Published in final edited form as:
Environ Mol Mutagen. 2021 March ; 62(3): 168–176. doi:10.1002/em.22423.

Inability of GSTT1 to Activate Iodinated Halomethanes to Mutagens in *Salmonella*

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Abstract

Drinking water disinfection by-products (DBPs), including the ubiquitous trihalomethanes (THMs), are formed during the treatment of water with disinfectants (e.g., chlorine, chloramines) to produce and distribute potable water. Brominated THMs (Br-THMs) are activated to mutagens via glutathione *S*-transferase theta 1 (GSTT1); however, iodinated THMs (I-THMs) have never been evaluated for activation by GSTT1. Among the I-THMs, only triiodomethane (iodoform) has been tested previously for mutagenicity in *Salmonella* and was positive (in the absence of GSTT1) in three strains (TA98, TA100, and BA13), all of which have error-prone DNA repair (pKM101). We evaluated five I-THMs (chlorodiiodomethane, dichloroiodomethane, dibromoiodomethane, bromochloroiodomethane, and triiodomethane) for mutagenicity in *Salmonella* strain RSJ100, which expresses GSTT1, and its homologue TPT100, which does not; neither strain has pKM101. We also evaluated chlorodiiodo-, dichloroiodo-, and dibromoiodo-methanes in strain TA100 +/− rat liver S9 mix; TA100 has pKM101. None were mutagenic in any of the strains. The I-THMs were generally more cytotoxic than their brominated and chlorinated analogues but less cytotoxic than analogous trihalonitromethanes tested previously. All five I-THMs showed similar thresholds for cytotoxicity at ~2.5 μmoles/plate, possibly due to release of iodine, a well-known

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AUTHOR CONTRIBUTIONS

Hannah K. Liberatore and David M. DeMarini conceived of and designed the study. Susan D. Richardson had the I-THMs synthesized and provided some of them for this study. Sarah H. Warren prepared media and maintained and grew cells, and David M. DeMarini performed the experiments. Weston J. Smith and David M. DeMarini conceived of the molecular mechanism by which iodoform is mutagenic. All the authors helped write the paper, and all approved of the manuscript.

CONFLICT OF INTERESTS

This article was reviewed by the Chemical Characterization and Exposure Division, Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. EPA, and approved for publication. Approval does not signify that the contents reflect the views of the agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The authors declare no financial conflict of interests.

antimicrobial. Although none of these I-THMs were activated by GSTT1, iodoform appears to be the only I-THM that is mutagenic in *Salmonella*, only in strains deficient in nucleotide excision repair (*uvrB*) and having pKM101. Given that only iodoform is mutagenic among the I-THMs and is generally present at low concentrations in drinking water, the I-THMs likely play little role in the mutagenicity of drinking water.

Keywords

mutagenicity; GSTT1; disinfection by-products; drinking water; DBPs

INTRODUCTION

Drinking water disinfected primarily by chlorine has been ubiquitous in most regions of the world for a century; however, the potential health effects of disinfection by-products (DBPs), which are formed by the reaction of chlorine with organics and halides in the water, were not recognized until the 1970s (DeMarini 2020). Among the >700 DBPs identified, >100 have been tested and found to be genotoxic *in vitro*, and 20 out of 22 that have been tested are carcinogenic in rodents (Richardson *et al.*, 2007; Wagner and Plewa 2017; Cortés and Marcos 2018; DeMarini 2020).

Chloramine (primarily NH_2Cl) is often used instead of chlorine because it produces lower concentrations of the regulated trihalomethanes (THMs) and haloacetic acids (HAAs) (Krasner *et al.,* 2006; Hong *et al.,* 2007; Richardson *et al.*, 2007; Liu *et al.,* 2017, 2019), allowing drinking water treatment plants to meet regulations more easily. Chloramine disinfection produces water that is generally less mutagenic in *Salmonella* (DeMarini *et al.*, 1995); however, with Br or I in the source water, it generates finished water that induces higher levels of DNA damage (comet assay) in mammalian cells (Plewa *et al.*, 2008; Yang *et al.*, 2014). As reviewed by Ersan *et al.* (2019), chloramination relative to chlorination typically promotes the formation of more unregulated DBPs, including iodinated DBPs (I-DBPs), when iodide is present in the source waters, which can result in higher cytotoxicity and genotoxicity of the treated water (Jeong *et al.*, 2012; Yang *et al.*, 2014; Plewa and Wagner 2015; Liu *et al.*, 2017, 2019).

As assessed by Richardson *et al.* (2008) in chlorinated and chloraminated drinking waters from 23 cities in the U.S. and Canada, as well as reviewed by Dong *et al.* (2019) and Postigo *et al.* (2019), various I-DBPs have been found in drinking water during the disinfection of waters containing iodide and/or organic iodine. The types and concentrations of I-DBPs are influenced by the concentration of iodide (Jones *et al.*, 2012; Richardson *et al.*, 2008; Postigo *et al.*, 2017), the molecular size of the natural organic matter (NOM) (Zhang *et al.*, 2016), and the presence of iodinated X-ray contrast media in the source water (Duirk *et al.,* 2011; Wendel et al., 2014; Xu *et al.,* 2017, Postigo *et al.*, 2018; Ackerson *et al.*, 2020). A U.S. nationwide DBP occurrence study found that the concentrations of I-THMs increased with chloramination more than with chlorination, with most I-THMs present at sub- to low-μg/L concentrations among various drinking water samples (Weinberg *et al.*, 2002; Krasner *et al.*, 2006). The increased formation of I-THMs with chloramine vs. chlorine is due to

faster rates of formation of I-THMs compared to competing reactions to form iodate (Bichsel and von Gunten 2000).

