Characterization of iodinated disinfection by-products in chlorinated and chloraminated waters using Orbitrap™ based gas chromatography-mass spectrometry

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Abstract

Recent developments in gas chromatography (GC) – mass spectrometry (MS) have opened up the possibility to use the high resolution- accurate mass (HRAM) Orbitrap mass analyzer to further characterize the volatile and semi-volatile fractions of environmental samples. This work describes the utilization of GC Orbitrap MS technology to characterize iodine containing disinfection by-products (iodo-DBPs) in chlorinated and chloraminated DBP concentrates. These DBP mixtures were generated in lab-scale disinfection reactions using Llobregat river water and solutions containing Nordic Lake natural organic matter (NOM). The DBPs generated were concentrated using XAD resins and extracts obtained were analyzed in full scan mode with the GC Orbitrap MS. Integration of high resolution accurate mass information and fragment rationalization allowed the characterization of up to 11 different iodo-DBPs in the water extracts analyzed, including one new iodo-DBP reported for the first time. Overall, formation of iodo-DBPs was enhanced during chloramination reactions, and water NOM characteristics and iodide and bromide content affected the amount and type of iodo-DBPs generated.

Keywords: iodinated disinfection by-products, chlorination, chloramination, gas chromatography, Orbitrap GC, high resolution mass spectrometry

INTRODUCTION

Chemical disinfection of water with strong oxidizing agents is a widely extended practice used to produce potable water in most developed countries. However, this process has the unintended consequence of forming disinfection byproducts (DBPs). Since the discovery of the first carcinogenic DBP, i.e., chloroform, in drinking water in 1974 [1, 2], concerns over DBP exposure have increased through the years due to epidemiologic studies showing associations between consumption of chlorinated water and bladder and colorectal cancer [3-6], and adverse reproductive outcomes, including spontaneous abortion, increased risk of low birth weight at term, still birth, and preterm delivery [7-16].

The formation of DBPs is mainly related to the type of the disinfection treatment applied, and the nature of the water source in terms of its natural organic matter (NOM) and bromide and iodide content. More than 600 DBPs have been identified in drinking water to date [17-20]. However, despite intense identification efforts, about 50% of the halogenated DBPs formed during water chlorination still remains unidentified, as assessed through total organic halogen (TOX) measurements [17]. Most iodine containing DBPs (iodo-DBPs) belong to the aforementioned unknown fraction and these chemicals are becoming a new focus because of their higher toxicity as compared to the brominated and chlorinated analogues [21-27]. In addition, one of these iodo-DBPs, iodoacetic acid, was recently shown to be tumorigenic in mice [28]. Furthermore, iodide levels in waters intended for drinking are likely to increase in the near future in coastal areas affected by climate-change (where water scarcity will lead to an increased use of groundwater resources, moving seawater intrusion inland, and desalinated water) and areas impacted by hydraulic fracturing activities that produce highly saline water with high iodine content (as recently reported by Parker et al [29]). Based on this, further research on iodo-DBPs, in terms of compound detection and confident chemical characterization, in addition to toxicity assessment, is required. This is critical in order to evaluate the potential risk of exposure to these compounds and the possible consequences on human health.

Characterization of iodo-DBPs in disinfected water is a challenging task due to the combined challenge of their occurrence at low concentrations and the complexity of the analytical matrix. This has been usually carried out using high resolution mass

spectrometry (HRMS) techniques. Magnetic sector mass spectrometry coupled to gas chromatography has been the technique of choice to discover semi-volatile iodo-DBPs in DBP mixtures [18, 30]. Operation of this type of analyzer in an untargeted manner (i.e., full scan mode) on one hand requires a high degree of expertise and on the other may not provide the required sensitivity and scan speed at a high mass resolving power. The recent development of Orbitrap based GC-MS benchtop instruments enables a unique opportunity to operate using full scan acquisition with very high mass resolving power and mass accuracy, whilst at the same time offering the ability to detect very low concentrations. Thus, the present work aimed at evaluating the application of Orbitrap GC-MS for the characterization of iodinated DBPs in chlorinated and chloraminated DBP mixtures. In order to accomplish this, DBP mixtures were generated in lab-scale disinfection reactions using waters with various NOM, iodide and bromide content, and were analyzed. Automatic peak deconvolution software was used to detect compounds, and putative identification of these compounds was performed using mass spectral matching against commercially available libraries combined with a comprehensive accurate mass based rationalization of the entire mass spectrum.

