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# Occurrence and Mammalian Cell Toxicity of Iodinated Disinfection Byproducts in Drinking Water

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An occurrence study was conducted to measure five iodo-acids (iodoacetic acid, bromoiodoacetic acid, (*Z*)-3-bromo-3-iodo-propenoic acid, (*E*)-3-bromo-3-iodo-propenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid) and two iodo-trihalomethanes (iodo-THMs), (dichloroiodomethane and bromochloro-iodomethane) in chloraminated and chlorinated drinking waters from 23 cities in the United States and Canada. Since iodoacetic acid was previously found to be genotoxic in mammalian cells, the iodo-acids and iodo-THMs were analyzed for toxicity. A gas chromatography (GC)/negative chemical ionization-mass spectrometry (MS) method was developed to measure the iodo-acids; iodo-THMs were measured using GC/high resolution electron ionization-MS with isotope dilution. The iodo-acids and iodo-THMs were found in waters from most plants, at maximum levels of 1.7 $\mu$g/L (iodoacetic acid), 1.4 $\mu$g/L (bromoiodoacetic acid), 0.50 $\mu$g/L ((*Z*)-3-bromo-3-iodopropenoic acid), 0.28 $\mu$g/L ((*E*)-3-bromo-3-iodopropenoic acid), 0.58 $\mu$g/L ((*E*)-2-iodo-3-methylbutenedioic acid), 10.2 $\mu$g/L (bromochloroiodomethane), and 7.9 $\mu$g/L (dichloroiodomethane). Iodo-acids and iodo-THMs were highest at plants with short free chlorine contact times (<1 min), and were lowest at a chlorine-only plant or at plants with long free chlorine contact times (>45 min). Iodide levels in source waters ranged from 0.4 to 104.2 $\mu$g/L (when detected), but there was not a consistent correlation between bromide and iodide. The rank order for mammalian cell chronic cytotoxicity of the compounds measured in this study, plus other iodinated compounds, was iodoacetic acid > (*E*)-3-bromo-2-iodopropenoic acid > iodoform > (*E*)-3-bromo-3-iodo-propenoic acid > (*Z*)-3-bromo-3-iodo-propenoic acid > diiodoacetic acid > bromoiodoacetic acid > (*E*)-2-iodo-3-methylbutenedioic acid > bromodiiodomethane > dibro-moiodomethane > bromochloroiodomethane ≈ chlorodi-iodomethane > dichloroiodomethane. With the exception of iodoform, the iodo-THMs were much less cytotoxic than the iodo-acids. Of the 13 compounds analyzed, 7 were genotoxic; their rank order was iodoacetic acid ≫ diiodoacetic acid > chlorodiiodomethane > bromoiodoacetic acid > *E*-2-iodo-3-methylbutenedioic acid > (*E*)-3-bromo-3-iodo-propenoic acid > (*E*)-3-bromo-2-iodopropenoic acid. In general, compounds that contain an iodo-group have enhanced mammalian cell cytotoxicity and genotoxicity as compared to their brominated and chlorinated analogues.

## Introduction

In a recent Nationwide Disinfection Byproduct (DBP) Occurrence Study (*1*), iodo-acids were identified for the first time as DBPs in drinking water disinfected with chloramines (*1, 2*). The iodo-acids included iodoacetic acid, bromoiodoacetic acid, (*Z*)-3-bromo-3-iodo-propenoic acid, (*E*)-3-bromo-3-iodo-propenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid (Figure 1). Gas chromatography (GC) with low- and high-resolution electron ionization (EI)-mass spectrometry (MS) was used to identify them, and they were confirmed through a match of mass spectra and GC retention times using authentic chemical standards (*2*). There is concern about these new iodo-acid DBPs because iodoacetic acid is highly cytotoxic and more genotoxic in mammalian cells than bromoacetic acid, the most genotoxic of the regulated haloacetic acids (HAAs) (*2*). Additionally, iodoacetic acid causes developmental abnormalities in mouse embryos (*3, 4*).

Iodo-trihalomethanes (iodo-THMs) have been predicted to be more toxic than chlorinated and brominated THMs (*5*), which are currently regulated in the United States (*6*). Iodo-THMs were included in the Nationwide DBP Occurrence Study and were highest in drinking waters treated with chloramines (*1*). In one plant, the summed concentrations of the six iodo-THMs was 81% of the sum of the four regulated THMs (*1*). Even though iodo-THMs have been known as DBPs since the mid-1970s (*7, 8*), there have been very few measurements in drinking water (*1, 9, 10*), and virtually no toxicity data.

Because chloramines produce significantly lower levels of the regulated THMs and HAAs (*11, 12*), many drinking water treatment plants in the United States have switched from chlorine to chloramines. However, evidence indicates that the formation of iodinated DBPs may be higher with chloramination than with chlorination (*1, 10, 13*). Our goals were to develop an analytical method to quantify five iodo-acids in drinking water, measure their occurrence in several waters treated with chloramination, and investigate the effect

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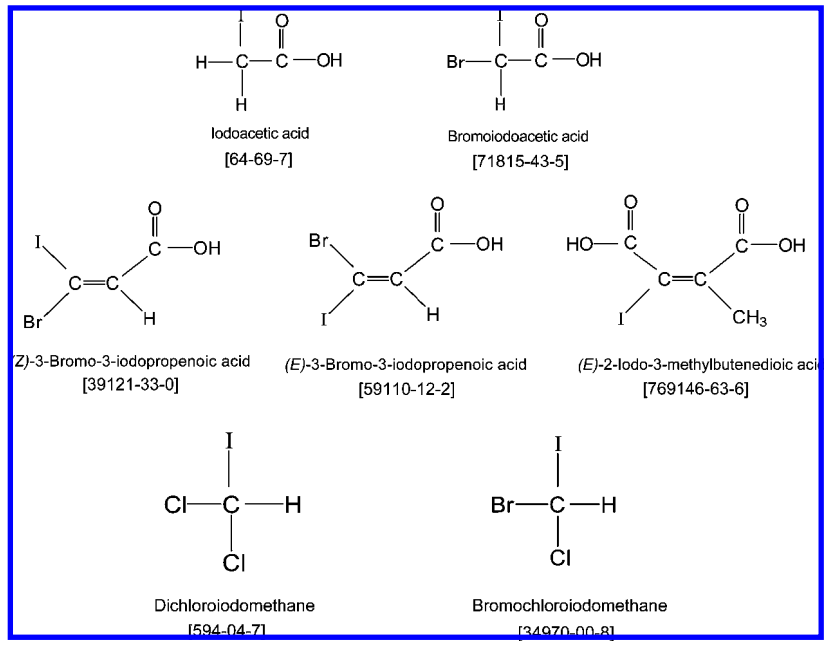


