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Chlorination of Source Water Containing Iodinated X-Ray Contrast Media: Mutagenicity and Identification of New Iodinated Disinfection By-products

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Abstract

Iodinated contrast media (ICM) are non-mutagenic agents administered for X-ray imaging of soft tissues. ICM can reach $\mu\text{g/L}$ levels in surface waters because they are administered in high doses, excreted largely un-metabolized, and poorly removed by wastewater treatment. Iodinated disinfection by-products (I-DBPs) are highly genotoxic and have been reported in disinfected waters containing ICM. We assessed the mutagenicity in *Salmonella* of extracts of chlorinated source water containing one of four ICM (iopamidol, iopromide, iohexol, and diatrizoate). We quantified 21 regulated and non-regulated DBPs, 11 target I-DBPs, and conducted a non-target, comprehensive broad-screen identification of I-DBPs. We detected one new iodo-methane (trichloriodomethane), three new iodo-acids (dichloriodoacetic acid, chlorodiodoacetic acid, bromochloriodoacetic acid), and two new nitrogenous I-DBPs (iodoacetoneitrile and chloriodoacetoneitrile). Their formation depended on the presence of iopamidol as the iodine source; identities were confirmed with authentic standards. This is the first identification in simulated drinking water of iodoacetoneitrile, which is highly cytotoxic and genotoxic in mammalian cells. Iopamidol ($5 \mu\text{M}$) altered the concentrations and relative distribution of several DBP classes, increasing total haloacetoneitriles by >10-fold. Chlorination of ICM-containing

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Supporting Information

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source water increased I-DBP concentrations but not mutagenicity, indicating that such I-DBPs were either not mutagenic or at concentrations too low to affect mutagenicity.

Keywords

iodinated disinfection by-products; DBPs; X-ray contrast media; chlorination; drinking water; mutagenicity; *Salmonella*

INTRODUCTION

Disinfecting source water used for drinking water or wastewater prior to discharge into distribution systems or the environment is important for inactivating pathogenic microorganisms. Disinfection by-products (DBPs) are formed by the reaction of oxidizing disinfectants, such as chlorine, chloramines, or chlorine dioxide with natural organic matter (NOM) and/or bromide/iodide in water. Although the use of alternative disinfectants has increased over time, chlorine remains the most frequently used disinfectant for both surface and ground waters in the United States¹. Many of the resulting DBPs are mutagenic, genotoxic, cytotoxic, and/or carcinogenic^{2–5}. Thus, water systems must operate their disinfection procedures to balance inactivating harmful pathogens with forming potentially harmful DBPs.

Epidemiologic studies have found a positive association between exposure to chlorinated water and increased risk for bladder cancer^{6–9}. Increased risks of colon cancer¹⁰ and adverse reproductive and developmental outcomes, including spontaneous abortion, heart defects, low birth weight at term, still birth, and preterm delivery^{11–21} have also been reported in some epidemiologic studies. Although the majority of DBPs that have been evaluated are genotoxic⁴, the iodine-containing DBPs (I-DBPs) are 10-1000 times more potent in Chinese hamster ovary (CHO) cells than their chlorinated and brominated counterparts^{3, 5, 22–25}, raising special concerns regarding waters with high concentrations of I-DBPs. This DBP class was also recently reported to show significant developmental toxicity in marine polychaete^{26–27} and in zebrafish embryos²⁸.

I-DBPs are formed during disinfection of waters containing iodide, including freshwaters impacted by seawater intrusion or fossilized seawater^{3, 5, 29–30}, wastewaters³¹, saline wastewater effluents³², or bromide-rich desalinated seawater³³, and also by iodine-based water disinfection treatments³⁴. Furthermore, they may also form during household cooking processes when using chloraminated tap water and iodized salt²⁷. A number of low- and high-molecular weight I-DBPs have been identified in recent years^{3, 5, 29, 35–40}, and their formation is influenced by a variety of factors, including the levels of iodide present in source waters^{41–42}, molecular size of NOM⁴³, presence of microbial organic matter⁴⁴, and the presence of other iodine sources, such as iodinated X-ray contrast media (ICM)^{45–46}.

