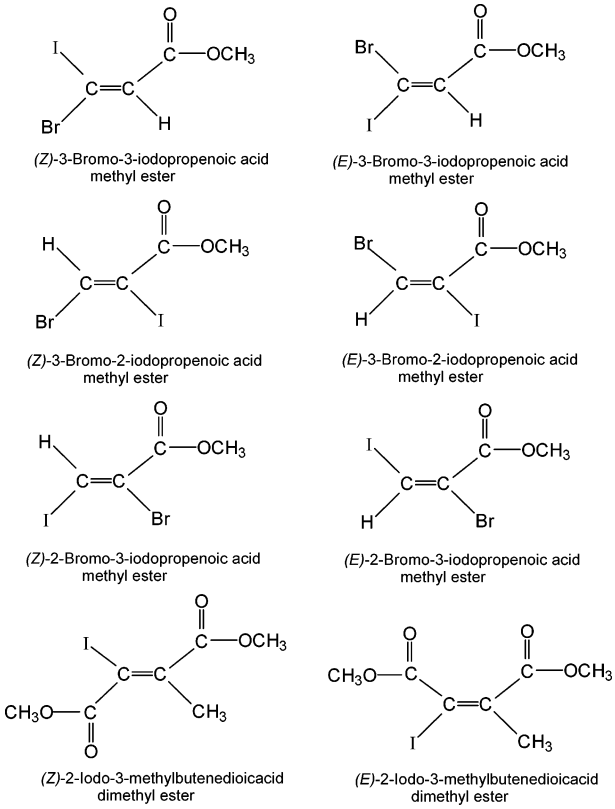

FIGURE 3. Possible structures for bromoiodopropenoic acid methyl ester and iodomethylbutenedioic acid dimethyl ester isomers.

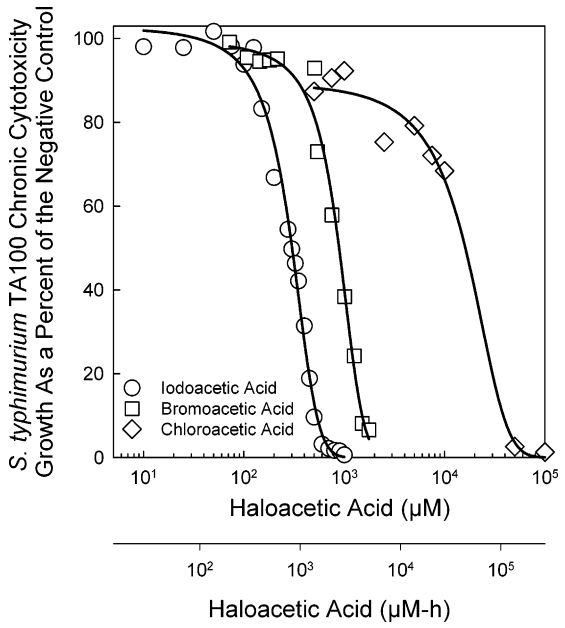

FIGURE 4. Induction of cytotoxicity in *S. typhimurium* by iodoacetic, bromoacetic, and chloroacetic acids. The ordinate is expressed (i) as the μM concentration of the haloacetic acid present in the reaction tube and (ii) as the μM-h, which included the 1-h exposure to the haloacetic acid concentration in the reaction tube plus the 210 min in the microplate well at half of its original concentration. The abscissa expresses the percent of bacterial growth as a percentage of the concurrent negative control for each haloacetic acid.

the mass spectra for the bromoiodopropenoic acid methyl ester isomers and the iodomethylbutenedioic acid dimethyl ester isomers were expected to be nearly identical and the GC retention times were expected to be very similar, all of the isomers except those of the 2-bromo-3-iodopropenoic acid and (*Z*)-2-iodo-3-methylbutenedioic acid were synthesized to ensure correct identifications. Results did reveal nearly identical mass spectra and similar retention times for the bromoiodopropenoic acid methyl ester isomers and the 2-iodo-3-methylbutenedioic acid dimethyl ester isomers. However, unequivocal matches were obtained for (*Z*)- and (*E*)-3-bromo-3-iodopropenoic acid methyl ester and (*E*)-2-iodo-3-methylbutenedioic acid dimethyl ester, confirming their identities. GC/MS retention times and mass spectra of iodoacetic acid methyl ester and bromoiodoacetic acid methyl ester also matched those in our drinking water samples, confirming their identities. After their initial discovery in drinking water samples collected for the nationwide occurrence study, we have since resampled this location (in December 2003) and continue to observe these five iodoacids in the chloraminated drinking water.