**FIGURE 1. Structures and CAS numbers of iodo-acids and iodo-THMs identified as DBPs in chloraminated drinking water and measured in this study.**

of free chlorine contact time on their formation; dichloroiodomethane and bromochloroiodomethane were also measured for comparison. The mammalian cell toxicity of seven synthesized iodo-acids and the six iodo-THMs was also investigated.

## Materials and Methods

**Chemicals and Reagents.** Bromoiodoacetic acid, diiodoacetic acid, (*E*)-3-bromo-3-iodo-propenoic acid, (*Z*)-3-bromo-3-iodo-propenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid were synthesized at purities of 97, 99, 97, 78, and 100%, respectively; their synthesis is reported elsewhere (*2*). (*E*)-3 bromo-2-iodopropenoic acid was prepared by the addition of iodine monobromide to propiolic acid (*14*). The purity was 93%. The methyl esters of iodoacetic acid, bromoiodoacetic acid, (*E*)-3-bromo-3-iodo-propenoic acid, (*Z*)-3-bromo-3-iodo-propenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid were synthesized at purities of 99, 90, 96.3, 60, and 95%, respectively (Supporting Information). Deuterated standards of bromochloroiodomethane and dichloroiodomethane (CDBrClI and $CDCl_2I$) were synthesized; their syntheses are reported elsewhere (*15*). All other chemicals were purchased at the highest level of purity from Sigma-Aldrich (Milwaukee, WI), VWR Scientific (Marietta, GA), ThermoFisher Scientific (Atlanta, GA), or CanSyn Chem. Corp. (Toronto, ON, Canada).

**Drinking Water Samples.** Drinking water samples were collected from full-scale water treatment plants in the United States (22 cities, representing 9 states and 6 geographic regions) and Canada (1 city). Twenty-one plants used chloramination for disinfection. Two plants used chlorination only; however, the source water for one plant contained substantial levels of natural ammonia, so chloramines were formed immediately. The initial sampling included 5 cities (May 2005); 22 cities in fall−winter 2005; and all 23 cities for the final 2006 sampling. Samples were collected headspace-free in 2-L Teflon bottles and shipped overnight to the U.S. EPA (Athens, GA) in coolers with icepacks. Four liters of finished water and two liters of raw, untreated source water were collected from each plant. Samples for iodo-acid analysis were extracted and derivatized immediately upon receipt of samples. Once derivatized, the iodo-acid methyl esters are quite stable when stored in the refrigerator in the dark. Because iodine-containing compounds can be light-sensitive (especially in their pure form), the extracts were stored in amber vials.

**Iodo-Acid Analysis.** Methods developed for quantifying the iodo-acids were similar to EPA Method 552.3 (*16*). Water samples (1 L) were acidified to pH 0.5, extracted using liquid−liquid extraction, methylated, and analyzed using GC/negative chemical ionization (NCI)-MS (selected ion monitoring of $m/z$ 127, representing the iodine fragment ion). Analyses were carried out on either an Agilent 6890 GC coupled to a 5973 MSD mass spectrometer (first two samplings) or an Agilent 6890 GC coupled to a Waters Micromass high resolution Autospec mass spectrometer (third sampling). Measurements were carried out in duplicate; method detection limits (MDLs) ranged from 0.20 to 20 ng/L (ppt). Further details are presented in the Supporting Information.

**Iodo-THM Analysis.** Dichloroiodomethane and bromochloroiodomethane were extracted from headspace above the water sample using solid-phase microextraction with a carboxen-polydimethylsiloxane fiber (*15*). GC/high resolution-EI-MS (10,000 resolution; multiple ion monitoring) was used with isotope dilution (deuterated standards of each analyte) for quantification. Analyses were carried out using an Agilent 6890 GC coupled to a Thermo MAT 95XP high-resolution magnetic sector mass spectrometer (*15*). Duplicate measurements resulted in an MDL of 2 ng/L for both iodo-THMs. Regulated THMs were also measured for comparison using the same method (isotope dilution with $^{13}C$ standards of each THM).

**Iodide Analysis.** Iodide was determined by cathodic stripping square wave voltammetry (*17*). Samples were filtered through 25-mm diameter, 0.45-$\mu$m pore size Nucleopore GD/XP Filters (Whatman, Florham Park, NJ). A model 310 hanging drop mercury electrode was used (Princeton Applied Research, Oak Ridge, TN) in conjunction with an AIS DLK100 electrochemical analyzer (Analytical Instrument Systems, Flemington, NJ) controlled via a laptop computer running AIS software. The scan was from −0.1 to −0.90 V, with a deposition potential of −0.1 V vs SCE, deposition time 30 s, equilibration time 5 s, scan rate 200 mV $s^{-1}$, pulse height 20 mV (for iodide concentrations lower than 10 nM, deposition time was increased to 90 s). The iodide wave appeared at −0.33 V. Calibration was with standard additions of a KI

solution. Measurements were performed in triplicate; the MDL was 0.13 $\mu$g/L (1.0 nM). Iodide was measured in the third sampling only.