ICM are commonly administered for medical imaging of soft tissues; they are administered in high doses (up to 200 g/person/day), excreted mostly unchanged, and poorly removed in wastewater treatment plants. Therefore, they can be present at levels in the µg/L range in surface waters impacted by effluents from wastewater treatment plants^{47–48}. For example,

diatrizoate (DTZ) has been quantified at levels between 20 and 100 µg/L⁴⁷, and ICM have also been detected in finished drinking waters^{46, 49–50}. Among the ICM, iopamidol (IPAM)^{40, 45–46, 51–55}, iohexol (IHX)^{45–46}, iopromide (IPR)^{45–46, 54}, DTZ^{45–46, 54}, iomeprol⁴⁶, iodixanol⁵⁴, and histodenz⁵⁴ have been investigated as potential precursors of I-DBPs during chlorination or chloramination of water. According to these studies, IPAM is the ICM that is most reactive with chlorine. Further, UV irradiation of ICM-containing waters also causes iodide to be released from ICM, increasing the possibility of I-DBP formation during subsequent disinfection treatments^{56–57}.

Under chlorination, IPAM has yielded the highest concentrations of I-DBPs, such as iodine-containing trihalomethanes (I-THMs) and iodine-containing haloacetic acids (I-HAAs). In contrast to chlorination, chloramination has been shown to enhance I-DBP formation in waters containing other ICM⁵⁴; however, the resulting concentrations are lower than those observed for chlorinated IPAM-containing waters.

Although ICM are not mutagenic or generally toxic *per se*^{58–59}, IPAM has been shown to induce DNA damage in CHO cells as measured by the comet assay⁵². In addition, chlorinating or chloraminating source waters containing IPAM increased the level of DNA damage relative to that of source water without IPAM^{45, 52, 58, 60}. Chlorination of source water containing IHX also increased the level of DNA damage relative to that of chlorinated source water without IHX⁵².

To explore this issue further, we determined the DBP concentrations and mutagenicity after laboratory-scale chlorination of source water containing one of four ICM: IPAM, IPR, DTZ, or IHX. We quantitatively analyzed the disinfected waters for 11 I-DBPs and 21 target non-I-DBPs. In addition, we also performed for the first time a non-target, comprehensive, broad-screen analysis in large-volume extracts of waters for detecting unknown I-DBPs that form in ICM-containing waters during chlorination. We determined the mutagenic potencies of the extracts in the *Salmonella* mutagenicity assay using strains TA98 and TA100 with or without metabolic activation (S9). We also used strain RSJ100, which expresses the *GSTT1* gene, to determine whether any portion of the activity was due to the presence of DBPs that are activated by GSTT1 enzyme, such as brominated trihalomethanes (Br-THMs)⁴. This is important because approximately 80% of the U.S. population carries this gene⁶¹, and results from a case-controlled epidemiologic study by Cantor et al.⁶ demonstrated an increased risk for DBP-related bladder cancer for such people who have sufficient exposure to chlorinated water.

This approach has resulted in the most extensive study to-date on the formation of DBPs and mutagenicity resulting from chlorination of ICM-containing waters, encompassing both quantitative analysis of 32 target DBPs and the identification of never-before-reported iodinated DBPs. In addition, this is the first mutagenicity study of chlorinated ICM-containing waters in the presence of NOM, which is an important factor in the formation of DBPs⁴⁵, and the first study to combine complementary chemical and mutagenicity analyses in the comparison of multiple ICMs.

EXPERIMENTAL METHODS

Chemicals and Reagents.

Chemicals and reagents were of the highest purity commercially available. Physical-chemical properties, purity, sources of the ICM and molecular structures are described in Supporting Information (SI) in Table S1 and Figure S1, respectively. Chloriodoacetonitrile was synthesized as described in SI and shown in Figure S2. Relevant information on the other chemicals and reagents used is provided in SI.

Disinfection Reactions.

The source water used in these experiments was collected at a water treatment plant after coagulation, flocculation, sedimentation, and filtration, but prior to disinfection. Water was collected by gravity flow through Teflon™ tubing into 35-gallon linear-low-density polyethylene, open-head barrels (Super Shipper, Bergan Barrel & Drum Co., Kearny, NJ) equipped with Teflon™ liners (24"x 24", open end 31.25", Welch Fluorocarbon Inc., Dover, NH) with a Teflon™ sheet between the water and the barrel lid. After collection, the water was stored at 4 °C in the dark until use. As reported in Table S2, the concentration of total organic carbon (TOC) was 2.1 mg/L, and both bromide and iodide were below their limits of detection.