Among five I-THMs evaluated, only chlorodiiodomethane (CDIM) induced DNA damage in Chinese hamster ovary (CHO) cells (Richardson *et al.*, 2008). Triiodomethane (iodoform) has also been evaluated for additional genotoxic endpoints in other mammalian cell lines. In mouse embryonic NIH3T3 cells, iodoform did not induce DNA damage detectable by the comet assay, double-strand DNA breaks, micronuclei, or cell transformation (Wei *et al.*, 2013). In Syrian hamster embryo cells, iodoform did not induce chromosome aberrations (Hikiba *et al.*, 2005). Thus, iodoform is not a chromosomal mutagen.

Consistent with its lack of ability to induce cell transformation (Wei *et al.*, 2013), iodoform was not carcinogenic in male or female rats and mice in a 78-week gavage study (NCI 1978). Although chloroform and bromoform are carcinogenic in rodents (Richardson *et al.*, 2007), chloroform is not mutagenic and requires repeated toxic doses causing necrosis to be carcinogenic in rodents; thus, it has been evaluated as only a possible (2B) human carcinogen (IARC 1999). Bromoform is unclassifiable as a human carcinogen due to limited data (IARC 1991), and iodoform has never been evaluated as a human carcinogen by the International Agency for Research on Cancer (IARC).

Although iodoform is not a chromosomal mutagen, it is a gene mutagen, being positive in *Salmonella* strains TA98 and TA100 +/− S9 (Haworth *et al.*, 1983; NTP 2020) and in the forward-mutation Ara assay in *Salmonella* strain BA13 +/− S9 (Roldán-Arjona *et al.*, 1991; Roldán-Arjona and Pueyo 1993). All three of these strains contain both a *uvrB* mutation that eliminates nucleotide excision DNA repair, as well as the pKM101 plasmid, which confers error-prone DNA repair to the cells due to the presence of the *mucA* and *mucB* genes on the plasmid (Walker *et al.*, 1985). The Br-THMs are activated to mutagens in *Salmonella* by GSTT1 (DeMarini *et al.*, 1997; Pegram *et al.*, 1997), as are five halonitromethanes (Kundu *et al.,* 2004b). Although iodoacetic acid is not activated to a mutagen by GSTT1 (Postigo *et al.*, 2018), it is the most genotoxic among the HAAs (Plewa *et al.*, 2002, 2004b; Attene-Ramos *et al.*, 2010; Zhang *et al.*, 2010) and induces cell transformation in NIH3T3 cells (Wei *et al.*, 2013). No other DBPs have been evaluated for their ability to be activated to mutagens in *Salmonella* by GSTT1, including the I-THMs.

To further characterize the mutagenicity of the I-THMs, we evaluated the mutagenicity of five I-THMs in *Salmonella* strain RSJ100, which expresses GSTT1, and strain TPT100, which does not; neither strain contains pKM101. The I-THMs were chlorodiiodomethane (CDIM), dichloroiodomethane (DCIM), dibromoiodomethane (DBIM), bromochloroiodomethane (BCIM), and iodoform. Because of the availability of sufficient quantities of standards, we also evaluated the first three compounds (CDIM, DCIM, DBIM) in strain TA100 with and without rat liver S9 mix. We describe the threshold for cytotoxicity exhibited by these compounds, explore possible mechanisms by which iodoform is mutagenic uniquely among the I-THMs, and discuss the limited role that the I-THMs may play in the mutagenicity of drinking water.

MATERIALS AND METHODS

Chemicals

We purchased three of the I-THMs from Toronto Research Chemicals, Inc., Toronto, ON, Canada: chlorodiiodomethane (CDIM), dichloroiodomethane (DCIM), and dibromoiodomethane (DBIM). We purchased iodoform, or triiodomethane (TIM), from Sigma-Aldrich, St. Louis, MO. Bromochloroiodomethane (BCIM) was purchased from CanSyn Chem Corp., Toronto, ON, Canada. Further details regarding the I-THM standards used in this study are shown in Table 1. HPLC-grade dimethyl sulfoxide (DMSO) was obtained from Burdick and Jackson, Muskegon, MI. General reagents for buffer solutions and positive controls (see below) were purchased from Sigma-Aldrich, St. Louis, MO. Stock solutions of each I-THM were prepared at 1000 μmoles/ml in DMSO, and 1:10 dilutions in DMSO of these stocks were made and stored at 4°C until use.

Mutagenicity assays and cytotoxicity

We evaluated all five I-THMs for mutagenicity in the absence of S9 in *Salmonella* strain RJS100, which expresses the rat *GSTT1–1* gene, and its homologue TPT100, which does not. These strains were derived by Thier *et al.* (1993) from the base-substitution strain TA1535, which contains a *uvrB* mutation, preventing nucleotide excision DNA repair; they do not contain the pKM101 plasmid, which confers error-prone trans-lesion DNA repair. These strains were provided by Dr. F.P. Guengerich, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN. These strains contain a plasmid containing the rat *GSTT1* gene, which is inserted in the correct orientation in RSJ100, permitting expression of the gene, but which is inserted in the opposite orientation in TPT100, preventing expression (Thier *et al.,* 1993).

Because of the availability of sample, we also evaluated three of the I-THMs (CDIM, DCIM, and DBIM) in strain TA100 of *Salmonella* [*his G46 chl-1005 (bio uvrB gal) rfa-1001* pKM101+ Fels-1^+ Fels-2^+ Gifsy-1^+ Gifsy-2^+] (Porwollik *et al.,* 2001), which was also derived from strain TA1535 (Maron and Ames 1983; Porwollik *et al.,* 2001). Although strains TA1535, RSJ100, TPT100, and TA100 all contain the *uvrB* mutation, only strain TA100 also contains the pKM101 plasmid. We obtained strain TA100 from Dr. Bruce N. Ames, University of California, Berkeley, CA. Strains were maintained and grown as recommended by Maron and Ames (1983).