MATERIALS AND METHODS

Chemicals and reagents

All reagents were purchased from Sigma-Aldrich (Barcelona, Spain) unless otherwise specified. Amberlite[®] XAD-2 and Supelite[™] DAX-8 resins were used for water extraction. Anhydrous Na₂SO₄ was used to dry the extracts. The list of solvents used includes: Chromasolv[®] grade methanol (≥99.9%, MeOH), and ethyl acetate for pesticide residue analysis (≥99.9%, EtAc). Potassium phosphate dibasic trihydrate (K₂HPO₄·H₂O) and potassium phosphate monobasic (KH₂PO₄) (≥98%) were used to buffer at pH 7.5 the disinfection reactions. Sulfuric acid (95-97%, H₂SO₄), hydrochloric acid (≥37%, HCl), and sodium hydroxide pellets (≥98%, NaOH) were ACS grade.

Reverse osmosis isolated NOM from Nordic Lake (NL) (Skarnes, Norway) was purchased from the International Humic Substances Society (IHSS) (St Paul, MN). Purified water (18 MΩ/cm) from an Aurum ultrapure water system (Sartorius, Madrid) was used to prepare all reagent solutions and to perform disinfection reactions with NL NOM.

Reagents used to measure free chlorine in the water samples and in the chlorine (HOCl/OCl⁻) and monochloramine (NH₂Cl) solutions by means of the *N,N*-diethyl-*p*-

phenylenediamine (DPD) ferrous titrimetric method [31] were: barium diphenylamine-4 sulfonate for redox titration, potassium dichromate (>99%, $\text{Cr}_2\text{K}_2\text{O}_7$), ethylenediaminetetraacetic acid disodium salt dehydrate (99-101%, EDTA), *N,N*-diethyl-*p*-phenylenediamine sulfate salt (>98%, DPD), ferrous ammonium sulfate hexahydrate (99%, FAS), orthophosphoric acid (85%, H_3PO_4), and sodium phosphate dibasic (99%, Na_2HPO_4).

Sodium hypochlorite (NaOCl) solution (10%, w/v, reagent grade) was purchased from Panreac (Barcelona, Spain). Ammonium chloride (>99.99%, NH_4Cl) was used to produce NH_2Cl .

Disinfection reactions

All disinfection reactions were performed in a Pyrex® glass reaction vessel filled up to the top (headspace-free; total volume of 17.1 L). Reactions were conducted over 72 h at room temperature (22-26°C) in the dark, under continuous stirring using a magnetic stir plate and a polytetrafluoroethylene (PTFE)-coated stir bar. In all experiments, water was buffered to pH 7.5 with phosphate buffer (10 mM) prior to oxidant addition. Either H_2SO_4 or NaOH (1N) was used to adjust the solution pH.

NOM solutions were prepared at a concentration of 5 mg/L by weighing the corresponding amount of NL NOM. Five hundred ppb of bromide (as KBr) and 50 ppb of iodide (as KI) were also added to this matrix in order to promote formation of iodinated and brominated DBPs during disinfection reactions. Similar bromide and iodide levels have been reported to occur in source waters used for drinking water production at different locations [32, 18, 33, 22].

In the case of the Llobregat River (LLOB) experiments, water was collected directly from the midpoint of the river at a time at one single location. The sampling point was located close to the intake of a drinking water treatment plant that gives service to part of Barcelona and its metropolitan area. Due to the high natural levels of iodide and bromide of this surface water, disinfection reactions were performed without the extra addition of iodide and bromide ions. Characteristics of the water matrices used for the experiments are summarized in Table S1 (see Electronic Supplementary Material (ESM)).

The chlorine dose was selected based on the specific chlorine demand of the NOM of each water matrix that resulted in approximately 0.5 mg/L of residual chlorine at the

end of the disinfection reaction. The disinfectant doses used to treat NL NOM solutions and LLOB water were 4 mg/L and 7.5 mg/L of Cl_2 , respectively.

Monochloramine reactions were carried out with freshly prepared pre-formed NH_2Cl . This solution was prepared at a 0.7 Cl/N molar ratio by dropwise addition of HOCl to a NH_4Cl solution at pH 8.5. The reaction was performed under continuous stirring with the reaction flask immersed in an ice bath for 30 min.

Two different procedural blanks were also analyzed: (a) Milli-Q water containing NL NOM, buffered but not treated (no disinfectant) and (b) LLOB water buffered but not treated. Both blanks were concentrated in the same manner as the treated samples, and they were used to investigate whether the compounds detected and identified were generated during disinfection treatments (i.e., DBPs) or were contaminants already present in the source waters or were artifacts generated during the sample preparation treatments.