Large-volume (up to 125 L) chlorination reactions were performed with source water brought to and held at room temperature (21-22°C) (Table S3), headspace-free, in the dark. Reactions were performed for each ICM using an ICM concentration of 5 µM (3.88, 3.95, 4.10, and 3.39 mg/L for IPAM, IPR, IHX, and DTZ, respectively) and chlorine doses of 100 µM (7.09 mg/L) as Cl₂. This ICM:Cl₂ molar ratio was selected based on previous experiments performed with IPAM⁴⁵. An advantage to higher chlorine concentrations than would typically be employed at water treatment plants, along with higher levels of ICM than those typically observed in actual impacted source waters, was the increased likelihood of formation of iodinated DBPs at concentrations above their limits of quantification. Chlorine-demand tests were performed with the source water to confirm that the selected amount of disinfectant was not completely consumed upon reaction time.

Reactions were conducted over 72 h with continuous stirring. Because we discovered that the stirring had ceased and was not continuous during the preparation of the first IPAM-containing water (IPAM1), we prepared a second sample (IPAM2). Consequently, we generated chemistry and mutagenicity data on both IPAM-containing water samples, which provided some opportunity to compare the reproducibility of the preparation method. In all cases, water was buffered with 10-mM phosphate buffer, using H₂SO₄ and NaOH to achieve pH 7.5 prior to disinfection. The pH was monitored with a pH 2100-benchtop meter (Oakton instruments, Vernon Hills, IL). The custom disinfection system used has been described in detail previously⁶².

The *N,N*-diethyl-*p*-phenylenediamine (DPD)-ferrous ammonium sulfate (FAS) titrimetric method⁶³ was used to measure both the chlorine concentration (as mg/L Cl₂) in the disinfectant solution prepared prior to each reaction and the free chlorine concentrations in the water after each experiment (mean residual free chlorine, 1.4 mg/L Cl₂).

XAD Resin Extractions.

DBPs were extracted from measured volumes of treated waters (up to 120 L) using XAD/ethyl acetate as published previously⁶⁴ and described in SI.

QA/QC.

Controls included source waters spiked with each of the four ICM (no disinfectant) and source water disinfected with chlorine (no ICM). In addition to these source water controls, an XAD resin blank (method blank) was also generated by passing Milli-Q® water through the XAD resins and eluting with ethyl acetate. The extract from the method blank was then analyzed for contaminants resulting from impurities in the solvent or the resin. Blanks consisting of disinfected water (Milli-Q® and source water) containing the individual ICM were also processed for chemical characterization of target iodo-DBPs. Efforts to ensure integrity of the source water samples and reduce the possibility of confounding are outlined in the SI (Characteristics of the Source Water).

Quantitative Analyses of 21 Target Non-I-DBPs.

Although only 9 DBPs (4 THMs and 5 HAAs) are required for monitoring by the U.S. EPA for water systems that disinfect with chlorine, we quantified a total of 21 DBPs that are measured routinely for research purposes in disinfected water by EPA Methods 552.2⁶⁵ and 551.1⁶⁶. Analytes included nine chloro-/bromo-acetic acids (HAA9); the four regulated trihalomethanes (THM4); chloral hydrate (CH); two halo ketones (dichloropropanone and trichloropropanone); four haloacetonitriles (dichloro-, bromochloro-, dibromo-, and trichloroacetonitrile); and trichloronitromethane.

Quantitative Analyses of 11 Target I-DBPs.

The 11 target I-DBPs and their physical-chemical properties are listed in Table S4. The unregulated I-DBPs (iodo-trihalomethanes (I-THMs), iodo-acids, and iodoacetaldehyde) were quantified using published methods^{35, 45}. Specific details, such as the instruments used and their operating conditions, are included in SI. Thus, a total of 32 target DBPs were measured quantitatively (21 non-I-DBPs and 11 I-DBPs). Formation of iodate, a non-toxic sink of iodide, was not monitored in the solutions. Previous studies have shown the absence of detectable levels of this inorganic iodine species in chlorinated ICM-containing source waters^{45, 51}.

Analysis of Non-Target I-DBPs.

To detect relevant I-DBPs not included in the list of target compounds, diazomethane-derivatized and non-derivatized XAD resin extracts (blanks and samples) were analyzed by GC-electron ionization (EI)-high-resolution mass spectrometry (HR-MS) using a LECO HRT time-of-flight mass spectrometer (St. Joseph, MI) in full-scan mode. Tentative molecular structures were proposed according to the evidence obtained from the National Institute of Standards and Technology (NIST) and Wiley mass spectral databases, and by manual interpretation when not present in the library. For manual interpretation of mass spectra, the presence of isotopic patterns and elemental composition of the molecular and fragment ions provided by EI-HR-MS data were taken into consideration. Tentative

identifications were confirmed by analysis of analytical standards (when available) and comparing the GC retention times and mass spectra.