Analytical Biology. We present new results of the cytotoxicity of IA and bromoacetic acid (BA) in *S. typhimurium*. Previously published cytotoxicity data for chloroacetic acid (CA) are used for comparison (*21*). The mutagenicity of IA in *S. typhimurium* was determined in this current study; comparative data for BA and CA were previously published (*21*). The CHO cell cytotoxicity of IA, BA, and CA were determined in this study. The SCGE analysis of IA in CHO cells was determined in the current study; the SCGE data for BA and CA were published (*22*).

Cytotoxicity and Mutagenicity in *S. typhimurium*. The *Salmonella* microplate cytotoxicity assay is a quantitative measurement of the repression of bacterial growth induced by a test agent. In Figure 4, the ordinate is expressed in two units of measurement. The concentration (expressed as μM) is the concentration of the haloacetic acid present in the reaction tube. The concentration (expressed as μM-h) included the 1-h exposure to the haloacetic acid concentration in the reaction tube plus the 210 min in the microplate well at half of its original concentration. This is due to the addition of 2× concentrated LB to each microplate well. The use of these two measurements allows for the comparison of the relative viability of the *S. typhimurium* cells between the cytotoxicity assay and the preincubation mutagenicity assay (*21*).

The chronic cytotoxicity of IA was evaluated within a concentration range from 10 μM to 1 mM. Each of the 20 concentrations was replicated from 4 to 12 times within two independent experiments (Figure 4). At concentrations above 100 μM, IA induced significant cytotoxicity as compared to the negative control; the $\%C^{1}/_{2}$ value was 303 μM (Table 1). The $\%C^{1}/_{2}$ value is the concentration of the test agent, determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative control. For BA the experiments were conducted in triplicate with 24 repeats at each concentration. The $\%C^{1}/_{2}$ value for BA was 881 μM with concentrations above 216 μM significantly different from the negative control (Table 1). The $\%C^{1}/_{2}$ value for CA was 16.2 mM (*21*).

IA was analyzed for its direct-acting mutagenicity with *S. typhimurium* strain TA100 for reversion at the *hisG46* allele. The mutagenicity of IA was assayed using a preincubation

TABLE 1. Chronic Cytotoxicity Analysis of Iodoacetic, Bromoacetic, and Chloroacetic Acids in *Salmonella typhimurium* TA100 or Chinese Hamster Ovary AS52 Cells

haloacetic acid	range of significant concentration−response (μM)	r^2 of regression analysis[a]	$\%C^{1}/_{2}$ (μM)[b]	ANOVA test statistic
		***Salmonella typhimurium* Cells**		
iodoacetic acid	100−1000	0.99	303	$F_{20,163} = 303.47$ $P < 0.001$
bromoacetic acid	507−2260	0.98	881	$F_{14,372} = 256.98$ $P < 0.001$
chloroacetic acid[c]	800−100 000	0.97	16 200	$F_{14,73} = 891.32$ $P < 0.001$
		CHO Cells		
iodoacetic acid	0.500−12.0	0.97	2.95	$F_{13,122} = 54.71$ $P < 0.001$
bromoacetic acid	2.00−25.0	0.99	9.56	$F_{10,165} = 94.45$ $P < 0.001$
chloroacetic acid	300−2000	0.99	848	$F_{8,127} = 37.62$ $P < 0.001$

[a] Regression analysis was used to determine the $\%C^{1}/_{2}$ value from the cytotoxicity concentration−response curve. [b] The $\%C^{1}/_{2}$ value is the concentration of the test agent that reduces the cell density by 50% as compared to the concurrent negative control. [c] Summarized from ref *21*.