Generation of DBP mixture concentrates

Up to 16 L of the treated water was concentrated using XAD resins. Details about the preparation and cleaning of these resins can be found elsewhere [34] and are provided in ESM. After acidification, the water samples ($\text{pH} < 1$) were passed through a resin column that contained a combination of DAX-8 resin over XAD-2 resin at a ratio of 770:1 (v/v) of water to resin to maximize adsorption of organic compounds and to minimize breakthrough. The combination of these two resins is effective for absorbing a wide range of organic compounds. Retained analytes were eluted with EtAc at a ratio of 9.5:1 (v/v) of solvent to resin. The EtAc extract was dried with the aid of a separator funnel and Na_2SO_4 , rotary-evaporated to approximately 5 mL, and further concentrated under a gentle stream of N_2 to 0.8 mL (see detailed extraction procedure in ESM). This extract was directly injected into the GC-Orbitrap MS for analysis of iodo-DBPs.

GC-Q/Orbitrap MS analyses

A total of six water concentrates were analyzed by means of GC Orbitrap based MS. The Q ExactiveTM GC was coupled to a TraceTM 1300 GC (Thermo Scientific). A volume of 1 μL of the DBP mixture extract was injected in splitless mode using a TriPlusTM RSH injector (Thermo Scientific) with a splitless time of 1 min. The injector temperature was set at 280°C.

In order to increase the resolution of chromatographic peaks, the GC separation of extracted components was carried out on a Thermo Scientific TR-5MS column, 60 m x 0.25 mm (I.D.) x 0.25 μ m (film thickness) using helium as carrier gas at a constant flow of 1.2 mL/min and the following GC temperature program: initial temperature of 40°C held for one min, increased at a rate of 15°C/min to 325°C and held for 10 min. The GC was interfaced with the Q Exactive GC instrument via a transfer line heated at 280°C. The source temperature was set at 250°C. MS analyses were performed using electron ionization (EI) at 70 eV in full-scan mode using a scan range of m/z 50-650 and a resolution of 60,000 (full width at half-maximum [FWHM] at m/z 200).

Data analysis

Data were both manually queried and automatically processed with TraceFinder software. TraceFinder provided automatic peak detection, spectral deconvolution, and compound identification based on spectral library matching and accurate mass information. In all cases, a mass window of ± 2 ppm was used to enable generation of highly selective extracted ion chromatograms with reduced chemical interferences from the matrix background.

RESULTS AND DISCUSSION

Compound identification workflow

Figure 1 summarizes the workflow applied for the detection and molecular structure characterization of iodo-DBPs. Data acquired in EI was sent to the TraceFinder that uses unique software workflows and algorithms to automate the discovery and identification of compounds. First, the software performs peak detection, spectral deconvolution, spectral library searching (e.g. NIST, Wiley or custom made) and validates candidate library hits using high resolution filtering (HRF) based on candidate elemental formulae [35]. This was simultaneously performed for all DBP mixtures generated with the same matrix (i.e., NL NOM, chlorinated NL NOM, and chloraminated NL NOM).

Data processing with TraceFinder was simultaneously performed for all DBP mixtures generated with the same matrix (i.e., NL NOM, chlorinated NL NOM, and chloraminated NL NOM) to visualize fold changes with respect to a particular analyte of interest. This process provides a heat map of components that facilitates the

187 identification of the most relevant compounds generated during each disinfection
188 process. Due to the large number of compounds detected following deconvolution
189 (>2500 peaks were found in the chloraminated NL NOM extract using a total ion
190 current (TIC) intensity threshold of 500,000 and a signal-to-noise (S/N) threshold of
191 10:1), a mass filter was employed to limit the chemicals of interest only to those
192 containing iodine (m/z 126.90392). This resulted in fewer compounds detected (less
193 than 15 major peaks in the chloraminated NL NOM extract), but with all of them
194 containing iodine in their mass spectra.

195 Moreover, despite the fact that iodine does not produce a distinctive mass spectral
196 isotopic pattern (it has only one stable isotope), iodo-DBPs may also include chlorine or
197 bromine atoms in their structures, which give distinctive isotopic patterns, due to their
198 two stable isotopes each (m/z 35 and 37 for chlorine and m/z 79 and 81 for bromine).
199 Consequently, this feature was also used for elucidation of iodo-DBP chemical
200 structures.