Mutagenicity.

XAD/ethyl acetate extracts were solvent-exchanged into dimethyl sulfoxide (DMSO). Appropriate dilutions with DMSO were done for evaluation of the mutagenicity of each extract with the *Salmonella* mutagenicity assay. For this we used the standard plate-incorporation method in strains TA100 and TA98 with and without metabolic activation (S9) provided by Aroclor-induced Sprague-Dawley rat liver (Moltox, Boone, NC)⁶⁷. These strains are commonly used to assess the mutagenicity of DBPs and drinking water⁴. In addition, we evaluated the extracts in *Salmonella* strains RSJ100 (*GSTT1+*) and TPT100 (*GSTT1-*) in the absence of S9 to assess if the mutagenicity of the extracts was enhanced by the presence of the GSTT1 enzyme, which activates brominated trihalomethanes (Br-THMs) to mutagens⁴. RSJ100 and TPT100 are otherwise isogenic to TA100 except that they do not contain the pKM101 plasmid.

We also evaluated the mutagenicity of chloro-, bromo-, and iodoacetic acid (Sigma, St. Louis, MO) in TA100, TA1535 (the parent strain of TA100 that does not have the pKM101 plasmid), as well as RSJ100 and TPT100 in the absence of S9 to assess the ability of the GSTT1 enzyme to activate these DBPs to mutagens. However, because these are semi-volatiles, we performed the pre-incubation assay, where the cells and each HAA were incubated at 37°C for 30 min, then the top agar was added, and then the mixture was vortexed and poured onto the bottom agar.

Extracts were tested at 1 plate per dose over a dose range of 0.01 to 1 L-equivalent (L-eq) per plate in TA100 and TA98, and at 0.05 to 0.5 L-eq per plate in RSJ100 and TPT100. The haloacetic acids were tested at 1 plate per dose over a dose range of 1 to 500 µg/plate. For the extracts, two experiments were performed in the absence of S9 in both TA98 and TA100; however, due to limited sample, experiments with S9 were conducted only once. Two to three experiments were performed with the extracts in RSJ100 and TPT100, and two experiments were performed with the haloacetic acids.

Plates were incubated for 3 days at 37 °C, and mutant colonies (revertants, rev) were counted on an automatic colony counter (Accura 1000, Manassas, VA). A dose-related response that approached or exceeded a twofold increase in rev/plate compared to the DMSO control was defined as a positive mutagenic response. Linear regressions were calculated over the linear portion of the dose-response curves as defined by the r^2 -value to calculate the mutagenic potencies of the water samples expressed as the slopes of the regressions (rev/L-eq)⁶⁷. Regressions with P -values ≤ 0.05 were considered a mutagenic response. Unpaired, 2-tailed t -tests were used to compare the mutagenic potencies, with $P \leq 0.05$ for significance.

RESULTS AND DISCUSSION

Quantification of 21 Target Non-Iodinated DBPs.

The occurrence in the samples of the regulated THMs, regulated and other chloro/bromo HAAs (Tables S5, S6) and investigated haloacetonitriles (HANs), trichloronitromethane,

haloketones, and chloral hydrate (Table S7) is summarized in Figure 1. As expected, and without exception, none of these DBPs was present above the detection limit in non-disinfected waters, including the source water (SW) as well as the SW containing any of the ICM.

ICM had no effect on the total concentrations or speciation of the four regulated THMs resulting from chlorination, with chloroform being the most abundant THM, followed by bromodichloromethane and dibromochloromethane. Of the four ICM tested, only IPAM, as expected due to its high reactivity with chlorine, increased the concentrations of the 9 HAAs quantified (5 regulated and 4 unregulated), especially dichloroacetic acid (DCAA) and bromoacetic acid (BAA). DCAA and trichloroacetic acid (TCAA) were the most abundant HAAs in the chlorinated source water, followed by either bromodichloroacetic acid (BDCAA) or BAA. These results are consistent with those of a recent study⁵¹, which also found chloroform and TCAA to be the predominant THM and HAA species formed in chlorinated IPAM-containing source water.