**Other Chemical Analyses.** Methods for measuring bromide, total organic carbon (TOC), total organic halogen (TOX), UV absorbance, the nine chloro-bromo-HAAs (chloro-, bromo-, dichloro-, dibromo-, bromochloro-, bromodichloro-, dibromochloro-, trichloro-, and tribromoacetic acid [HAA9]), and the regulated THMs (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane) are provided in the Supporting Information. TOC was measured in all samplings, bromide and TOX were measured in the last 2 samplings, and UV absorbance and HAA9 (10 plants) were measured in the third sampling only.

**Chinese Hamster Ovary Cells.** Chinese hamster ovary (CHO) cells, line AS52, clone 11-4-8 were used for toxicity studies (*18*). This cell line that was used in previous DBP toxicity studies was maintained in Ham's F12 medium containing 5% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 5% $CO_2$.

**Mammlian Cell Cytotoxicity.** This assay measures the reduction in cell density as a function of DBP concentration over a period of approximately three cell divisions (72 h) (*2, 19*−*23*). This system was calibrated (*23*), and detailed procedures are in the Supporting Information. In general, within each experiment, 10 DBP concentrations were analyzed with 8 replicates per concentration. Each experiment was repeated 2−3×. A concentration−response curve was generated and regression analysis was used to calculate the $\%C^1/_2$ value, which is analogous to a $LC_{50}$ value. The $\%C^1/_2$ value is the DBP concentration that induced a cell density that was 50% of the negative control. A one-way analysis of variance (ANOVA) test was conducted to determine whether the DBP induced a significant level of cell killing (*24*). If a significant $F$ value ($P \leq 0.05$) was obtained, a Holm−Sidak multiple comparison versus the control group analysis was conducted. The power of the test statistic was maintained as ≥0.8 at $\alpha = 0.05$.

**Mammalian Cell Genotoxicity.** Single-cell gel electrophoresis (SCGE) quantitatively measures genomic DNA damage induced in individual nuclei of treated cells (*23*). This assay was used in previous DBP genotoxicity studies (*2, 19*−*23*, Supporting Information). CHO cells were exposed to a DBP for 4 h at 37 °C, 5% $CO_2$. Each experiment included a negative control, a positive control (3.8 mM ethylmethanesulfonate), and 9 DBP concentrations. The concentration range was determined by measuring acute cytotoxicity with trypan blue vital dye (*26*). After treatment, cells were harvested, embedded in an agarose microgel, and lysed; the DNA was denatured and electrophoresed under alkaline conditions. Each concentration was evaluated with 2 microgels and 25 randomly chosen nuclei per microgel. The experiments were repeated 3 times and usually had a total of 6 microgels per concentration. Using Komet 3.1 software, the primary measure of DNA damage was the tail moment (integrated value of DNA density multiplied by migration distance). Within the concentration range that allowed for 70% or greater viable cells, a concentration−response curve was generated, and a regression analysis was used to fit the curve. The SCGE genotoxic potency value was determined as the midpoint of this curve. The median tail moment value for each microgel was determined, and the data were averaged among all of the microgels for each DBP concentration. Averaged median values express a normal distribution according to the central limit theorem (*24*). The averaged median tail moment values were analyzed with a one-way ANOVA test (*27*). If a significant $F$ value ($P \leq 0.05$) was obtained, a Holm−Sidak multiple comparison versus the control group analysis was conducted. The power of the test statistic was maintained as ≥0.8 at $\alpha = 0.05$.

## Results

**Overview.** The cities chosen for this study were expected to have bromide/iodide in their source waters (based on levels of regulated brominated THMs and HAAs). The majority used chloramination for treatment (primarily to lower the levels of regulated THMs and HAAs), with two chlorination plants for comparison. In practice, chloramination plants produce monochloramine (the active disinfectant) by reacting chlorine and ammonia at ratios of approximately 4:1 or 5:1 chlorine to ammonia-nitrogen (by weight). In our study, some water treatment plants added ammonia and chlorine simultaneously, others allowed an amount of free chlorine contact time before adding ammonia, and some added ammonia only at the very end. One plant (Plant 19) had natural ammonia in its source water (at 1.2 mg/L), so that when chlorine was added, chloramines were immediately formed, resulting in chloramine levels similar to other chloramination plants in this study (2 mg/L). There was a broad range of TOC levels in the source waters (0.7−16.1 mg/L), with an average of 5.0 mg/L. Specific UV absorbance (SUVA) levels ranged from 1.3 to 4.9 L/mg-m (Supporting Information). SUVA was measured because it provides an indication of humic content in the natural organic matter (NOM) and is often a good predictor of the levels of DBPs formed (*28*).

Bromide ranged from 24 to 1120 $\mu$g/L (median of 109 $\mu$g/L) in source waters; iodide ranged from 0.4 to 104.2 $\mu$g/L (when detected) (median of 10.3 $\mu$g/L). Higher bromide and iodide levels would be expected in source waters from coastal cities (due to salt water intrusion). However, the highest iodide level was not from a coastal city, and five of the six inland plants had iodide levels above 10 $\mu$g/L. Generally, source waters with the highest bromide levels contained the highest iodide levels, however, there was not a consistent correlation. The molar iodide/bromide ratios ranged from 0.24 to 21.6%, and the corresponding weight ratios varied from 0.42 to 34.7%. On average, the iodide was 4.3% (molar ratio) of the bromide, and 7.5% by weight. This variation in the bromide/:iodide ratio is not unexpected, as salt deposits can change composition as they dissolve and reprecipitate over time (*29, 30*). Because bromide is a conservative species in natural waters compared to iodine, which undergoes various redox and biological processes that can change its behavior and ratio to bromine, bromide and iodide levels vary according to location. In particular, iodide can be found in excess due to bacterial decomposition of organic matter containing iodine (*31*).