201 Following the discussion started by Schymanski et al. [36] on identification confidence
202 levels for small molecules, three different confidence levels for iodo-DBP identification
203 were considered (Figure 1). If no analytical standards are available, but enough
204 evidence is obtained through fragment rationalization (assisted by the Mass Frontier
205 software) and the presence of isotopic patterns in the molecular ion, an identification
206 confidence level 3 for the chemical structure proposed was assigned. If there is also
207 evidence provided by a mass spectral library match (such as NIST), the confidence level
208 for the chemical structure proposed rises from level 3 to level 2. The confidence level is
209 risen to 1 if the chemical structure proposed is confirmed with the analysis of a
210 reference analytical standard by a match of retention times and mass spectra.

211
212 Figure 1.

213 214 **Iodinated DBPs in disinfected water extracts**

215 According to the literature [18, 30, 37-39], iodo-DBP formation (as well as subsequent
216 cytotoxicity and genotoxicity of treated water [24]) is enhanced during chloramination
217 reactions when iodide is present in source waters prior to disinfection. This was also
218 observed in the experiments performed here (normalized extracted ion chromatograms
219 [XICs] at m/z 126.90392 for the different analyzed extracts are provided in Figures S1
220 and S2, ESM). Interestingly, this was more noticeable in NL NOM extracts as

compared to the LLOB water extracts. Profiles of formed iodo-DBPs in NL NOM and LLOB water samples were also different, with a higher number of peaks containing iodine in NL NOM than in LLOB extracts, which could be attributed to the difference in NOM characteristics of the waters tested. According to SUVA measurements (see ESM Table S1), which indicate the aromatic content per unit concentration of carbon [40], the organic matter of the LLOB water has a relatively high content of hydrophobic, aromatic, and high molecular weight fractions, whereas NL NOM contains mainly non-humic, hydrophilic, and low molecular weight materials. Thus, different reactivity with disinfecting agents is expected. The increased formation of iodo-DBPs in NL NOM extracts is consistent with three other studies showing increased formation of iodo-trihalomethanes (I-THMs) from hydrophilic and low molecular weight precursors [39, 41, 42]. However, LLOB water extracts are expected to contain overall higher amounts of total organic iodine (TOI) (parameter not measured in this study) and high-molecular weight iodo-DBPs (less amenable to GC/MS analysis than low and medium molecular weight compounds) than NL NOM extracts, since hydrophobic and high MW precursors (related to high SUVA values) were observed to be more reactive with iodine in the formation of TOI and unknown TOI (not attributed to I-THMs) [42]. Furthermore, different levels of bromide and iodide in the tested waters (Br^-/I^- concentration ratios of 8 and 46 in NL NOM solutions and LLOB water, respectively, see Table S1 in ESM) may also rule iodine and bromine incorporation into NOM, and consequently, iodo-DBP formation. In this respect, Jones et al. reported an increased iodo-DBP formation (I-THMs) in waters with low Br^-/I^- ratio when compared to waters with high Br^-/I^- ratio (2 vs 10). Additionally, I^-/DOC ratio has been also pointed as an important factor in iodo-DBP (I-THMs) formation, e.g., increased formation of I-THMs in waters presenting high I^-/DOC ratios [43].

A total of 11 different iodo-DBPs, most of them iodine containing halomethanes, were detected and identified in the extracts analyzed. Chemical structures were proposed for all compounds with a minimum confidence level of 2 after applying the workflow described in the previous section, with the exception of ethyl iodoacetate and ethyl β -iodopropionate (confidence level 3). Experimental and theoretical masses of molecular and fragment ions, the mass difference (in ppm) between them, the assigned elemental compositions for each diagnostic ion, and the proposed chemical structures for the identified DBPs are shown in Tables 1 and 2.

Table 1

Table 2

For some DBPs, the molecular ion was not observed due to extensive fragmentation under EI, as shown in Figure 2 for dichloriodomethane. To determine the elemental composition, the presence of the molecular ion is essential for correct mass spectrum interpretation. In this respect, the use of softer ionization sources, e.g., negative/positive chemical ionization (NCI or PCI) or atmospheric pressure chemical ionization (APCI) for GC may help to identify and confirm additional iodo-DBPs in these extracts.