We evaluated the I-THMs in a pre-incubation assay as we have done with other halomethanes (Kundu *et al.,* 2004a) because they are semi-volatile and should be tested in a closed system. We evaluated all five I-THMs in RSJ100 and TPT100 in amber glass 8-ml screw-cap tubes as described previously (Kundu *et al.,* 2004a), and we evaluated CDIM, DCIM, and DBIM in TA100 in 6-ml screw-cap tubes due to lack of availability of the 8-ml tubes. Tubes were kept in an ice bath while the following were added to each tube: 100 μl of the appropriate concentration of each I-THM, 500 μl of either 15-mM phosphate buffer or S9 mix prepared from Aroclor-induced Sprague Dawley rat liver S9 (Moltox, Boone, NC), and 100 μl of an overnight culture of cells. Thus, the total reaction volume was 700 μl.

After securing the caps, the tubes were vortexed and incubated at 37°C for 30 min without shaking in a water bath. We then added 2.5 ml of molten top agar to each tube, replaced the cap, vortexed the tube, and then poured the contents onto the surface of Vogel-Bonner medium E (VBME) plates. We incubated the plates at 37°C for 3 days and then counted the colonies on an automatic colony counter (ProtoCOL 3, Synbiosis, Frederick, MD). Due to the limited amounts of halomethanes available, we performed all experiments at one tube per dose and performed two experiments with each I-THM. We evaluated 8–10 doses for each compound, spanning 2–3 orders of magnitude; depending on the compound, the doses ranged from 0.001 to 10 μmoles/plate. We used three plates for the DMSO control and for each of the positive controls (also dissolved in DMSO): sodium azide (3 μg/plate) in the absence of S9 and 2-aminoanthracene (0.5 μg/plate) in the presence of S9.

As with past studies, compounds were determined to be mutagenic if they produced a dose-related increase in revertants (rev)/plate that approached or exceeded a twofold increase relative to the DMSO control and gave a significant ($P \leq 0.05$) trend test (Prism, GraphPad, San Diego, CA). The minimum cytotoxic doses were those causing a reduction in the number of rev/plate and/or a thinning of the background lawn of cells. Thus, cytotoxicity was assessed indirectly as is done when using the plate-incorporation assay, and efforts were made to test the I-THMs at doses that caused cytotoxicity as defined above.

RESULTS AND DISCUSSION

Mutagenicity

The mutagenicity data (rev/plate) for all five I-THMs in strains RSJ100 and TPT100 showed that none of the compounds were mutagenic (Table 2); all were tested to toxicity as recommended by OECD TG471 (OECD 2020). Thus, as with chloroform, the I-THMs were not activated to mutagens by GSTT1, in contrast to the Br-THMs (DeMarini *et al.*, 1997; Pegram *et al.*, 1997) and some halonitromethanes (Kundu *et al.*, 2004b), which are activated by GSTT1. The high amount of GSTT1 activity exhibited by RSJ100 (18,900 units) relative to TPT100 (<4 units) (Ross and Pegram 2003) was insufficient to activate these I-THMs, although RSJ100 activates the Br-THMs and other dihalomethanes (Thier *et al.*, 1993).

The mutagenicity data (rev/plate) for three of the I-THMs in strain TA100 showed that none of these compounds were mutagenic in either the presence or absence of S9 (Table 3); all were tested to toxicity as recommended by OECD TG471 (OECD 2020). As noted above, the Ames strains contain only a small amount of GSTT1 activity. Thier *et al.* (1993) showed that addition of S9 to the standard Ames strains did not activate the dihalomethanes, presumably because the activated form of the THM was too unstable to pass through the cell membrane, prompting the construction of strain RSJ100, which over-expresses GSTT1 internally. Likely for this reason, S9 had no impact on the mutagenic potency of iodoform in TA98 or TA100 (Haworth *et al.*, 1983), as we demonstrated by calculating the mutagenic potencies (rev/μg) of iodoform based on the slopes of the linear regressions of the dose-response data from Haworth *et al.* (1983). We found the rev/μg to be 0.54 (-S9) and 0.55 (+ rat liver S9) in TA100, and 0.51 (-S9) and 0.45 (+S9) in TA98; thus, neither S9 nor GSTT1 influences the mutagenicity of iodoform.

Studying the ability of GSTT1 to activate agents to genotoxic forms is equally challenging in many mammalian cells lines, such as Chinese hamster ovary (CHO) cells, which do not express GST activity; even primary cell lines can pose problems when studying the role of GSTT1 to activate agents to genotoxic compounds. For example, we showed that freezing or culturing primary human lung cells prevented the expression of GSTT1 activity even in cells that had the *GSTT1* gene based on PCR analysis, preventing an assessment of the role of GSTT1 on the induction by THMs of DNA damage as determined by the comet assay (Landi *et al.*, 2003).

Cytotoxicity

In RSJ100 and TPT100, all five I-THMs were tested to toxicity as evidenced by the reduced number of rev/plate relative to the controls (in some cases to complete cell killing as evidenced by clear plates and no colonies) (Table 2). Interestingly, none of the I-THMs showed a dose-related decrease in rev/plate in these strains but, instead, exhibited a threshold, generally starting at 2.5 μmoles/plate (~3 mM in the 700-μl reaction).

In TA100, all three I-THMs were tested to toxicity as evidenced by the reduced number of rev/plate relative to the controls except for CDIM in the absence of S9 due to a limited amount of compound (Table 3). In TA100, these three I-THMs exhibited a reduction in rev/plate that started at 0.5 to 5 μmoles/plate, whereas this reduction began at ~3 to ~12 μmoles/plate by the non-iodinated THM analogues of these compounds (Kundu *et al.*, 2004a), making the I-THMs ~6 times more cytotoxic than their brominated or chlorinated analogues.