**Occurrence of Iodo-Acids and Iodo-THMs.** The iodo-acids and iodo-THMs were found in finished drinking waters from most of the plants, at ng/L (ppt) to low-$\mu$g/L (ppb) levels, with a high of 1.7 $\mu$g/L (iodoacetic acid), 1.4 $\mu$g/L (bromoiodoacetic acid), 0.50 $\mu$g/L ((*Z*)-3-bromo-3-iodopropenoic acid), 0.28 $\mu$g/L ((*E*)-3-bromo-3-iodo-propenoic acid), 0.58 $\mu$g/L ((*E*)-2-iodo-3-methylbutenedioic acid), 10.2 $\mu$g/L (bromochloroiodomethane), and 7.9 $\mu$g/L (dichloroiodomethane). Data from the third sampling are shown in Table 1; other data are available in the Supporting Information.

The iodo-acids and iodo-THMs were generally not detected in the corresponding source waters. Iodo-THM concentrations were higher than those of the corresponding iodo-acids (Table 1), and iodo-THM levels were higher than most measurements reported previously (including the Nationwide DBP Occurrence Study (*1*)). This is likely because this study focused on chloramination plants, where iodo-DBP formation is expected to be the highest (*2, 10, 13*).

An example of a GC/MS chromatogram is shown in the Supporting Information. It is interesting to note that the methyl ester of bromochloroacetic acid, a commonly measured HAA, also forms an $m/z$ 127 ion and as a result, is detected with the iodinated compounds (eluting just after

**TABLE 1. Concentrations of Iodo-Acids, Iodo-THMs, TOX, TOC, Bromide, and Iodide in the Third Sampling Event (2006), in $\mu$g/L[a]**

| plant | disinfectant | TOX ($\mu$g/L as Cl) | TOC (mg/L) | bromide | iodide | IAA | BIAA | *Z* | *E* | diacid | BCIM | DCIM | sum iodo-acids | sum iodo-THMs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | $NH_2Cl$ | 138 | 5.1 | 699 | 65.0 | 0.093 | 0.29 | 0.085 | 0.28 | 0.064 | 5.4 | 1.5 | 0.81 | 6.9 |
| **2** | $NH_2Cl$ | 99 | 5.6 | 133 | 1.0 | 0.015 | 0.091 | 0.050 | 0.13 | 0.085 | 1.4 | 3.5 | 0.37 | 4.9 |
| 3 | $NH_2Cl$ | 113 | 4.2 | 230 | 10.3 | 0.002 | 0.013 | 0.008 | 0.005 | 0.002 | 0.64 | 0.40 | 0.03 | 1.0 |
| 4 | $NH_2Cl$ | 259 | 3.3 | 96 | ND (<0.13) | 0.031 | 0.028 | 0.010 | 0.019 | 0.009 | 0.42 | 0.77 | 0.10 | 1.2 |
| 5 | $NH_2Cl$ | 117 | 4.2 | 230 | 10.3 | 0.002 | 0.003 | 0.003 | 0.005 | 0.002 | NA | NA | 0.02 | NA |
| **6** | $NH_2Cl$ | 22 | 3.3 | 96 | 0.4 | 0.007 | 0.026 | 0.032 | 0.013 | 0.027 | 0.23 | 1.1 | 0.11 | 1.3 |
| 7 | $NH_2Cl$ | 232 | 3.4 | 105 | ND (<0.13) | 0.014 | 0.005 | 0.010 | 0.003 | 0.004 | 0.13 | 0.30 | 0.04 | 0.43 |
| 8 | $NH_2Cl$ | 60 | 5.1 | 67 | ND (<0.13) | 0.008 | 0.008 | 0.011 | 0.009 | 0.006 | 0.10 | 0.15 | 0.04 | 0.25 |
| 9 | $NH_2Cl$ | 116 | 0.7 | 277 | 1.9 | 0.023 | 0.13 | 0.019 | 0.015 | 0.052 | 0.20 | ND (<0.062) | 0.24 | 0.20 |
| 10 | $Cl_2$ | 89 | 3.5 | 214 | 7.3 | 0.033 | 0.005 | 0.005 | 0.003 | 0.003 | 0.25 | 0.22 | 0.05 | 0.47 |
| 11 | $NH_2Cl$ | 145 | 3.2 | 104 | 1.5 | 0.046 | 0.035 | 0.034 | 0.053 | 0.043 | 0.66 | 1.6 | 0.21 | 2.3 |
| **12** | $NH_2Cl$ | 164 | 5.2 | 204 | 10.3 | 0.078 | 0.048 | 0.003 | 0.032 | 0.031 | 0.98 | 5.1 | 0.19 | 6.1 |
| **13** | $NH_2Cl$ | 211 | 5.1 | 186 | 22.3 | 0.070 | 0.044 | 0.010 | 0.037 | 0.028 | 2.1 | 5.7 | 0.19 | 7.8 |
| 14 | $NH_2Cl$ | 119 | 2.9 | 107 | 1.1 | 0.015 | 0.008 | 0.008 | 0.008 | 0.010 | 0.42 | 0.91 | 0.05 | 1.3 |
| **15** | $NH_2Cl$ | 73 | NR | 107 | ND (<0.13) | 0.050 | 0.056 | 0.038 | 0.013 | 0.012 | 0.19 | 2.2 | 0.17 | 2.4 |
| 16 | $NH_2Cl$ | 273 | 6.1 | 24 | ND (<0.13) | 0.061 | 0.013 | 0.003 | 0.001 | 0.003 | 0.062 | 0.35 | 0.08 | 0.41 |
| **17** | $NH_2Cl$ | 41 | 3.9 | NR | 22.4 | 0.018 | 0.038 | 0.064 | 0.11 | 0.048 | 3.5 | 0.64 | 0.28 | 4.1 |
| 18 | $NH_2Cl$ | 116 | 3.2 | 35 | 10.4 | 0.033 | 0.035 | 0.009 | 0.005 | 0.009 | ND (<0.062) | 0.46 | 0.09 | 0.46 |
| **19** | $Cl_2$ (but natural ammonia present to form $NH_2Cl$) | 152 | 5.0 | 300 | 104.2 | 0.67 | 0.29 | 0.082 | ND (<0.020) | 0.017 | 0.72 | 1.1 | 1.1 | 1.8 |
| 20 | $NH_2Cl$ | 52 | 16.1 | 193 | ND (<0.13) | 0.022 | 0.048 | 0.011 | ND (<0.020) | 0.031 | 0.33 | 0.97 | 0.11 | 1.3 |
| 21 | $NH_2Cl$ | 96 | 5.3 | 65 | 0.4 | 0.082 | 0.051 | 0.014 | 0.023 | 0.010 | 0.14 | 0.36 | 0.18 | 0.50 |
| 22 | $NH_2Cl$ | 138 | 1.4 | 103 | 10.8 | 0.040 | 0.045 | 0.024 | 0.007 | 0.016 | 0.34 | 0.64 | 0.13 | 0.98 |
| 23 | $NH_2Cl$ | 99 | 2.6 | 37 | 2.7 | ND (<0.0002) | 0.007 | 0.012 | 0.004 | 0.004 | ND (<0.062) | 0.090 | 0.03 | 0.09 |