Figure 2

During this study, the scan range was not extended below m/z 50, as such, some lower mass fragments of these compounds were not measured. This was the case for the peaks appearing at 8.03 min, as shown in Figure 3. The elemental composition of the molecular ion and the fragments observed suggests these peaks to be ethyl iodoacetate. As it can be observed in the mass spectral library records for this compound, m/z 29 ($C_2H_5^+$) and m/z 42 ($COCH_2^+$) in the case of peak at 8.03 are additional fragments of this compound that contribute to increase the confidence level in the molecular structure proposed. The remaining fragments observed fit with the proposed structure; however, because the fragments' relative abundance is not in complete agreement with that provided by a NIST mass spectral library search (see Figure 3), the molecular structure assigned to the elemental composition of $C_4H_7O_2I$ cannot be explained with a confidence level higher than 3. Differences in fragments' relative abundance could be attributed to differences in the type of mass analyzers used to construct the spectral library used.

Peak eluting at time 8.14 was suggested to be and ethyl β -iodopropionate. Our identification was based on the longer retention time of these peak in the column compared to ethyl iodoacetate ($t_R=8.07$), and the fragments observed, as shown in figure 4. This compound also presents an unstable molecular ion under electron ionization.

287 Ethyl iodoacetate and and ethyl β -iodopropionate were present in all investigated
288 extracts (including the blanks), and therefore it may be formed during the experimental
289 procedure, due to the use of ethyl acetate as extraction solvent. However, the intensity
290 of this peak in the chloraminated NL extract was 10 times higher (60×10^6 counts per
291 second (cps)) than in the other samples (6×10^6 cps), and therefore, it was considered to
292 be also formed during chloramination of the NL NOM solutions. A similar situation
293 was observed for the peak appearing at retention time 3.7 min, tentatively identified as
294 iodomethane (confidence level 2) (see ESM Fig. S3). Iodomethane was present in all
295 NL NOM extracts, but its concentration noticeably increases during chlorination and
296 chloramination of NL NOM solutions.

297 In order to confirm that these compounds were DBPs and not formed during the
298 extraction process, an additional blank of the chloramination process with purified
299 water spiked with 500 ppb of bromide and 50 ppb of iodide (as KBr and KI,
300 respectively) was performed. Both, ethyl iodoacetate and ethyl β -iodopropionate were
301 found in this blank extract, however, the peak areas in chloraminated and chlorinated
302 extracts were comparatively much higher than in the blank (10 times and 5 times,
303 respectively), and therefore, it was concluded that these compounds also were generated
304 during the disinfection process.

305 Ion mass spectra for additional iodo-DBPs identified are provided as supplementary
306 material (see ESM Figures S4-S11). All iodo-DBPs identified in NL NOM and LLOB
307 disinfected waters were generated during both chlorination and chloramination
308 treatments, except chlorodiiodomethane and bromodiiodomethane, which were
309 exclusively formed during chloramination. Similar levels of iodo-DBPs were found in
310 chloraminated and chlorinated LLOB waters, whereas, iodo-DBP formation was
311 enhanced during chloramination of NL NOM solutions compared to chlorination (i.e.,
312 8-66 fold higher base peak areas at XIC 126.90392 in the chloraminated extract
313 compared to the chlorinated extract, up to 145 in the case of diiodomethane).

314 Despite the fact that most of the characterized iodo-DBPs belong to the well-known
315 iodo-DBP class of I-THMs, this work reveals the formation of chloriodomethane and
316 ethyl iodoacetate in chloraminated waters for the first time, and points to ethyl β -
317 iodopropionate as a novel iodo-DBP that may generate during disinfection of waters
318 containing iodine.

319

Figure 3

CONCLUSIONS

This work has shown the successful application of GC-Orbitrap MS for the characterization of iodinated DBPs in disinfected water extracts.

By using this novel GC-MS technology, further scientific insights into the formation of volatile and semi-volatile DBPs generated during disinfection treatments are possible.

To the authors' knowledge, this work would represent the first evidence on the formation of the tentatively identified iodo-DBPs, i.e., chloriodomethane, and ethyl iodoacetate, in chloraminated water, despite the fact that their presence in chlorinated waters is well known [30]. In the case of ethyl β -iodopropionate, no previous records on its formation during water disinfection processes were found in the peer-reviewed literature.

The consistent sub-ppm mass accuracy even for compounds present at low concentrations, and the power of high resolution allows for clear mass spectrum interpretation and ultimately for compound identification and chemical structure elucidation. Further investigations using softer ionization techniques, such as chemical ionization, may help to identify and confirm the presence of additional iodo-DBPs in these extracts.

Ultimately, the workflow described here can be also applied to discover "unknown" chlorine and bromine containing DBPs in DBP mixtures, as the identification of all major components of DBP mixtures generated during disinfection treatments is essential to further minimize the potential risks of exposure.