Considering the doses at which the various analogues of the THMs initiated a reduction in rev/plate in *Salmonella* TA100, the trihalonitromethane (nitro-THM) homologues were at least 7 times more cytotoxic than the I-THMs, initiating a reduction rev/plate at ~0.07 to ~3.39 μmoles/plate (Kundu *et al.*, 2004a). Based on this metric, the cytotoxic potencies of the various classes of THMs in *Salmonella* TA100 rank as follows: nitro-THMs > I-THMs > Br-THMs > Cl-THMs (Figure 1), which is generally consistent with their ranking for cytotoxic potency in mammalian (CHO) cells (Plewa *et al.*, 2004a; Plewa and Wagner 2009; Richardson *et al.*, 2008; Wagner and Plewa 2007).

The reduction in rev/plate induced by these I-THMs, including the killing of all the cells on the plate by some of them, may be due to cytotoxic reaction products resulting from the probable scission of iodine from the I-THMs. Iodine is the strongest leaving group of all the halogens (Cottrell 1958; Danen and Winter 1971); however, it is unclear what potentially cytotoxic reaction products might be formed after removal of iodine from the I-THMs. Given the high cytotoxicity of the iodinated DBPs noted here in *Salmonella* and in mammalian cells (Richardson *et al.*, 2008), as well as the potential exposure of people to such compounds in drinking water (Krasner *et al.*, 2006; Richardson *et al.* 2008), research is needed to understand more fully the formation and types of potentially cytotoxic reaction products resulting from the scission of iodine from the I-THMs.

Interpretation of X-ray contrast media study

Recently, we assessed the impact of four iodinated contrast media, which are used to enhance medical X-ray imaging for soft tissues, on the mutagenicity of source water after

the contrast media-containing water was chlorinated (Postigo *et al.,* 2018). We found that organic extracts of the chlorinated waters containing either of two of the contrast media, iopamidol (IPAM) and iohexol (IHX), were more mutagenic in the GSTT1-expressing strain of *Salmonella* (RSJ100) than in the non-expressing strain (TPT100). The concentrations of Br-THMs as well as I-THMs were increased in these waters; however, there were no correlations between the concentrations of these DBPs and the mutagenicity of the waters.

Because nothing was known at the time about the ability of the I-THMs to be activated by GSTT1, we ascribed the enhanced mutagenicity of these water extracts by GSTT1 to the Br-THMs, which we had detected in the waters and that are activated by GSTT1. Beyond five halonitromethanes (Kundu *et al.*, 2004b), none of the other halonitromethanes are activated by GSTT1, nor is iodoacetic acid (Postigo *et al.*, 2018); no other DBPs have been evaluated for their ability to be activated to mutagens by GSTT1. The I-THM data reported here support our original conclusion, eliminating the I-THMs as a contributor to the enhanced mutagenicity of the IPAM- or IHX-containing waters in RSJ100 that we reported in Postigo *et al.* (2018).

Relative to their chlorinated and brominated analogues, the I-HAAs, like the I-THMs, are generally more cytotoxic. However, unlike all but CDIM among the I-THMs, all the I-HAAs induce DNA damage in CHO cells as assessed by the comet assay (Plewa *et al.*, 2010; Richardson *et al.*, 2008) and are more genotoxic than their brominated or chlorinated analogues (Wagner and Plewa 2017); they are also 8 times more mutagenic at the *Hprt* locus in CHO cells than their chlorinated or brominated analogues (Zhang *et al.,* 2010). We have shown that iodoacetic acid (IAA) is also mutagenic in *Salmonella* TA100 and not activated by GSTT1 (Postigo *et al.*, 2018).

Proposed mechanism for the mutagenicity of iodoform in *Salmonella* TA98, TA100, and BA13

Among the I-THMs, only iodoform is mutagenic in *Salmonella*, and only in strains containing both the *uvrB* mutation, which eliminates nucleotide excision DNA repair, and the pKM101 plasmid, which provides error-prone, trans-lesion DNA repair synthesis (Haworth *et al.*, 1983). In addition, neither S9 (Haworth *et al.*, 1983) nor GSTT1 (Table 2) influences the mutagenicity of iodoform. The DNA damage induced by iodoform that results in mutagenesis in *Salmonella* strains containing both *uvrB* and pKM101 does not induce other types of genotoxicity, as evidenced by the fact that iodoform in mammalian cells does not produce DNA damage detectable by the comet assay (Wagner and Plewa 2017) and does not induce cell transformation, DNA strand breaks, or micronuclei (Wei *et al.*, 2013) or chromosome aberrations (Hikiba *et al.*, 2005). These results show that iodoform does not induce chromosomal mutations but, instead, induces gene mutations in *Salmonella* strains TA98, TA100, and BA13, likely by forming a DNA adduct that in these strains is not repaired due to the *uvrB* mutation and is then available to be misread by the error-prone DNA polymerase provided by pKM101, resulting in a mutation.

It is unclear why only an I-THM with three iodine moieties might do this, whereas those with just one or two iodine moieties might not. This is especially the case given that the carbon-iodine bond is the weakest between carbon and any of the halogens (Cottrell, 1958),

promoting a high probability of bond scission (Danen and Winter 1971), which would likely occur among all the I-THMs. However, we speculate that scission of an iodine from iodoform would result in a carbon-centered radical whose remaining two iodine moieties would provide sufficient resonance stability to confer a lifetime long enough to form a stable DNA adduct. This would be consistent with the processing of such a DNA adduct into a gene mutation in cells containing both *uvrB* and pKM101, which are required for mutagenesis by iodoform. In contrast, scission of an iodine from either the mono- or di-I-THMs would not leave two iodine moieties available to create the necessary resonance stability to form a stable DNA adduct.