[a] Concentrations represent the average of duplicate measurements. Plant numbers in bold indicate chloramination plants with very short free chlorine contact times (<1 min). ND = not detected; NA = not analyzed; NR = not reported. IAA = iodoacetic acid, BIAA = bromoiodoacetic acid, *Z* = (*Z*)-3-bromo-3-iodopropenoic acid, *E* = (*E*)-3-bromo-3-iodopropenoic acid, Diacid = (*E*)-2-iodo-3-methylbutenedioic acid, BCIM = bromochloroiodomethane, DCIM = dichloroiodomethane. TOC, bromide, and iodide were measured in raw source waters; Iodo-DBPs were measured in finished drinking waters. Drinking water samples were collected from the distribution system for Plants 7, 9, 11, 13, 20, and 22; other finished drinking water samples were from the plant effluent.

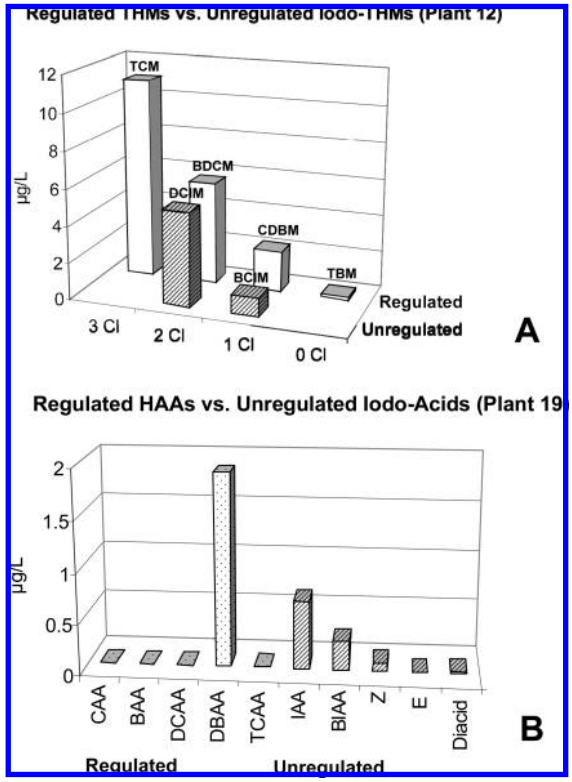


**FIGURE 2. (A) Regulated THMs vs unregulated iodo-THMs for Plant 12 (third sampling; for the iodo-THMs, only DCIM and BCIM were measured in this study); (B) regulated haloacetic acids vs unregulated iodo-acids for Plant 19 (third sampling). Legend: TCM = trichloromethane (chloroform), BDCM = bromodichloromethane, CDBM = chlorodibromomethane, TBM = tribromomethane (bromoform), DCIM = dichloroiodomethane, BCIM = bromochloroiodomethane, CAA = chloroacetic acid, BAA = bromoacetic acid, DCAA = dichloroacetic acid, DBAA = dibromoacetic acid, TCAA = trichloroacetic acid, IAA = iodoacetic acid, BIAA = bromoiodoacetic acid, *Z* = (*Z*)-3-bromo-3-iodopropenoic acid, *E* = (*E*)-3-bromo-3-iodopropenoic acid, and diacid = (*E*)-2-iodo-3-methylbutenedioic acid.**

iodoacetic acid methyl ester). Bromochloroiodomethane elutes just before iodoacetic acid methyl ester.

The sum of the 5 iodoacetic acids (IAA5) ranged from 0.02% (Plant 5) to 23% (Plant 19) of the HAA9 levels. The ratio of the 2 iodo-THMs measured (ITHM2) to the 4 regulated THMs (THM4) ranged from 0.5% (Plant 16) to 43% (Plant 19). Figure 2 provides an illustration of regulated THMs vs unregulated iodo-THMs and regulated HAAs (HAA5) vs unregulated iodo-acids. It is also of note that iodo-acids and iodo-THMs were detected at all six plants that had no detectable iodide in their source waters. It is possible that there were other inorganic or organic sources of iodine beyond the inorganic iodide measured in the source waters.

**Effect of Iodide and Free Chlorine Contact Time on Iodo-DBP Formation.** Generally, increasing iodide levels in the source waters increased iodo-DBP formation (Figure S6, Supporting Information). This trend was more evident with the iodo-acids (where the complete set of identified iodo-acids was measured). It is likely that a similar trend would be observed for the iodo-THMs if the complete set of 6 had been measured.

Previous controlled laboratory studies hypothesized that a longer free chlorine contact time results in decreased formation of iodo-DBPs, due to the rapid oxidation of iodide by chlorine to form iodate (that serves as a sink for iodide) ((*10*), Supporting Information). Conversely, a shorter free chlorine contact time is expected to increase the formation of iodo-DBPs because the oxidation of iodide to iodate by monochloramine is much slower than the reaction with NOM.