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Fig. 1 Workflow applied for identification of iodo-DBPs and confidence levels of the iodo-DBP molecular structures proposed

Fig. 2 Mass spectrum of dichloriodomethane ($t_R=6.33$ min) (a) in an analytical standard solution and (b) in the chloraminated NL NOM

Fig. 3 Fragment rationalization for mass spectra of peak appearing at $t_R=8.03$ min in the chloraminated NL NOM extract and comparison with mass spectra of the tentatively assigned molecular structure provided by the MS library (NIST chemistry webbook)

Fig. 4. Fragment rationalization for mass spectra of peak appearing at $t_R=8.14$ min in the chloraminated NL NOM

Table 1. Iodo-DBPs characterized in disinfected NL NOM waters.

Analyte	t_R (min)	Molecular ion and fragment ions	Elemental composition				Compound ID	Identification confidence level**
			Experimental (m/z)	Theoretical (m/z)	Error (ppm)	Molecular formula		
DBP1	3.71	142	141.92743	141.92739	0.3	CH ₃ I	Iodomethane	2
		141	140.91963	140.91956	0.5	CH ₂ I		
		139	138.90395	138.90391	0.3	CI		
		127	126.90396	126.90392	0.4	I		
DBP2	5.36	175	175.88844	175.88842	0.1	C ³⁵ ClH ₂ I	Chloriodomethane	2
		174	174.88063	174.88059	0.2	C ³⁵ ClHI		
		141	140.91963	140.91956	0.5	CH ₂ I		
		127	126.90396	126.90392	0.4	I		
DBP3	5.76	170	169.92235	169.92231	0.3	C ₂ H ₃ OI	Iodoacetaldehyde	1
		142	141.92749	141.92739	0.7	CH ₃ I		
		141	140.91953	140.91956	0.3	CH ₂ I		
		127	126.90399	126.90392	0.5	I		
DBP4	6.33	210	n.d.	209.84945	-	C ³⁵ Cl ₂ HI	Dichloriodomethane	1
		175	174.88061	174.88060	0.1	C ³⁵ ClHI		
		127	126.90399	126.90392	0.5	I		
		87	86.93908	86.93908	0.0	C ³⁷ Cl ₂ H		
		85	84.94202	84.94203	0.1	C ³⁵ Cl ³⁷ Cl H		
		83	82.94502	82.94498	0.0	C ³⁵ Cl ₂ H		
DBP5	7.35	268	267.82416	267.82405	0.4	CH ₂ I ₂	Diiodomethane	2
		254	253.80847	253.80838	0.2	I ₂		
		141	140.91953	140.91956	0.3	CH ₂ I		
		127	126.90399	126.90392	0.6	I		

Analyte	t _R (min)	Molecular ion and fragment ions	Elemental composition				Compound ID	Identification confidence level**
			Experimental (m/z)	Theoretical (m/z)	Error (ppm)	Molecular formula		
DBP6	8.04	214	213.94844	213.94908	0.4	C ₄ H ₇ O ₂ I	Ethyl iodoacetate	3
		186	185.91718	185.91722	0.2	C ₂ H ₃ O ₂ I		
		169	168.91444	168.91448	0.2	C ₂ H ₂ OI		
		142	141.92735	141.92739	0.3	CH ₃ I		
		141	140.91957	140.91956	0.0	CH ₂ I		
		127	126.90397	126.90392	0.4	I		
DBP7	8.14	228		227.96420		C ₅ H ₉ IO ₂	Ethyl β-iodopropionate	3
		154	153.92739	153.92739	0.0	C ₂ H ₃ I		
		155	154.93519	154.93522	-0.2	C ₂ H ₄ I		
		141	140.91966	140.91956	0.0	CH ₂ I		
		127	126.90397	126.90392	0.4	I		
		87	87.04409	87.044057	0.4	C ₄ H ₇ O ₂		
DBP8*	8.77	302	301.78513	301.78507	0.3	C ³⁵ ClHI ₂	Chlorodiiodomethane	1
		267	266.81625	266.81622	0.1	CHI ₂		
		254	253.80847	253.80838	0.3	I ₂		
		175	174.88060	174.88059	0.0	C ³⁵ ClHI		
		127	126.90399	126.90392	0.6	I		
DBP9*	9.85	346	345.73459	345.73455	0.1	C ⁷⁹ BrHI ₂	Bromodiiodomethane	1
		267	266.81638	266.81621	0.1	CHI ₂		
		254	253.80844	253.80838	0.2	I ₂		
		219	218.83009	218.83008	0.0	C ⁷⁹ BrHI		
		127	126.90399	126.90392	0.6	I		
		79	78.91781	78.91778	0.4	⁸¹ Br		

*Iodo-DBP present only in chloraminated extract.