Two of the I-THMs contained bromine (DBIM and BCIM), and we have shown previously that Br-THMs are activated to mutagens by GSTT1 (DeMarini *et al.*, 1997; Pegram *et al.*, 1997). Our finding that neither of these brominated I-THMs was activated by GSTT1 may be due to the scission of iodine and the subsequent formation of cytotoxic products prior to any potential activation by GSTT1. Another possibility is that the presence of iodine in these THMs made them sterically incompatible with the GSTT1 enzyme.

CONCLUSIONS

The five I-THMs evaluated here were not activated to mutagens in *Salmonella* by GSTT1; however, all five showed the same threshold for a reduction in rev/plate at ~2.5 μmoles/plate. Iodoform appears to be the exception among the I-THMs in that it is a gene mutagen, being positive in *Salmonella* strains lacking nucleotide excision DNA repair but having error-prone DNA repair. However, it is not a chromosomal mutagen, being negative for induction of DNA strand breaks, micronuclei, and chromosome aberrations in mammalian cells. In addition, iodoform was not carcinogenic in a 78-week gavage study in rats and mice. The two iodinated Br-THMs evaluated here were not activated by GSTT1. The I-THMs have been found at low concentrations in many drinking-water samples, and because only iodoform among the I-THMs is mutagenic, the I-THMs as a class of DBPs likely play little role in the mutagenicity of drinking water.

ACKNOWLEDMENTS

We thank Rex Pegram (U.S. EPA) and James McCord (U.S. EPA) for their helpful comments on the manuscript; John Ferry (University of South Carolina) and Ann Richard (U.S. EPA) for helpful discussions; and Josh Allen (University of South Carolina) for helping us to obtain the I-THMs. This research was funded by the intramural research program of the Office of Research and Development of the U.S. Environmental Protection Agency, Research Triangle Park, NC. Susan Richardson acknowledges support from the National Science Foundation (NSF CBET 1705206).

REFERENCES

Ackerson NOB, Liberatore HK, Plewa MJ, Richardson SD, Ternes TA and Duirk SE (2020) Disinfection byproducts and halogen-specific total organic halogen speciation in chlorinated source water—the impact of iopamidol and bromide. Journal of Environmental Sciences (China), 89, 90–101. [PubMed: 31892405]

Attene-Ramos MS, Wagner ED and Plewa MJ (2010) Comparative human cell toxicogenomic analysis of monohaloacetic acid drinking water disinfection byproducts. Environmental Science & Technology, 44, 726–7212.

Bichsel Y and von Gunten U (2000) Formation of iodo-trihalomethanes during disinfection and oxidation of iodide containing waters. Environmental Science & Technology, 34, 2784–2791.

Cortés C and Marcos R (2018) Genotoxicity of disinfection byproducts and disinfected waters: A review of recent literature. Mutation Research, 831, 1–12. [PubMed: 29875071]

Cottrell TL (1958) The Strengths of Chemical Bonds. Academic Press, New York.

Danen WC and Winter RL (1971) Halogen abstraction studies. II. Free-radical abstraction of iodine from aliphatic iodides. Evidence to support anchimeric assistance by neighboring halogen in homolytic reactions. Journal of the American Chemical Society, 93, 716–720.

DeMarini DM (2020) A review on the 40th anniversary of the first regulation of drinking water disinfection by-products. Environmental and Molecular Mutagenesis, 61, 588–601. [PubMed: 32374889]

DeMarini DM, Abu-Shakra A, Felton CF, Patterson KS and Shelton ML (1995) Mutation spectra in Salmonella of chlorinated, chloraminated, or ozonated drinking water extracts: Comparison to MX. Environmental and Molecular Mutagenesis, 26, 270–285. [PubMed: 8575416]

DeMarini DM, Shelton ML, Warren SH, Ross TM, Shim J-Y, Richard AM and Pegram RA (1997) Glutathione *S*-transferase-mediated induction of GC to AT transitions by halomethanes in Salmonella. Environmental and Molecular Mutagenesis, 30, 440–447. [PubMed: 9435885]

Dong H, Qiang Z and Richardson SD (2019) Formation of iodinated disinfection byproducts (I-DBPs) in drinking water: emerging concerns and current issues. Accounts of Chemical Research, 52, 896–905. [PubMed: 30919613]

Duirk SE, Lindell C, Cornelison CC, Kormos J, Ternes TA, Attene-Ramos M, Osiol J, Wagner ED, Plewa MJ and Richardson SD (2011) Formation of toxic iodinated disinfection by-products from compounds used in medical imaging. Environmental Science & Technology, 45, 6845–6854. [PubMed: 21761849]

Ersan MS, Liu C, Amy G, Plewa MJ, Wagner ED and Karanfil T (2019) Chloramination of iodide-containing water: Formation of iodinated disinfection byproducts and toxicity correlation with total organic halides of treated waters. Science of the Total Environment, 697, 134142.

Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E (1983) Salmonella mutagenicity test results for 250 chemicals. Environmental Mutagenesis 5 (Suppl 1), 1–142.

Hikiba H, Watanabe E, Barrett JC and Tsutsui T (2005) Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. Journal of Pharmacological Sciences, 97, 146–152. [PubMed: 15665446]

Hong Y, Liu S, Song H and Karanfil T (2007) HAA formation during chloramination—significance of monochloramine's direct reaction with DOM. Journal of the American Water Works Association, 99, 57–59.

IARC (1991) Chlorinated drinking-water; chlorination by-products; some other halogenated compounds; cobalt and cobalt compounds. In: IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 52. Lyon, France: International Agency for Research on Cancer, p. 553.

IARC (1999) Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. In: IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 73. Lyon, France: International Agency for Research on Cancer, p. 683.