Data from this study support this hypothesis (Table 1). The iodo-DBP levels for Plant 10 (chlorine only) were among the lowest measured (0.049 and 0.47 $\mu$g/L, for IAA5 and ITHM2, respectively) even with moderate iodide levels (7.3 $\mu$g/L) and TOC (3.5 mg/L) in its source water. While the SUVA for this water was somewhat lower than that for other plants (1.3 L/mg-m), the regulated DBP concentrations were sufficiently high (among the 4 highest of the 23 plants), such that it is unlikely the lower SUVA value contributed to the low iodo-DBP levels found. Chloramination plants with the longest free chlorine contact times (>45 min) (Plants 7, 8, 18, 20, and 23) had lower levels of iodo-DBPs. For example, Plant 18 had a free chlorine contact time of approximately 1080 min and contained very low levels of iodo-acids (0.09 $\mu$g/L) and iodo-THMs (0.46 $\mu$g/L), despite moderate iodide levels (10.4 $\mu$g/L) in its source water. Chloramination plants with short free chlorine contact times (<1 min) (Plants 1, 2, 6, 12, 13, 15, 17, and 19), had the highest iodo-DBP levels (Table 1). For example, the three plants with highest iodo-acid levels (Plants 1, 17, and 19) had no free chlorine contact time, due to either chlorine and ammonia added simultaneously to form chloramines or to natural ammonia in the source waters (which instantly forms chloramines upon addition of chlorine). This supports the hypothesis that chloramination with short free chlorine contact times maximizes the formation of iodo-DBPs when natural iodide is present in the source waters.

**Mammalian Cell Cytotoxicity and Genotoxicity.** The CHO cell chronic cytotoxicity concentration−response curves for the iodo-acids are presented in Figure 3A. Table 2 presents the concentration range, the lowest concentration that induced a significant increase in toxicity, and the $\%C^1/_2$ ($\sim LC_{50}$) value for each compound. The rank order of the chronic cytotoxicity of the haloacetic acids was IAA > DIAA > BIAA; IAA was approximately 113× and 304× more cytotoxic than DIAA and BIAA, respectively. When compared with other HAAs analyzed under the same conditions, IAA was 3× and 287× more cytotoxic than bromoacetic and chloroacetic acid, respectively (*2*). DIAA was approximately 2× more cytotoxic than dibromoacetic acid and 34× more cytotoxic than dichloroacetic acid (*23*). The HAAs with at least one iodo group were 1.3× more cytotoxic than HAAs with bromo or bromo-chloro groups; this factor increased to 24× when compared with HAAs containing only chloro groups (*2, 23, 32*). Among the other iodo-acids analyzed, the descending rank order of chronic cytotoxicity was *E*3B2IPPA > *E*3B3IPPA > *Z*3B3IPPA > *E*2I3MBDA. Of interest was the finding that the *E* isomer of 3-bromo-3-iodopropenoic acid was more cytotoxic than the *Z* isomer (Table 2). Iodoform was the most potent cytotoxic iodomethane analyzed (Figure 3B). The rank order for cytotoxicity of the iodo-THMs was IF > BDIM > DBIM > BCIM ≈ CDIM > DCIM (Table 2). The iodo and iodo-bromo-THMs were more toxic than their iodo-chloro analogues. Compared to bromoform and chloroform, IF was 60× and 146× more cytotoxic, respectively (*32*). BDIM was 8× more cytotoxic than bromodichloromethane; DBIM was 3× more cytotoxic than chlorodibromomethane (*32*). Clearly cytotoxicity is enhanced in both the haloacid and halomethane DBPs containing one or more iodo-groups.

Under the conditions of the CHO cell SCGE assay, *Z*3B3IPPA, IF, DBIM, DCIM, BCIM, and BDIM were not genotoxic (Table 3). The concentration−response curves of the 7 genotoxic iodinated compounds are presented in Figure 4. Using SCGE genotoxic potency values, the rank order of the iodo-acetic acids was IAA ≫ DIAA > BIAA, and *E*2I3MBDA > *E*3B3IPPA > *E*3B2IPPA for the other iodo-acids. IAA was approximately 2× and 47× more genotoxic than bromoacetic

**FIGURE 3. Concentration−response curves of mammalian cell cytotoxicity of iodo-acids (A) and iodo-THMs (B) (72 h exposure time). IAA = iodoacetic acid, DIAA = diiodoacetic acid, BIAA = bromoiodoacetic acid, *E*3B3IPPA = (*E*)-3-bromo-3-iodopropenoic acid, *Z*3B3IPPA = (*Z*)-3-bromo-3-iodopropenoic acid, *E*3B2IPPA = (*E*)-3-bromo-2-iodopropenoic acid, *E*2I3MBDA = (*E*)-2-iodo-3-methylbutenedioic acid, IF = iodoform, BDIM = bromodiiodomethane, DCIM = dichloroiodomethane, BCIM = bromochloroiodomethane, DBIM = dibromoiodomethane, CDIM = chlorodiiodomethane.**

and chloroacetic acid, respectively (*2*). In a carbon−halogen bond, iodine is a stronger leaving group than bromine and chlorine. The SN2 reactivity of an alkyl iodide is 3−5× greater than an alkyl bromide, and is ∼50× greater than an alkyl chloride (*33*). The relative SN2 reactivity and the genotoxicity of the monohaloacetic acids are very similar. Haloacetic acids have been examined for developmental toxicity in mammalian whole embryo cultures (*3, 4, 34*). The pattern of potency in the induction of mouse neural tube damage (*3, 4, 34*) was highly correlated with cytotoxicity ($r = 0.82$) and genotoxicity ($r = 0.83$) in CHO cells. From the present study, an interesting observation was that the *E* isomer of 3-bromo-3-iodopropenoic acid was genotoxic, while the *Z* isomer was not (Table 3). Except for CDIM, the iodo-THMs were not genotoxic, which may be due to solubility or metabolism (Table 3). Brominated THMs require glutathione-*S*-transferase-theta1-1 (GSTT1-1) mediated metabolism to form mutagenic intermediates (*35, 36*). It is not known whether the CHO cells in this study express GSTT1-1 (*37*). In previous studies, other brominated and chlorinated halomethanes were not genotoxic in CHO cells (*32*). Since CDIM is genotoxic, it may be through a different metabolic activation pathway than the other iodo-THMs analyzed. Generally, haloacid and halomethane DBPs that contain an iodo-group are more cytotoxic and genotoxic in mammalian cells than their brominated and chlorinated analogues (*32*). This order of toxicity correlates with the leaving tendency of the halogens in SN2 reactions: I > Br ≫ Cl.