When source water without IPAM was chlorinated, the HAAs and THMs contributed the most and equally to the total DBP concentrations, followed by chloral hydrate and HANs. However, this contribution pattern of DBP classes was altered considerably after chlorination of source water containing IPAM, resulting in the initial total HAN concentration of 7.8 µg/L increasing to 83.7 µg/L. Although ICM reactivity to form HANs has not been studied previously, IPAM is the ICM that is generally most reactive with chlorine^{45, 51}. This is borne out in the lower amounts of residual free chlorine remaining after the 72-h reaction for IPAM than for the other three ICM evaluated (Table S3). In addition to IPAM being an iodine source for iodo-DBP formation, it may also serve as a source of nitrogen (three secondary amides) in the formation of N-DBPs such as DCAN.

Quantification of 11 Target I-DBPs.

The concentration of the 11 target I-DBPs (Figure 2A, Table S8) in the chlorinated samples represented <10% of the total concentration of the total quantified 32 target DBPs (Figure 2B). Overall, the addition of each ICM increased slightly the concentrations of the different classes of I-DBPs in the disinfected waters. The highest increase in the concentration of I-DBPs was observed in chlorinated source water containing IPAM, as previously reported⁴⁵ (Figure 2A and Table S8). Only a few I-DBPs were formed in reaction blanks (chlorinated IPAM solutions in the absence of NOM), 0.1 µg/L of iodoacetic acid (IAA) and 3.9 µg/L of dichloriodomethane, and at much lower concentrations than in the presence of NOM (Table S8), which is consistent with previous findings from Duirk et al⁴⁵.

As for the classes of I-DBPs formed (Figure 2A, Table S8), I-THMs were always more abundant than I-HAAs and iodoacetaldehyde (IAL). Of all the targeted I-THMs, dichloriodomethane (>50-fold increase with IPAM compared to SW-Cl), followed by chlorodiiodomethane (>8-fold increase with IPAM) and bromochloriodomethane were the most abundant in chlorinated source waters containing ICM.

In the case of I-HAAs, chloriodoacetic acid and IAA were produced at low levels (<300 ng/L) in chlorinated source waters in the absence of ICM and at higher concentrations in

chlorinated source waters containing IPR and IPAM. Chlorinated IPAM-containing waters produced higher levels of these I-HAAs (up to 5.9 µg/L in total) than did chlorinated IPR-containing waters (0.7 µg/L). IAL was detected only in chlorinated IPAM-containing waters (3.2 µg/L). The high I-DBP concentrations formed by chlorinated IPAM-containing waters could be attributed to the potentially high reactivity of chlorine towards IPAM. For example, chlorine is known to attach a specific amide side chain in the molecule that leads to the further release of iodine and other reactions³⁶.

Identification and Quantification of Non-Target I-DBPs.

Because ICM may serve as iodine sources in water, we performed a non-target screening to potentially identify new I-DBPs in the extracts. To aid in the identification of iodine-containing peaks, we utilized extracted ion chromatograms of the m/z 126.904 fragment ion (iodine; I^+) from GC/HR-EI-MS full-scan data obtained for each extract. New I-DBPs, namely iodoacetoneitrile (Figure 3A), chloriodoacetoneitrile (Figure 3B), and trichloriodomethane (Figure 3C), were tentatively identified using HR-MS data in the chlorinated IPAM-containing water (Figure S3), which was also the water sample with the highest concentrations of the 11 target I-DBPs. Iodoacetoneitrile and trichloriodomethane both had high similarity library matches with the NIST mass spectral database; however, chloriodoacetoneitrile did not have any sufficient matches. In all three cases, especially for chloriodoacetoneitrile, structures were elucidated via mass spectral interpretation of the EI fragmentation patterns, and molecular formulas for each molecular ion and fragment ion were determined from the accurate mass, as shown in Figure 3. The presence of chlorine(s) was determined from characteristic isotopic patterns⁶⁸. Common fragment ions included the losses of chlorine (m/z between successive fragments of 34.9688 and 36.9659) and iodine (m/z of 126.9045), as well as the presence of iodine fragment ions (I^+ ; m/z 126.9039).

The identities of iodoacetoneitrile and chloriodoacetoneitrile were confirmed through the analysis of pure standards that were available commercially for the former and synthesized for the latter (Figures 3 and S2). Although trichloriodomethane had a high-similarity NIST mass spectral library match, its identity is still tentative because a pure standard could not be obtained.