Jeong CH, Wagner ED, Siebert VR, Anduri S, Richardson SD, Daiber EJ, McKague AB, Kogevinas M, Villanueva CM, Goslan EH, Luo W, Isabelle LM, Pankow JF, Grazuleviciene R, Cordier S, Edwards SC, Righi E, Nieuwenhuijsen MJ and Plewa MJ (2012) The occurrence and toxicity of disinfection byproducts in European drinking waters in relation with the HIWATE epidemiology study. Environmental Science & Technology, 46, 12120–12128. [PubMed: 22958121]

Jones DB, Saglam A, Song H and Karanfil T (2012) The impact of bromide-iodide concentration and ratio on iodinated trihalomethane formation and speciation. Water Research, 46, 11–20. [PubMed: 22078225]

Krasner SW, Weinberg HS, Richardson SD, Pastor SJ, Chinn R, Sclimenti MJ, Onstad GD, Thruston AD Jr. (2006) Occurrence of a new generation of disinfection byproducts. Environmental Science & Technology, 40, 7175–7185. [PubMed: 17180964]

Kundu B, Richardson SD, Granville CA, Shaughnessy DT, Hanley NM, Swartz PD, Richard AM and DeMarini DM (2004a) Comparative mutagenicity of halomethanes and halonitromethanes in *Salmonella* TA100: structure-activity analysis and mutation spectra. Mutation Research, 554, 335–350. [PubMed: 15450430]

Kundu B, Richardson SD, Swartz PD, Matthews PP, Richard AM and DeMarini DM (2004b) Mutagenicity in *Salmonella* of halonitromethanes: a recently recognized class of disinfection by-products in drinking water. Mutation Research, 562, 39–65. [PubMed: 15279829]

Landi S, Naccarati A, Ross MK, Hanley NM, Dailey L, Devlin RB, Vasquez M, Pegram RA and DeMarini DM (2003) Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells. Mutation Research, 538, 41–50. [PubMed: 12834753]

Liu S, Ersan MS, Plewa MJ, Amy G and Karanfil T (2019) Formation of iodinated trihalomethanes and noniodinated disinfection byproducts during chloramination of algal organic matter extracted from *Microcystis aeruginosa*. Water Research, 162, 115–126. [PubMed: 31255781]

Liu S, Li Z, Dong H, Goodman BA and Qiang A (2017) Formation of iodo-trihalomethanes, iodo-acetic acids, and iodo-acetamindes during chloramination of idodide-containing waters: factors influencing formation and reaction pathways. Journal of Hazardous Material, 321, 28–36.

Maron DM and Ames BN (1983) Revised methods for the Salmonella mutagenicity test. Mutation Research, 113, 173–215. [PubMed: 6341825]

NCI (National Cancer Institute) (1978) Bioassay of iodoform for possible carcinogenicity. Technical Report Series 110. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr110.pdf?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tr110 [Accessed 30 July 2020].

NTP (National Toxicology Program) (2020) Genetic Toxicity Evaluation of Iodoform in Salmonella/E.coli Mutagenicity Test or Ames Test. Study 367919. https://manticore.niehs.nih.gov/cebssearch/study/002-02292-0003-0000-0 [Accessed 30 July 2020].

OECD (2020) Test guidelines for testing of chemicals: bacterial reverse mutation test 471. https://www.oecd-ilibrary.org/docserver/9789264071247-en.pdf?expires=1609616898&id=id&accname=guest&checksum=B3891C7ED1842F9376211774984A6EAA [Accessed 2 January 2020].

Pegram RA, Andersen ME, Warren SH, Ross TM and Claxton LD (1997) Glutathione *S*-transferase-mediated mutagenicity of trihalomethanes in *Salmonella typhimurium*: contrasting results with bromodichloromethane off chloroform. Toxicology and Applied Pharmacology, 144, 183–188. [PubMed: 9169083]

Plewa MJ, Kargalioglu Y, Vankerk D, Minear RA and Wagner ED (2002) Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products. Environmental and Molecular Mutagenesis, 40, 134–142. [PubMed: 12203407]

Plewa MJ, Simmons JE, Richardson SD and Wagner ED (2010) Mammalian cell cytotoxicity and genotoxicity of the haloacetic acids, a major class of drinking water disinfection by-products. Environmental and Molecular Mutagenesis, 51, 871–878. [PubMed: 20839218]

Plewa MJ and Wagner ED (2009) Mammalian cell cytotoxicity and genotoxicity of disinfection by-products. Denver, CO: Water Research Foundation. Mammalian cell cytotoxicity and genotoxicity of disinfection by-products. Water Research Foundation: Denver, CO. https://www.waterrf.org/research/projects/mammalian-cell-cytotoxicity-and-genotoxicity-disinfection-products

Plewa MJ and Wagner ED (2015) Charting a new path to resolve the adverse health effects of DBPs. In: Karanfil T, Mitch W, Westerhoff P and Xie Y (Eds.) Occurrence, formation, health effects, and control of disinfection by-products. Washington, DC: American Chemical Society, pp. 3–23.

Plewa MJ, Wagner ED, Muellner MG, Hsu KM and Richardson SD (2008) Comparative mammalian cell toxicity of N-DBPs and C-DBPs. In: Karanfil T, Krasner SW, Westerhoff P and Xie Y (Eds.) Occurrence, formation, health effects and control of disinfection by-products in drinking water. Vol. 995. Washington, DC: American Chemical Society, pp. 36–50.