**Future Research and Impact on Drinking Water Treatment.** The analytical and toxicology data provided through this study are important for prioritizing future health effects

**TABLE 2. Comparison of the CHO Cell Chronic Cytotoxicity of the Iodo-Acids and Iodo-THMs Analyzed in This Study**

| chemical | concentration range (M) | lowest tox. conc. (M)[a] | %C$^1/_2$ (M)[b] | $R^2$ [c] | ANOVA test statistic[d] | abbreviation |
|---|---|---|---|---|---|---|
| iodoacetic acid | $1-120 \times 10^{-7}$ | $5.00 \times 10^{-7}$ | $2.95 \times 10^{-6}$ | 0.97 | $F_{13, 122} = 54.7$; $P \leq 0.001$ | IAA |
| diiodoacetic acid | $1-1000 \times 10^{-6}$ | $1.00 \times 10^{-4}$ | $3.32 \times 10^{-4}$ | 0.97 | $F_{20, 239} = 49.9$; $P \leq 0.001$ | DIAA |
| bromoiodoacetic acid | $1-25 \times 10^{-4}$ | $2.50 \times 10^{-4}$ | $8.97 \times 10^{-4}$ | 0.96 | $F_{20, 239} = 59.2$; $P \leq 0.001$ | BIAA |
| (*Z*)-3-bromo-3-iodopropenoic acid | $1-100 \times 10^{-5}$ | $7.50 \times 10^{-5}$ | $2.08 \times 10^{-4}$ | 0.98 | $F_{13, 247} = 68.8$; $P \leq 0.001$ | *Z*3B3IPPA |
| (*E*)-3-bromo-3-iodopropenoic acid | $3-1000 \times 10^{-6}$ | $2.50 \times 10^{-5}$ | $1.45 \times 10^{-4}$ | 0.99 | $F_{13, 250} = 101.7$; $P \leq 0.001$ | *E*3B3IPPA |
| (*E*)-3-bromo-2-iodopropenoic acid | $5-3700 \times 10^{-6}$ | $1.75 \times 10^{-5}$ | $4.36 \times 10^{-5}$ | 0.98 | $F_{13, 137} = 106.8$; $P \leq 0.001$ | *E*3B2IPPA |
| (*E*)-2-iodo-3-methylbutenedioic acid | $5-20 \times 10^{-4}$ | $7.00 \times 10^{-4}$ | $9.44 \times 10^{-4}$ | 0.98 | $F_{11, 255} = 55.2$; $P \leq 0.001$ | *E*2I3MBDA |
| iodoform | $1-40 \times 10^{-5}$ | $1.00 \times 10^{-5}$ | $6.60 \times 10^{-5}$ | 0.91 | $F_{13, 250} = 84.2$; $P \leq 0.001$ | IF |
| dibromoiodomethane | $1-250 \times 10^{-5}$ | $1.50 \times 10^{-3}$ | $1.90 \times 10^{-3}$ | 0.92 | $F_{18, 102} = 42.8$; $P \leq 0.001$ | DBIM |
| dichloroiodomethane | $5-47.5 \times 10^{-4}$ | $2.00 \times 10^{-3}$ | $4.13 \times 10^{-3}$ | 0.93 | $F_{11, 177} = 57.4$; $P \leq 0.001$ | DCIM |
| bromochloroiodomethane | $5-24 \times 10^{-4}$ | $2.20 \times 10^{-3}$ | $2.40 \times 10^{-3}$ | 0.95 | $F_{9, 155} = 12.8$; $P \leq 0.001$ | BCIM |
| bromodiiodomethane | $5-25 \times 10^{-4}$ | $1.50 \times 10^{-3}$ | $1.40 \times 10^{-3}$ | 0.90 | $F_{10, 89} = 27.9$; $P \leq 0.001$ | BDIM |
| chlorodiiodomethane | $5-40 \times 10^{-4}$ | $1.00 \times 10^{-3}$ | $2.41 \times 10^{-3}$ | 0.92 | $F_{11, 172} = 25.3$; $P \leq 0.001$ | CDIM |

[a] Lowest toxic concentration was the lowest concentration of the iodoacid or iodomethane in the concentration−response curve that induced a significant reduction in cell density as compared to the negative control. [b] The %C$^1/_2$ value (∼$LC_{50}$) is the concentration of the compound determined from a regression analysis of the data, that induced a cell density of 50% as compared to the concurrent negative control. [c] $R^2$ is the coefficient of determination for the regression analysis upon which the %C$^1/_2$ 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.