Iodoacetoneitrile is extremely cytotoxic and genotoxic in mammalian cells and has the highest genotoxicity among an entire class of seven haloacetoneitriles². Both iodoacetoneitrile and chloriodoacetoneitrile are nitrogen-containing DBPs (N-DBPs), which are generally much more toxic than DBPs without nitrogen^{4, 69}. Iodinated DBPs are also consistently more toxic than brominated and chlorinated DBPs⁴. Thus, it is highly likely that chloriodoacetoneitrile (having both nitrogen and iodine in its structure) is highly cytotoxic and genotoxic in mammalian cells.

New iodo-acids were also tentatively identified in the derivatized IPAM-Cl extract by selected reaction monitoring (SRM) of expected fragment ions and MS/MS transitions for these compounds. These were the following trihaloiodoacetic acids: dichloriodoacetic acid (DCIAA), chlorodiiodoacetic acid (CDIAA), and bromochloriodoacetic acid (BCIAA), identified in their methyl ester forms (Figure 4). The predicted SRM transitions utilized for their detection included losses of the methyl ester group ($-COOCH_3$; -59), methoxy group ($-$

OCH₃; -31), iodine (-I; -127), and a rearrangement resulting in a loss of iodine and CO (-I, CO; -155). For example, the exhibited transitions from *m/z* 268>209 (-COOCH₃) and 268>237 (-OCH₃) at the same retention time of 13.72 min should be characteristic of DCIAA, whereas transitions from *m/z* 312>185 (-I) and 312>157 (-I, CO) at 18.38 min could be attributed to BCIAA.

Although their identities could not be confirmed, due to lack of available chemical standards, their GC retention times occurred in regions that would be expected for these compounds based on comparison to mono- and di-haloacetic acids. It is likely that these never-before-reported trihalo-I-HAAs were formed at low levels because they were not detected in the comprehensive full scan-MS analyses, but were seen using the much more sensitive and selective SRM tandem mass spectrometry method. The detection of these six new I-DBPs in the IPAM-Cl water samples, and not in the corresponding controls without IPAM, confirmed IPAM as the source of iodine in their formation.

Mutagenicity of Chlorinated Waters.

We analyzed the mutagenicity data in strain TA98 and TA100 (Table S9) by linear regression analysis (Figure S4) to calculate the slope values, which were the mutagenic potencies (rev/L-eq) of the samples in those strains (Table S10). The method blank (an ethyl acetate extract of the XAD that had been solvent-exchanged into DMSO) was not mutagenic relative to the DMSO control (Table S9), indicating that the mutagenicity observed throughout the study was not due to mutagens from the extraction procedure itself.

Statistical analyses of the mutagenic potency of the chlorinated source water (no ICM) (Figure 5A and 5B) confirmed a previous study on disinfected waters⁶⁷ in that the extract (a) was more mutagenic in the absence than the presence of S9, (b) was more mutagenic in TA100 than in TA98, and (c) had a mutagenic potency in TA100 -S9 (1388.0 rev/L-eq) that was similar to that found in previous studies (~1200 rev/L-eq)^{4, 67, 70-71}.

Overall, both the mutagenicity and DBP data confirmed that the source water was typical of other source waters studied previously and that the laboratory-based disinfection procedure simulated those of commercial drinking water plants⁷⁰. Although sample limitation prevented us from assessing the mutagenicity of the source water prior to disinfection, previous studies have shown most source waters (85-100%) are weakly or not mutagenic^{67, 70, 72}. Supporting this is the absence of detectable DBPs in the non-disinfected source water with or without the addition of the ICM (Figure 1 and Tables S5 and S7).

Mutagenicity of Chlorinated Source Water Containing ICM.

None of the ICM increased the mutagenicity of the source water after the waters were chlorinated, and this was the case in both TA98 and TA100 of *Salmonella* with or without S9 (Figure 5A and 5B). Other than IHX, none of these four ICM enhanced the genotoxicity (DNA damage measured by the comet assay in CHO cells) of chlorinated ICM-containing source water relative to chlorinated source alone⁵². On the contrary, in this study, the discovery of a highly cytotoxic and genotoxic compound (e.g., iodoacetone nitrile)² in chlorinated IPAM-containing water could increase the cytotoxicity and genotoxicity of chlorinated source water.

In contrast, chlorinated water containing IPAM was less mutagenic than chlorinated source water alone in some strain/S9 combinations (Figure 5A and 5B). A similar phenomenon was found for IPR for DNA damage; chlorinated water containing IPR was less genotoxic than chlorinated water alone⁵². Although the basis for these findings is unclear, it is possible that the observed lower mutagenicity is due to lower formation of highly mutagenic DBPs in chlorinated IPAM-containing source waters. This finding could also be due to the competitive reaction of free chlorine with IPAM (versus NOM) to form high molecular weight DBPs, which have been found to be significantly less toxic than iodo-DBPs measured in this study^{40, 60}.