Plewa MJ, Wagner ED, Jazwierska P, Richardson SD, Chen PH and McKague AB (2004a) Halonitromethane drinking water disinfection byproducts: chemical characterization and mammalian cell cytotoxicity and genotoxicity. Environmental Science & Technology, 38, 62–68. [PubMed: 14740718]

Plewa MJ, Wagner ED, Richardson SD, Thruston AD Jr., Woo YT and McKague AB (2004b) Chemical and biological characterization of newly discovered iodoacid drinking water disinfection byproducts. Environmental Science & Technology, 38, 4713–4722. [PubMed: 15487777]

Porwollik S, Wong RM-Y, Sims SH, Schaaper RM, DeMarini DM and McClelland M (2001) The *ΔuvrB* mutations in the Ames strains of *Salmonella* span 15–119 genes. Mutation Research, 483, 1–11. [PubMed: 11600126]

Postigo C, DeMarini DM, Armstrong MD, Liberatore HK, Lamann K, Kimura SY, Cuthbertson AA, Warren SH, Richardson SD, McDonald T, Sey Y, Ackerson NOB, Duirk SE and Simmons JE (2018) Chlorination of source water containing iodinated X-ray contrast media: mutagenicity and identification of new iodinated disinfection by-products. Environmental Science & Technology, 52, 13047–13056. [PubMed: 30339747]

Postigo C, Richardson SD and Barcelo D (2017) Formation of iodo-trihalomethanes, iodo-haloacetic acids, and haloacetaldehydes during chlorination and chloramination of iodine containing waters in laboratory-controlled reactions. Journal of Environmental Science (China), 58, 127–134.

Postigo C and Zonja B (2019) Iodinated disinfection byproducts: formation and concerns. Current Opinion in Environmental Science & Health, 7, 19–25.

Richardson SD, Fasano F, Ellington JJ, Crumley FG, Buettner KM, Evans JJ, Blount BC, Silva LK, Waite TJ, Luther GW, McKague AB, Miltner RJ, Wagner ED and Plewa MJ (2008) Occurrence and mammalian cell toxicity of iodinated disinfection byproducts in drinking water. Environmental Science & Technology, 42, 8330–8338. [PubMed: 19068814]

Richardson SD, Plewa MJ, Wagner ED, Schoeny R and DeMarini DM (2007) Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. Mutation Research, 636, 178–242. [PubMed: 17980649]

Roldán-Arjona T, García-Pedrajas MD, Luque-Romero FL, Hera C and Pueyo C (1991) An association between mutagenicity of the *Ara* test of *Salmonella typhimurium* and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons. Mutagenesis, 6, 199–205. [PubMed: 1881351]

Roldán-Arjona T and Pueyo C (1993) Mutagenic and lethal effects of halogenated methanes in the *Ara* test of *Salmonella typhimurium*: quantitative relationship with chemical reactivity. Mutagenesis, 8, 127–131. [PubMed: 8464381]

Ross MK and Pegram RA (2003) [^{35}S]-Labeling of the *Salmonella typhimurium* glutathione pool to assess glutathione-mediated DNA binding by 1,2-dibromoethane. Chemico-Biological Interactions, 146, 39–49. [PubMed: 12902151]

Thier R, Taylor JB, Pemble SE, Humphreys WG, Persmark M, Ketterer B and Guengerich FP (1993) Expression of mammalian glutathione *S*-transferase 5–5 in *Salmonella typhimurium* TA1535 leads to base-pair mutations upon exposure to dihalomethanes. Proceedings of the National Academy of Sciences USA, 90, 8576–8580.

U.S. EPA (2020) CompTox Chemicals Dashboard. https://comptox.ega.gov/dashboard [Accessed 7 January 2021].

Wagner ED and Plewa MJ (2017) CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: An updated review. Journal of Environmental Sciences (China), 58, 64–76. [PubMed: 28774627]

Walker GC, Marsh L and Dodson L (1985) Cellular responses to DNA damage. Environmental Health Perspectives, 62, 115–117. [PubMed: 3910414]

Wei X, Wang S, Zheng W, Wang X, Liu X, Jiang S, Pi J, Zheng Y, He G and Qu W (2013) Drinking water disinfection byproduct iodoacetic acid induces tumorigenic transformation of NIH3T3 cells. Environmental Science & Technology, 47, 5913–5920. [PubMed: 23641915]

Weinberg HS, Krasner SW, Richardson SD and Thruston AD Jr. (2002) The occurrence of disinfection by-products (DBPs) of health concern in drinking water: results of a nationwide DBP occurrence study. EPA/600/R02/068.

Wendel FM, Lütke Eversloh C, Machek EJ, Duirk SE, Plewa MJ, Richardson SD, Ternes TA (2014) Transformation of iopamidol during chlorination. Environmental Science & Technology, 48, 12689–12697. [PubMed: 25325766]

Xu ZF, Li X, Hu XL and Yin DQ (2017) Distribution and relevance of iodinated X-ray contrast media and iodinated trihalomethanes in an aquatic environment. Chemosphere, 184, 253–260. [PubMed: 28601007]

Yang Y, Komaki Y, Kimura SY, Hu HY, Wagner ED, Marinas BJ and Plewa MJ (2014) Toxic impact of bromide and iodide on drinking water disinfected with chlorine or chloramines. Environmental Science & Technology, 48, 12362–12369. [PubMed: 25222908]

Zhang J, Chen DD, Li L, Li WW, Mu Y and Yu HQ (2016) Role of NOM molecular size on iodo-trihalomethane formation during chlorination and chloramination. Water Research, 102, 533–541. [PubMed: 27423047]

Zhang S-H, Miao DY, Liu AL, Zhang L, Wei W, Xie H and Lu WQ (2010) Assessment of the cytotoxicity and genotoxicity of haloacetic acids using microplate-based cytotoxicity test and CHO/HGPRT gene mutation assay. Mutation Research, 703, 174–179. [PubMed: 20801231]

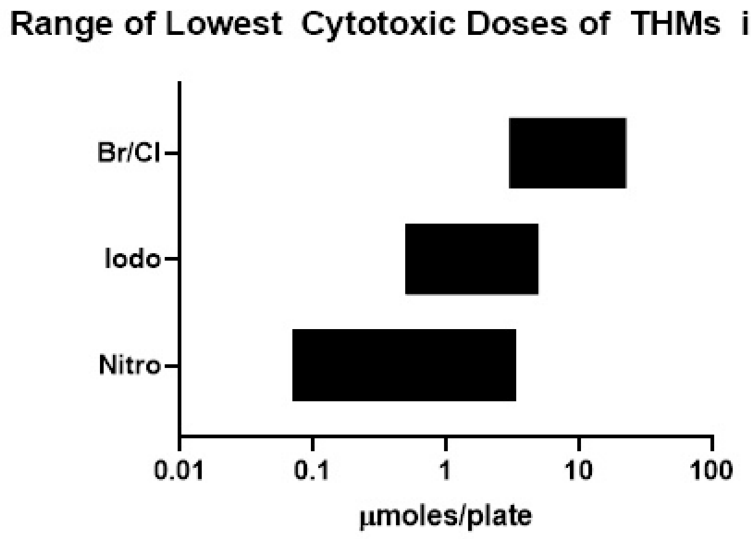

Figure 1.