TABLE 2. Genotoxicity of Iodoacetic, Bromoacetic, and Chloroacetic Acids in *Salmonella typhimurium* TA100 or Chinese Hamster Ovary AS52 Cells

haloacetic acid	r^2 of regression analysis[a]	genotoxic potency[b]	ANOVA test statistic
	***Salmonella typhimurium* TA100 Mutagenicity**		
iodoacetic acid	0.82	14 129 revertants/μmol	$F_{13, 79} = 59.02$ $P < 0.001$
bromoacetic acid[c]	0.98	5 465 revertants/μmol	$F_{8, 69} = 72.12$ $P < 0.001$
chloroacetic acid[c]	0.92	27 revertants/μmol	$F_{12, 104} = 53.49$ $P < 0.001$
	CHO Cell Single Cell Gel Electrophoresis		
iodoacetic acid	0.99	8.70 μM	$F_{9, 49} = 33.90$ $P < 0.001$
bromoacetic acid[d]	0.99	17.0 μM	$F_{8, 50} = 77.47$ $P < 0.001$
chloroacetic acid[d]	0.99	411 μM	$F_{8, 23} = 20.13$ $P < 0.001$

[a] The regression analysis represents the linear section of the concentration−response curve for *S. typhimurium*. For the CHO cell SCGE assay, the r^2 represents the fit of the regression analysis for the concentration−response curve used to calculate the genotoxic potency value. [b] For *S. typhimurium*, the mutagenic potency is expressed as the induced revertants per μmole of the test agent. For the CHO cell SCGE assay, the genotoxic potency is the concentration of the test agent at the midpoint of the tail moment concentration−response curve. [c] Summarized from ref *21*. [d] Summarized from ref *22*.

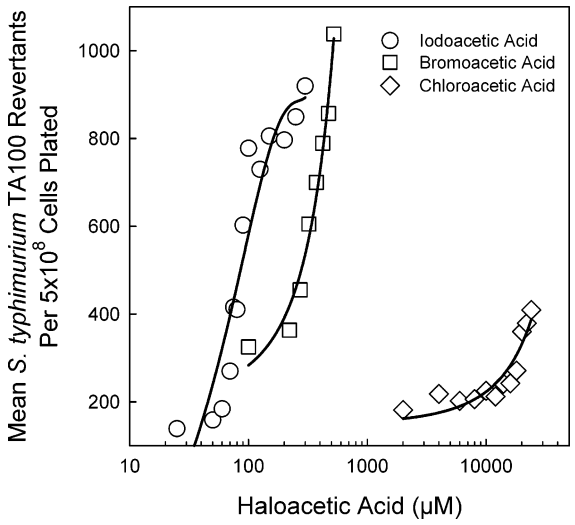

FIGURE 5. Concentration−response curves illustrating the relative mutagenicity of iodoacetic, bromoacetic, and chloroacetic acids in *S. typhimurium* strain TA100.

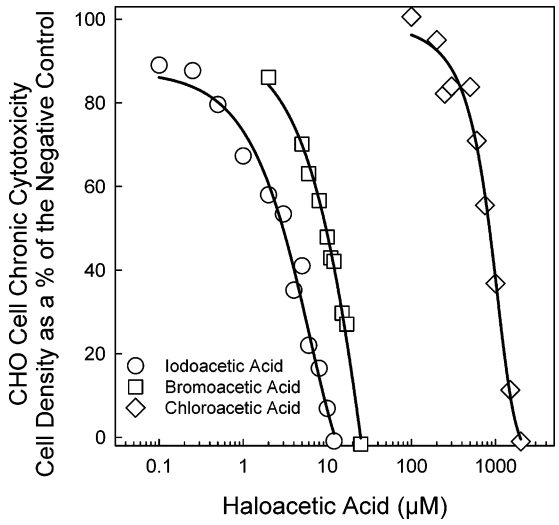

FIGURE 6. Concentration−response curves illustrating the chronic cytotoxicity of iodoacetic, bromoacetic, and chloroacetic acids in CHO cells after 72-h exposure.