As an indication of the reproducibility of our laboratory-based chlorination procedure, the mutagenic potencies of the extracts from two independently conducted chlorination reactions of IPAM-containing waters (IPAM1 and IPAM2) were not significantly different from each other in either strain with or without S9 ($P > 0.217$); however, due to lack of sample, no data were generated in TA98 +S9 with the IPAM1 extract (Table S10).

Although we did not find that any of the ICM increased the bacterial cell mutagenicity of the water extracts after chlorination, Duirk et al.⁴⁵ found a significant, but modest (1.3-fold) increase in DNA damage induced by extracts of chlorinated IPAM-containing waters in mammalian cells *in vitro*. The *Salmonella* mutagenicity assay detects mutations (i.e., a change in DNA sequence), whereas the comet assay used by Duirk et al.⁴⁵ detects DNA damage (i.e., a DNA strand break or a molecule bound covalently to the DNA). Such DNA damage can be either repaired by the cell or processed by the cell into a mutation. Thus, it is possible that the small amount of DNA damage induced by chlorinating IPAM-containing water may be repaired and not result in mutation detectable in the *Salmonella* mutagenicity assay.

Correlations between Mutagenic Potencies and Concentrations of Target DBPs.

Using the data in Table S10 to calculate Pearson r correlation coefficients, we found no significant ($P > 0.05$) positive correlations between the mutagenic potencies of any of the 11 water extracts and the total concentrations of the 21 target non-iodinated DBPs, the 11 target I-DBPs, or all 32 target DBPs (data not shown). In contrast, we found strong correlations between the concentrations of the 21 target non-iodinated DBPs and the mutagenicity of chlorinated or brominated finished, tap, swimming pool, or spa (hot tub) waters in a previous study⁷⁰. Some of the pool and spa water samples of our previous study had concentrations of the 21 target DBPs and levels of mutagenicity that were nearly twice as high as those reported here. In addition, our present study had only one chlorinated water sample without ICM, so we could not perform a correlation analysis to confirm the correlation between DBP concentrations and mutagenicity that we found in our pool and spa study.

Chlorinated IPAM-containing waters generated the highest DBP concentrations, especially I-DBPs (Table S8); however, as stated earlier, IPAM did not increase the mutagenicity of chlorinated water (Figure 5A and 5B). This is likely because IAA is only weakly mutagenic in TA100 (2.8 rev/ μ g) (Table S12). Other I-DBPs have not been studied extensively for mutagenicity.

A previous study showed that methanol extracts of chlorinated IPAM-containing buffer or purified water (no NOM) were mutagenic in *Salmonella*⁵⁸. However, the authors identified several high-molecular weight structures that structure-activity relationship models predicted to be mutagenic^{55, 58}. Wendel et al.⁴⁰ found that <2% of the IPAM in chlorinated IPAM-containing water in the absence of NOM was converted to low-molecular weight I-DBPs, and they identified a variety of high-molecular weight I-DBPs. However, of the five high-molecular weight DBPs evaluated for induction of DNA damage in mammalian cells *in vitro* (comet assay), none were genotoxic⁶⁰.

Role of *GSTT1*.

The mutagenicity of the extracts in strain RSJ100 (*GSTT1*+) and strain TPT100 (*GSTT1*-) is shown in Table S11. We subjected these data to linear regression analysis (Figure S5) to determine the slope values, which were the mutagenic potencies (Table S11 and Figure 6). The *GSTT1* enzyme enhanced the mutagenic potencies of the extracts of the chlorinated SW+IPAM by 4.6-fold and of the chlorinated SW+IHx by 2-fold. Again, the two preparations of SW+IPAM replicated well. These results imply that some of the mutagenicity of these samples is due to the formation of Br-THMs and/or possibly the I-THMs. No I-THMs have been evaluated in the *GSTT1*-expressing strain of *Salmonella* to know if they are activated to mutagens by this enzyme.

We also examined the ability of the *GSTT1* enzyme to activate the I-DBP iodoacetic acid (IAA) to a mutagen, as well as its homologues, bromoacetic acid (BAA) and chloroacetic acid (CAA). The results (Table S12) showed that the *GSTT1* enzyme did not activate any of these three DBPs. For comparison, we showed that only IAA was mutagenic in the standard strain, TA100 (Table S12). Thus, it seems that the enhancements by the *GSTT1* enzyme of the selected water extracts noted above were due to the presence of Br-THMs and/or possibly I-THMs.