**Confirmation level: 1- Elemental composition and fragment rationalization based on accurate mass measurements, mass spectral library match, and MS and retention time comparison with analytical reference standard; 2- Elemental composition, and fragment rationalization based on accurate mass measurements and mass spectral library match 3 - Elemental composition and fragment rationalization based on accurate mass measurements.

Table 2. Iodo-DBPs characterized in disinfected LLOB waters.

Analyte	t_R (min)	Molecular ion and fragment ions	Elemental composition			Molecular formula	Compound ID	Identification confidence level*
			Experimental (m/z)	Theoretical (m/z)	Error (ppm)			
DBP1	6.34	210	n.d.	209.84945	-	$C^{35}Cl_2HI$	Dichloriodomethane	1
		175	174.88062	174.88060	0.1	$C^{35}ClHI$		
		127	126.90400	126.90392	0.7	I		
		87	86.93906	86.93908	0.3	$C^{37}Cl_2H$		
		85	84.94206	84.94203	0.3	$C^{35}Cl^{37}ClH$		
		83	82.94499	82.94498	0.0	$C^{35}Cl_2H$		
DBP2	7.34	254	253.79907	253.79893	0.5	$C^{35}HCl^{79}BrI$	Bromochloro iodomethane	1
		219	218.83007	218.83009	0.0	$C^{79}BrHI$		
		175	174.88062	174.88060	0.1	$C^{35}ClHI$		
		127	126.90400	126.90392	0.7	I		
		127	126.89230	126.89242	0.9	$C^{79}Br^{35}ClH$		
DBP3	8.44	298	297.74862	297.74897	0.6	$CH^{79}Br_2I$	Dibromiodomethane	1
		219	218.83014	218.83008	0.3	$C^{79}BrHI$		
		206	205.82209	205.82281	0.8	^{79}BrI		
		173	172.84188	172.84191	0.2	$C^{79}Br^{81}BrI$		
		127	126.90400	126.90392	0.7	I		

*Confirmation level: 1- Elemental composition and fragment rationalization based on accurate mass measurements, mass spectral library match, and MS and retention time comparison with analytical reference standard, 2- Elemental composition, and fragment rationalization based on accurate mass measurements and mass spectral library match, 3 - Elemental composition and fragment rationalization based on accurate mass measurements.

Fig. 1

23jul15_009 #1412-1416 RT: 6.31-6.31 AV: 5 SB: 134 6.38-6.56 , 6.14-6.28 NL: 8.58E7
T: FTMS + p EI Full lock ms [50.00-650.00]

Fig. 2

Fig. 3

Fig 4. Fragment rationalization for mass spectra of peak appearing at $t_R=8.14$ min in the chloraminated NL NOM.

Electronic Supplementary Material

Characterization of iodinated disinfection by-products in chlorinated and chloraminated waters

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I- Characteristics of the source waters

Total organic carbon (TOC) measurements were carried out by means of a Shimadzu TOC-VCSH analyzer (Shimadzu Europa GmbH, Duisburg, Germany). UV absorbance at 254 nm to calculate the specific UV absorbance (SUVA) was measured with an Agilent 8453 UV-Visible spectrophotometer. Bromide and iodide measurements were carried out by means of ion chromatography and inductively coupled plasma (ICP)-MS, respectively. Average values of TOC, SUVA, bromide and iodide content in the source waters used for the experiments are summarized in Table S1.

Table S1 – Characteristics of the source waters

Source water	Abbrev.	TOC (mg/L)	SUVA ₂₅₄ (L/mg-M)	Bromide (µg/L)	Iodide (µg/L)
Nordic Lake (n=2) spiked with Br ⁻ and I ⁻	NL	5.2±1.4	1.4±0.4	433.0±0.0	52.6±0.7
Llobregat River (n=2)	LLOB	7.2±0.4	4.3±0.2	832.5±45.9	18.0±0.5
Method limits of detection (LODs): TOC – 0.05 mg/L, Br – 10 µg/L, I – 1 µg/L					

II- Generation of DBP mixture concentrates.

IIa. Purification of XAD resins.

As reported by Richardson et al. (1994), resins were cleaned and purified prior to use. One hundred g of resins were sequentially washed with 150 mL NaOH (0.1M), 150 mL Milli-Q water, and 150 mL MeOH under stirring for 30 min. Then, they were placed into a glass thimble and were sequentially Soxhlet extracted with 400 mL of MeOH, 400 mL of ethyl acetate, and 400 mL of MeOH during 24 hours each time. After purification with Soxhlet extraction, they were kept in the dark at 4°C for no more than 3 months.