procedure with a treatment time of 1 h in a concentration range of 25−300 μM with three independent experiments. As illustrated by the μM-h scale in Figure 4, this concentration range was not cytotoxic. A significant increase in the induced revertants per 5×10^8 cells plated was observed at IA concentrations of 70 μM and above (Table 2). The average spontaneous reversion frequency was 130 revertants per 5×10^8 cells plated; 300 μM IA induced 919 revertants per 5×10^8 cells plated (Figure 5).

To directly compare the mutagenicity of IA with BA and CA in *S. typhimurium*, we calculated the induced mutagenic potency of each agent. The mutagenic potency was calculated for the haloacetic acid concentrations that expressed a significant increase over their corresponding negative control. The mutagenicity concentration−response curves were analyzed by running a linear regression for specific regions of the curve and the coefficient of linearity (r^2) for each agent is presented in Table 2. The induced revertants per plate were calculated by subtracting the average negative control revertant frequency from the treatment revertant frequency. Using these data the induced revertants per reaction tube were determined. The induced revertants per micromole were calculated for each concentration in the linear region of the DBP concentration−response curve and an average mutagenic potency value was determined (Table 2). With a mutagenic potency of 14 129 revertants/μmol, IA is the most potent haloacetic acid analyzed in *S. typhimurium*.

Cytotoxicity and Genotoxicity in CHO Cells. Although BA and CA were previously evaluated for chronic cytotoxicity in CHO cells (*22*), we made a modification to the microplate assay and analyzed the monohalogenated acetic acids. IA was analyzed in a concentration range from 100 nM to 12 μM and induced a significant amount of cytotoxicity at concentrations of 500 nM and above. BA was cytotoxic in the concentration range from 2 to 25 μM, while CA was cytotoxic in a range from 300 μM to 2 mM. The $\%C^1/_2$ values for IA, BA, and CA were 2.95, 9.56, and 848 μM, respectively (Table 1, Figure 6).

IA was a potent inducer of genomic DNA damage in CHO cells. We measured two indices of DNA damage, the % tail DNA (the amount of DNA migrating from the nucleus into the gel), and the SCGE tail moment (the product of the amount of migrated DNA and the distance). IA induced a significant concentration response in the mean % tail DNA

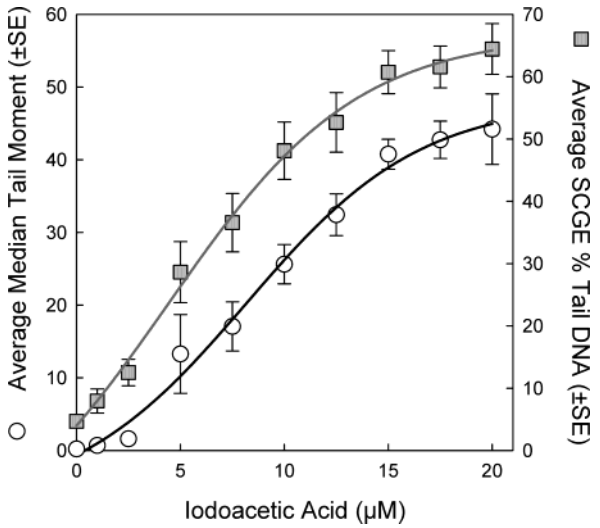

FIGURE 7. Comparison of the genomic DNA damage induced by iodoacetic acid using two SCGE measurement parameters: median tail moment and the percentage of fragmented DNA migrated into the gel (% tail DNA).