**TABLE 3. Comparison of SCGE Genomic DNA Damage in CHO Cells Induced by the Iodo-Acids and Iodo-THMs Analyzed in This Study**

| chemical | concentration range (M) | lowest genotox. conc. (M)[a] | SCGE genotox. potency value (M)[b] | $R^2$ [c] | ANOVA test statistic[d] | abbreviation |
|---|---|---|---|---|---|---|
| iodoacetic acid | $1-20 \times 10^{-6}$ | $5.00 \times 10^{-6}$ | $8.70 \times 10^{-6}$ | 0.99 | $F_{9,\ 49} = 33.9$; $P \leq 0.001$ | IAA |
| diiodoacetic acid | $1-700 \times 10^{-5}$ | $1.00 \times 10^{-3}$ | $1.98 \times 10^{-3}$ | 0.97 | $F_{13,\ 60} = 13.2$; $P \leq 0.001$ | DIAA |
| bromoiodoacetic acid | $5-800 \times 10^{-5}$ | $2.50 \times 10^{-3}$ | $3.16 \times 10^{-3}$ | 0.97 | $F_{14,\ 43} = 14.0$; $P \leq 0.001$ | BIAA |
| (*Z*)-3-bromo-3-iodopropenoic acid | $5-100 \times 10^{-4}$ | NS[e] | NS[e] | - | $F_{9,\ 30} = 0.86$; $P = 0.42$ | *Z*3B3IPPA |
| (*E*)-3-bromo-3-iodopropenoic acid | $5-100 \times 10^{-4}$ | $5.00 \times 10^{-3}$ | $6.35 \times 10^{-3}$ | 0.98 | $F_{10,\ 29} = 27.3$; $P \leq 0.001$ | *E*3B3IPPA |
| (*E*)-3-bromo-2-iodopropenoic acid | $1-80 \times 10^{-4}$ | $7.50 \times 10^{-3}$ | $7.58 \times 10^{-3}$ | 0.98 | $F_{9,\ 28} = 8.4$; $P \leq 0.001$ | *E*3B2IPPA |
| (*E*)-2-iodo-3-methylbutenedioic acid | $0.5-7.5 \times 10^{-3}$ | $6.00 \times 10^{-3}$ | $6.00 \times 10^{-3}$ | 0.98 | $F_{12,\ 53} = 20.7$; $P \leq 0.001$ | *E*2I3MBDA |
| iodoform | $1-50 \times 10^{-5}$ | NS[e] | NS[e] | - | $F_{10,\ 39} = 0.54$; $P = 0.85$ | IF |
| dibromoiodomethane | $5-100 \times 10^{-4}$ | NS[e] | NS[e] | - | $F_{9,\ 30} = 0.77$; $P = 0.65$ | DBIM |
| dichloroiodomethane | $0.5-10 \times 10^{-3}$ | NS[e] | NS[e] | - | $F_{9,\ 29} = 1.52$; $P = 0.19$ | DCIM |
| bromochloroiodomethane | $5-100 \times 10^{-4}$ | NS[e] | NS[e] | - | $F_{9,\ 30} = 0.70$; $P = 0.70$ | BCIM |
| bromodiiodomethane | $0.5-10 \times 10^{-3}$ | NS[e] | NS[e] | - | $F_{16,\ 43} = 0.77$; $P = 0.71$ | BDIM |
| chlorodiiodomethane | $0.5-7 \times 10^{-3}$ | $2.00 \times 10^{-3}$ | $2.95 \times 10^{-3}$ | 0.96 | $F_{10,\ 39} = 38.4$; $P \leq 0.001$ | CDIM |

[a] The lowest genotoxic concentration was the lowest concentration of the iodoacid or iodomethane in the concentration–response curve that induced a significant amount of genomic DNA damage as compared to the negative control. [b] The SCGE genotoxic potency value is the concentration that was calculated, using regression analysis, at the midpoint of the curve within the concentration range that expressed above 70% cell viability. [c] $R^2$ is the coefficient of determination for the regression analysis upon which the genotoxic potency 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] Not significantly different from the negative control.




**FIGURE 4. Concentration–response curves of mammalian cell genotoxicity of iodo-DBPs (4 h treatment time). Iodoform, bromochloroiodomethane, dibromoiodomethane, dichloroiodomethane, bromodiiodomethane, and (*Z*)-3-bromo-3-iodopropenoic acid were not genotoxic. IAA = iodoacetic acid, DIAA = diiodoacetic acid, BIAA = bromoiodoacetic acid, *E*3B3IPPA = (*E*)-3-bromo-3-iodopropenoic acid, *E*3B2IPPA = (*E*)-3-bromo-2-iodopropenoic acid, *E*2I3MBDA = (*E*)-2-iodo-3-methylbutenedioic acid, CDIM = chlorodiiodomethane.**

research for these DBPs. Because some of these iodo-acids and iodo-THMs are significantly genotoxic and/or cytotoxic to mammalian cells (iodoacetic acid is the most genotoxic DBP in mammalian cells studied to-date), new in vivo toxicology studies and human cell toxicogenomic studies (*38*) are currently being planned. A controlled laboratory study is also planned to understand the kinetics and mechanism of iodo-acid DBP formation with chloramination.

Overall, the levels of iodo-DBPs were reasonably low (the highest iodo-acid concentration was 1.7 $\mu$g/L, and the highest iodo-THM concentration was 10.2 $\mu$g/L, but most levels were sub-ppb or ppt). Because these drinking waters were from cities with high iodide/bromide and TOC levels in their source waters, it is likely that the concentrations would capture the upper range of occurrence for these iodo-DBPs. Despite the increased formation of iodo-DBPs with chloramination in these plants, it should be noted that if these plants only use chlorine for disinfection, it is likely that the regulated maximum contaminant levels (MCLs) would have been exceeded for the regulated THMs and HAAs (due to high TOC levels in many of the source waters). As a result, there can be competing issues such as these—how to maintain regulated DBPs below the required limits and produce quality drinking water that is free of other potentially hazardous byproducts, while at the same time, producing microbially safe drinking water. At this point, we cannot address the human health risk of these iodo-DBPs; further in vivo (whole animal) toxicology research is needed to determine whether these iodo-DBPs that were toxic in vitro would also be toxic in vivo.

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## Supporting Information Available

Synthesis of iodo-acid methyl esters; detailed analytical methods for iodo-acids, HAA9, bromide, TOC, TOX, UV absorbance; occurrence data for first and second sampling; water quality data. This information is available free of charge via the Internet at http://pubs.acs.org.

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