IIb. Water extraction with XAD resins.

For the extraction of 16 L of water, 24 mL of DAX-8 resins was placed onto 24 mL of XAD-2 resins in a 1-L liquid chromatography glass column with a 1-L glass reservoir with the aid of MeOH for resin transfer. Glass wool (Sigma Aldrich) was used for column packing. Before extraction, resins were conditioned with three resin volumes of Milli-Q water to remove MeOH, two resin volumes of HCl (0.1M), one resin volume of NaOH (0.1M), one resin volume of HCl (0.1 M), and two resin volumes of Milli-Q water. Water extraction was performed by pouring 1 L of disinfected water acidified with 25 mL of concentrated H₂SO₄. Upon water extraction, resins are eluted with 225 mL of ethyl acetate. Water present in the ethyl acetate extract is removed with the aid of a separatory funnel and sodium sulfate. The dried extract was rotary-evaporated with a Buchi Rotavapor R-215 (Postfach, Switzerland) at 35°C and 140 rpm to approximately 5 mL and then further evaporated under nitrogen with a TurboVap LV from Biotage (Uppsala, Sweden) to a final volume of 0.8 mL. This extract was analysed by means of gas chromatography (GC)-electron ionization (EI)-high resolution (HR)-mass spectrometry (MS) with the Q Exactive GC-Orbitrap-MS.

III- Screening of iodine containing peaks in the investigated extracts

Figure S1. Normalized extracted ion chromatograms (XICs) of m/z 126.90392 of the sample extracts obtained with NL NOM.

RT: 0.00 - 26.00 SM: 7B

NL:
1.00E8
m/z=
126.90367-
126.90417
MS
13jul15_03
1

NL:
1.00E8
m/z=
126.90367-
126.90417
MS
13jul15_03
3

NL:
1.00E8
m/z=
126.90367-
126.90417
MS
13jul15_03
5

Figure S2. Normalized extracted ion chromatograms (XICs) at m/z 126.90392 of extracts obtained with LLOB river water.

IV- Mass spectra of iodo-DBPs present in NL NOM extracts.

Figure S3. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of iodomethane (t_R 3.71 min) (a) in the chloraminated NL NOM extract and (b) MS library match.

Figure S4. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of chloriodomethane (t_R 5.36 min) (a) in the chloraminated NL NOM extract and (b) MS library match.

23/01/15_009 #1178 RT: 5.75 AV: 1 SB: 354 5.85-6.31, 5.31-5.68 NL: 1.15E7
T: FTMS + p EI Full lock ms [50.00-650.00]

Figure S5. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of iodoacetaldehyde (t_R 5.76 min) (a) in an analytical standard solution and (b) in the chloraminated NL NOM extract.

Figure S6. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of diiodomethane (t_R 7.36 min) (a) in the chloraminated NL NOM extract and (b) MS library match.

Figure S7. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of chlorodiiodomethane (t_R 8.77 min) (a) in the chloraminated NL NOM extract and (b) MS library match.

Figure S8. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of bromodiiodomethane (t_R 9.85 min) (a) in the chloraminated NL NOM extract and (b) MS library match.

23k#15_009 #1414 RT: 6.31 AV: 1 SB: 94 6.34-6.46, 6.17-6.27 NL: 9.57E7
T: FTMS + p EI Full lock ms [50.00-650.00]

(a)

(b)

Figure S9. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of dichloriodomethane (t_R 6.34 min) (a) in an analytical standard solution and (b) in the chloraminated LLOB river water extract.

23k4y15_009 #1841 RT: 7.33 AV: 1 SB: 159 7.38-7.54 , 7.08-7.30 NL: 1.52E8
T: FTMS + p EI Full lock ms [50.00-650.00]

(a)

(b)

Figure S10. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of bromochloriodomethane (t_R 7.34 min) (a) in an analytical standard solution and (b) in the chloraminated LLOB river water extract.

23July15_009 #2291-2294 RT: 8.41-8.41 AV: 4 SB: 92 8.44-8.55, 8.29-8.39 NL: 6.04E7
T: FTMS + p EI Full lock ms [50.00-650.00]

Figure S11. Mass spectrum and corresponding accurate mass (ppm) and elemental composition of dibromiodomethane (t_R 8.44 min) (a) in an analytical standard solution and (b) in the chloraminated LLOB river water extract.