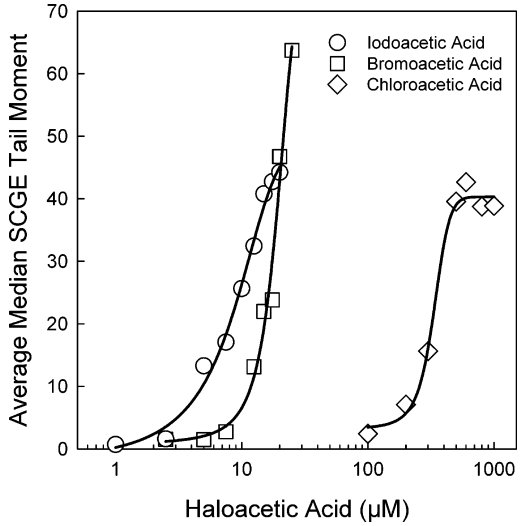

FIGURE 8. Concentration−response curves illustrating the relative levels of genomic DNA damage induced by iodoacetic, bromoacetic, and chloroacetic acids.

in the range from 5 to 20 μM (Figure 7). Likewise a significant increase in the average median tail moment values was observed from 5 to 20 μM (Figure 7, Table 2). The SCGE genotoxic potency is the concentration of the test agent that is at the midpoint of the rising slope of the tail moment concentration−response curve. This value was determined after regressing the data within the concentration range that was not acutely cytotoxic. The genotoxic potency of IA was 8.70 μM (Table 2), and it expressed the highest genotoxicity of the three monohaloacetic acids (Figure 8).

Comparative Responses and Possible Mechanisms of Action. The cytotoxicity and genotoxicity of the monohaloacetic acids is expected to be related to the cellular uptake and transport of the chemicals and their subsequent chemical interaction with cellular macromolecules. Table 3 summarizes several relevant physicochemical properties and parameters that may be useful in understanding the mechanistic basis of the observed data.

The ability of the monohaloacetic acids to cross cell membranes is dependent on their lipophilicity, the degree of ionization, and possible transport mechanisms. Consistent with the rank order of their cytotoxicity and genotoxicity in

TABLE 3. Summary of Physicochemical Properties of Monohaloacetic Acids

physicochemical properties of monohaloacetic acids[a]				bond length, bond dissociation energy, and relative S_N2 reactivity of C−X bond based on alkyl halides[b]			
compd	log *P*	pK_a	E_{LUMO}	C−X	length (Å)	dis. energy (kcal/mol)	relative S_N2
CA	0.38	2.82	0.126	C−Cl	1.77	78.5	1
BA	0.52	2.90	0.111	C−Br	1.93	65.9	50
IA	0.91	3.12	0.091	C−I	2.14	57.4	150−250

[a] Calculated log *P* and E_{LUMO} and measured pK_a summarized from ref *42*. The measured log *P* of CA and BA are 0.22 and 0.41, respectively (*54*). [b] Summarized from ref *41*.

TABLE 4. Pearson Correlation Analysis of Physicochemical and Toxicological Measurements of Monohaloacetic Acids

physicochemical parameters	cytotoxicity (*r*)		genotoxicity (*r*)	
	Salmonella	CHO	*Salmonella*	CHO
log *P*	−0.73	−0.71	0.99	−0.72
pK_a	−0.73	−0.71	0.99	−0.72
E_{LUMO}	0.84	0.83	−0.99	0.83
bond length (Å)	−0.84	−0.83	−0.99	−0.84
C−X dissociation energy	0.07	0.09	0.53	0.08
relative S_N2	−0.71	−0.69	0.99	−0.70

S. typhimurium and CHO cells, the log *P* of the un-ionized monohaloacetic acids follows the order of IA > BA > CA. The correlation coefficients (*r*) for cytotoxicity ($\%C^1/_2$) and the log *P* values were similar for *S. typhimurium* and CHO cells (Table 4). A direct correlation also results between genotoxicity and log *P*. The higher mutagenic potency values for *S. typhimurium* express increased mutagenic activity. Lower SCGE genotoxic potency values for CHO cells indicate stronger DNA-damaging capacity and thus an inverse correlation with log *P*.