Range of lowest cytotoxic doses of THMs in *Salmonella* TA100. Data for Br-, Cl-, and nitro-THMs are from Kundu *et al.* (2004a); data for I-THMs are from the present study.

TABLE 1

Iodinated trihalomethane compound information

Chemical	Acronym	CAS	MW	Purity (%)	DTXSID[a]
Chlorodiiodomethane	CDIM	638-73-3	302.28	95	20213251
Dichloroiodomethane	DCIM	594-04-7	210.83	95	7021570
Dibromoiodomethane	DBIM	593-94-2	299.73	>90	60208040
Bromochloroiodomethane	BCIM	34970-00-8	255.28	95	9021502
Triiodomethane (iodoform)	TIM	75-47-8	393.73	99	4020743

[a]DSSTox substance identifier (U.S. EPA 2020).

TABLE 2

Mutagenicity (rev/plate) of I-THMs in *Salmonella* RSJ100 and TPT100[a]

		RSJ100		TPT100	
Compound	**Dose (μmoles/plate)**	**Exp 1**	**Exp 2**	**Exp 1**	**Exp 2**
Dichloroiodomethane (DCIM)	0.000	22	15	22	16
	0.100	23	21	-	11
	0.250	18	20	4	15
	0.500	21	10	24	7
	1.000	24	18	23	16
	2.500	4[b]	14	1[b]	0
Dibromoiodomethane (DBIM)	0.000	22	15	22	16
	0.010	19	-	10	-
	0.025	21	-	13	-
	0.050	19	-	19	-
	0.075	23	-	16	-
	0.100	18	5	2	9
	0.250	-	16	-	17
	0.500	-	37	-	19
	1.000	-	22	-	12
	2.500	-	24	-	7
Chlorodiiodomethane (CDIM)	0.000	22	15	22	16
	0.100	27	16	21	19
	0.250	20	18	15	15
	0.500	30	19	25	33
	1.000	28	20	3	8
	2.500	9	0	0	4
Bromochloroiodomethane (BCIM)	0.000	22	15	22	16
	0.010	21	-	6	-
	0.025	22	-	11	-
	0.050	12	-	8	-
	0.100	19	19	0	13
	0.250	22	22	0	13
	0.500	20	22	8	12
	1.000	33	33	5	17
	2.500	-	0	-	11
Triiodomethane (TIM)	0.000	22	15	22	16
	0.010	25	-	3	-
	0.025	19	-	14	-
	0.050	31	-	16	-
	0.100	24	23	18	18
	0.250	27	21	18	11

		RSJ100		TPT100	
Compound	**Dose (μmoles/plate)**	**Exp 1**	**Exp 2**	**Exp 1**	**Exp 2**
	0.500	39	32	17	10
	1.000	31	33	26	12
	2.500	-	2[b]	-	7[b]
Sodium azide[c]	3 μg/plate	756	769	871	889

[a] Values represent counts from single plates except for the zero (0) DMSO controls and positive controls, which represent the average counts from three plates. Cells within the table with dashes represent doses that were not tested.

[b] These plates showed a thinning of the background lawn, indicative of cytotoxicity.

[c] Positive control.

TABLE 3

Mutagenicity (rev/plate) of I-THMs in *Salmonella* TA100[a]

		−S9		+S9	
Compound	**Dose (μmoles/plate)**	**Exp 1**	**Exp 2**	**Exp 1**	**Exp 2**
Dichloroiodomethane (DCIM)	0.000	82	78	87	99
	0.010	-	74	-	-
	0.025	-	70	-	-
	0.050	-	82	-	-
	0.100	80	52	94	-
	0.500	72	-	81	-
	1.000	55	-	-	-
	2.500	65	-	86	-
	5.000	12[b]	-	80	77[b]
	7.500	-	-	-	43[b]
	10.00	-	-	-	51[b]
Dibromoiodomethane (DBIM)	0.000	82	78	87	99
	0.001	-	86	-	97
	0.003	-	66	-	97
	0.005	-	85	-	87
	0.010	-	78	89	101
	0.020	86	-	-	-
	0.025	-	79	-	86
	0.050	86	73	110	92
	0.100	17	-	94	-
	0.250	23	-	81	-
	0.500	8[b]	-	15[b]	-
Chlorodiiodomethane (CDIM)	0.000	82	78	87	99
	0.010	81	-	91	-
	0.050	98	-	99	-
	0.100	82	82	95	109
	0.250	-	82	-	103
	0.500	-	91	115	101
	1.000	-	58	-	-
	2.500	-	83	-	-
	5.000	-	-	17[b]	-
Sodium azide[c]	3 μg/plate	1159	807	-	-
2-Aminoanthracene[c]	0.5 μg/plate	-	-	444	503

[a]Values represent counts from single plates except for the zero (0) DMSO controls and positive controls, which represent the average counts from three plates. Cells within the table with dashes represent doses that were not tested.

[b] These plates showed a thinning of the background lawn, indicative of cytotoxicity.

[c] Positive control.