Implications for Public Health.

This study confirms IPAM as a relevant iodine source of I-DBPs in drinking water. However, the DBPs formed in the presence of IPAM or any of the other three ICM did not increase the mutagenicity of chlorinated water. Further characterization of I-DBPs formed after disinfection of ICM-containing waters is warranted given the generally potent genotoxicity of such DBPs relative to chlorinated or brominated DBPs.

DBP formation and mutagenicity of disinfected waters will depend on the type of NOM present in the source waters, which may produce different results than those reported here. Nonetheless, the potential associated risk could be diminished by reducing ICM release into drinking water sources. This requires advanced treatment of municipal and hospital wastewaters, which are the main sources of ICM into the environment. In addition, a feasibility study has demonstrated collection of ICM from hospitalized patients through decentralized urine collection⁷³, and decreasing the amount of ICM entering hospital wastewater could potentially decrease requirements for advanced treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Concentration ($\mu\text{g/L}$) of (A) regulated trihalomethanes (THM4), chloral hydrate, selected chlorine-containing haloketones, (B) haloacetic acids (HAA9), and (C) trichloronitromethane and chloro-bromo-containing haloacetonitriles in blank and chlorinated waters. TCM: Chloroform or trichloromethane, BDCM: Bromodichloromethane, DBCM: dibromochloromethane, TBM: tribromomethane or bromoform, CH: chloral hydrate or trichloroacetaldehyde, DCP: dichloropropanone, TCP: trichloropropanone, CAA: chloroacetic acid, DCAA: dichloroacetic acid, TCAA: trichloroacetic acid, BCAA:

bromochloroacetic acid, BDCAA: bromodichloroacetic acid, CDBAA: chlorodibromoacetic acid, BAA: bromoacetic acid, DBAA: dibromoacetic acid, TBAA: tribromoacetic acid, BCAN: bromochloroacetonitrile, DBCAN: dibromochloroacetonitrile, DCAN: dichloroacetonitrile, TCAN: trichloroacetonitrile, TCNM: trichloronitromethane. SW = source water, and SW-Cl = chlorinated source water.

Figure 2.

(A) Concentration ($\mu\text{g/L}$) of the target I-DBPs and (B) contribution of I-DBPs (%) to the total mass of the 32 target DBPs measured in the samples. DCIM: dichloriodomethane, CDIM: chlorodiiodomethane, BCIM: bromochloriodomethane, CIAA: chloriodoacetic acid, IAA: iodoacetic acid, IAL: iodoacetaldehyde; n/a: not analyzed.

Figure 3.

High resolution EI mass spectra of (A) iodoacetonitrile ($t_R = 9.12$ min), (B) chloriodoacetonitrile ($t_R = 10.27$ min), and (C) trichloriodomethane ($t_R = 9.15$ min) in chlorinated IPAM-containing source water.

[†]Electron ionization of trichloriodomethane (TCIM; CCl_3I) does not produce a molecular ion; therefore, mass accuracy ($\Delta m/m$) is shown for its highest m/z fragment (CCl_2I^+).

*Due to chromatographic co-elution of TCIM with iodoacetonitrile (IAN), the high-abundance IAN molecular ion ($\text{C}_2\text{H}_2\text{IN}^+$) is observed in the mass spectrum of TCIM.

Figure 4. Selected reaction monitoring (SRM) of expected fragment ions and MS/MS transitions for new iodo-acids tentatively identified in the derivatized extract of chlorinated IPAM-containing source water. Possible identifications are dichloroiodoacetic acid (DCIAA), bromochloroiodoacetic acid (BCIAA), and chlorodiiodoacetic acid (CDIAA).

Figure 5. Mutagenic potencies (rev/L-eq) in TA100 (A) and TA98 (B) of extracts of chlorinated source water and chlorinated source water containing each of the four ICM. Asterisks indicate a significantly different mutagenic potency relative to that of the comparable chlorinated source water ($P < 0.05$).

Figure 6. Mutagenic potencies of extracts in *Salmonella* strains either expressing (+) or not (–) the *GSTT1* gene. Higher mutagenic potencies in *GSTT1*± imply that some of the mutagenesis of that sample may be due to the presence of Br-THMs and/or I-THMs. $P < 0.05$.