The lipophilicity of and cell permeability to monohaloacetic acids can be substantially decreased by ionization, which is determined by their pK_a and the pH of the medium. The fraction (*f*) of un-ionized monohaloacetic acids can be calculated by the formula (*38*):

$$f = 1/(1 + 10^{(\mathrm{pH}-\mathrm{p}K_a)})$$

Chemicals with higher pK_a values are less likely to be ionized. For the monohaloacetic acids the ranking of pK_a follows the order of IA > BA > CA. A correlation among the pK_a and the cytotoxicity and genotoxicity of the monohalogenated acetic acids was observed (Table 4). However, the relatively minor difference in pK_a (Table 3) does not appear to be a major contributing factor to the substantial difference in the relative genotoxic/cytotoxic potency. Furthermore, since the experimental conditions were maintained around neutral pH, all three monohaloacetic acids should be mostly in the ionized form. It is possible that facilitated or active membrane transport of anionic monohaloacetates may be an additional mechanism for cellular uptake. The experimental conditions were maintained around neutral pH, thus the monohaloacetic acids should be in an ionized form, and facilitated or active membrane transport of anionic monohaloacetates may be a mechanism for cellular uptake. There is some evidence of active transport of CA and BA across synthetic membranes (*39*), but the relevance to biological membranes remains to be studied. Tissue distribution studies in rats (*40*) showed significant accumulation of CA and IA in the kidney and liver. However, a percutaneous absorption study using human skin sections showed very poor permeability to CA and BA around neutral pH (*38*).

The chemical reactivity of monohaloacetic acids is expected to be similar to that of methyl halides. With the exception of methyl fluoride, methyl halides are potent alkylating agents that react by an S_N2 type of mechanism. The reactivity of methyl halides is primarily dependent on the carbon−halogen bond dissociation energy, which in turn is related to the bond length. Since the atomic size of the halogen follows the order I > Br > Cl, the length of carbon−halogen bond increases and the bond dissociation energy decreases (Table 3) accordingly. Polarizability and delocalization of the electron cloud also contribute to making iodine a better leaving group than bromine and a much better leaving group than chlorine. Typically, the S_N2 reactivity of an alkyl iodide is 3−5× greater than an alkyl bromide, which in turn is 50× greater than an alkyl chloride (*41*). Interestingly, this relative S_N2 reactivity is moderately correlated with cytotoxicity in *S. typhimurium* and CHO cells, as well as genotoxicity in CHO cells and mutagenic potency in *S. typhimurium* (Table 4). This correlation is remarkably similar to that observed in developmental toxicity using mammalian whole embryo cultures (*24*, *42*). A comparison of the potency of developmental toxicity with the calculated lowest unoccupied molecular orbital (E_{LUMO}) of monohaloacetic acids showed an inverse relationship, supporting the view that electrophilic reactivity played an important role (*42*). This relationship was also observed with the cytotoxicity and genotoxicity expressed in both cell types (increased revertants/μmole for *S. typhimurium* and decreased μM concentration for the genotoxic potency measurement with CHO cells) (Table 4).

The toxic consequence of chemical interaction between monohaloacetic acids and cellular constituents is dependent on the sensitivity of the target tissue and the overall effect on the whole organism. Like methyl halides, monohaloacetic acids are expected to be relatively soft electrophiles, which preferentially react with soft nucleophiles, such as thiol groups of cysteinyl residues in proteins and glutathione (*43*). IA is well-known as a metabolic inhibitor of glycolysis because of inhibition of sulfhydryl rich glyceraldehyde-3-phosphate dehydrogenase and can cause acute toxicity (*40*) via poisoning of the heart and nerve cells (*44*, *45*) as well as chromosome aberrations due to the depletion of adenosine 5′-triphosphate (ATP) (*46*, *47*). Both IA and methyl iodide are potent depletors of cellular glutathione (*40*, *48*, *49*), which is the key protective nucleophile against cytotoxicity, oxidative stress, and electrophilic attacks on the hard nucleophilic sites on purines and pyrimidines. The level of intracellular glutathione is also a key regulator for the induction of stress-activated signal transduction pathways (*50*).

The finding that IA is the most potent genotoxic DBP in mammalian cells may raise substantial concern as a potential health hazard. IA has also been shown to be the most potent haloacid in vitro toxicant in mammalian embryo cultures (*42*). The carcinogenic potential of IA remains to be studied. A limited study in 1953 showed that IA was a tumorigenesis promoter or cocarcinogen on mouse skin (*51*). No other long-term carcinogenesis studies have been conducted. The closely related CA has been tested by the National Toxicology Program (NTP) (*52*) (1992) by oral exposure in rats and mice and shown to have no carcinogenic activity. The ability to detect any potential carcinogenic activity of CA may have been complicated by its acute myocardial toxicity. Although IA is 1.8× more acutely toxic than CA in rats (*40*), the substantially higher genotoxicity of IA (around 2 orders of magnitude) may shift the delicate balance toward higher potential for carcinogenic activity. Another closely related compound, methyl iodide, is carcinogenic (*53*). Beyond concern for IA as an individual chemical, the marked depletion of cellular glutathione by IA is also a great concern in the presence of other carcinogenic DBPs. Cells stripped of protective nucleophiles are rendered much more susceptible to other electrophilic carcinogens. In this respect, it would be important to closely monitor the occurrence of IA in drinking water and study the combination effects of IA with other DBP genotoxins.

Implications and Future Directions. Given the high cytotoxic and genotoxic potency of IA in *S. typhimurium* and CHO cells, further work is planned for the other four iodoacids that have been identified as DBPs. Efforts are currently underway to purify the synthetic standards of bromoiodoacetic acid, (*Z*)-3-bromo-3-iodopropenoic acid, and (*E*)-3-bromo-3-iodopropenoic acid, so that their purities will be sufficiently high to conduct definitive cytotoxicity and genotoxicity studies. We are in the process of synthesizing (*E*)-2-iodo-3-methylbutenedioic acid in a pure, diacid form for biological analysis (initially, the dimethyl ester was obtained due to difficulty in synthesizing the diacid). Research is planned to obtain quantitative concentration data for these iodoacids in drinking water. In the nationwide occurrence study, these iodoacids were found only in drinking water from one location that was treated with chloramines (and whose source waters were high in bromide/iodide) (*23*). Interestingly, the highest levels of iodinated THMs (trihalomethanes) (dichloroiodomethane, bromochloroiodomethane, dibromoiodomethane, chlorodiiodomethane, and bromodiiodomethane) were also observed in this same drinking water at a total of 18.7 μg/L (which was 81% of the concentration of the four regulated THMs—chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) (*23*). These findings are consistent with earlier work by Bichsel and von Gunten that predicted an increased formation of iodinated DBPs in chloraminated drinking water, based on the very slow rate of iodide oxidation to iodate by chloramines, which allows hypoiodous acid (HOI) to accumulate and react with natural organic matter to form iodinated DBPs (*36*, *37*). Thus, it is likely that the highest levels of iodoacids (and other iodo-DBPs) will not be in chlorinated drinking waters but in chloraminated drinking waters. This is in stark contrast to the effect chloramine disinfection has on the formation of the regulated chloro/bromo-THMs and haloacetic acids, where chloramination significantly reduces their levels compared to chlorination. As a result, chloraminated drinking waters that have high bromide and iodide source waters will be targeted for this future quantitative occurrence work to determine whether the iodoacids commonly occur in high-iodide, chloraminated drinking waters and at what levels they are present. Ultimately, it will be important to know the levels at which these iodoacids occur in order to assess the potential for adverse environmental and human health risks. It should be noted that the iodoacids identified in this study were from a plant using chloramines only. For plants that have a significant free chlorine contact time before the addition of ammonia (to form chloramines), iodo-DBP formation may not be a problem.

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Note Added after ASAP Posting

This paper was released ASAP on August 17, 2004. Two sentences were added to the last paragraph of the paper, and the corrected version was posted on August 19, 2004.

Supporting Information Available

Information on the methodology of the GC/MS analysis and the *S. typhimurium* and CHO cell cytotoxicity and genotoxicity assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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