Total organic halogen (TOX) in human urine: A halogen-specific method for human exposure studies

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**Abstract**

Disinfection by-products (DBPs) are a complex mixture of compounds unintentionally formed as a result of disinfection processes used to treat drinking water. Effects of long-term exposure to DBPs are mostly unknown and were the subject of recent epidemiological studies. However, most bioanalytical methods focus on a select few DBPs. In this study, a new comprehensive bioanalytical method has been developed that can quantify mixtures of organic halogenated compounds, including DBPs, in human urine as total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI). The optimized method consists of urine dilution, adsorption to activated carbon, pyrolysis of activated carbon, absorption of gases in an aqueous solution, and halide analysis with ion chromatography and inductively coupled plasma-mass spectrometry. Spike recoveries for TOCl, TOBr, and TOI measurements ranged between 78% and 99%. Average TOCl, TOBr, and TOI concentrations in five urine samples from volunteers who consumed tap water were 1850, 82, and 21.0 μg/L as X−, respectively. Volunteers who consumed spring water (control) had TOCl, TOBr, and TOI average concentrations in urine of 1090, 88, and 10.3 μg/L as X−, respectively. TOCl and TOI in the urine samples from tap water consumers were higher than the control. However, TOBr was slightly lower in tap water urine samples compared to mineral water urine samples, indicating other sources of environmental exposure other than drinking water. A larger sample population that consumes tap water from different cities and mineral water is needed to determine TOCl, TOBr, and TOI exposure from drinking water.

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**Introduction**

Water disinfection has been widely used around the world to protect human health against pathogens that cause waterborne diseases like cholera and typhoid. However, disinfectants can further react with other constituents found in natural waters (i.e., natural organic matter, halide ions) and unintentionally form disinfection by-products (DBPs). While only a selected few DBPs are regulated or monitored worldwide, including trihalomethanes (THMs) and haloacetic acids (HAAs), they represent only a small fraction of DBPs formed in disinfected waters. Other classes of DBPs that have been found in disinfected waters include haloacetonitriles, haloacetamides, haloaldehydes, haloketones, halonitromethanes, iodinated-THMs, iodinated acids, halobenzoquinones, and nitrosamines (Brass et al., 1977; Cancho et al., 2000; Mitch and Sedlak, 2002; 

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Total organic halogen (TOX) is a surrogate measurement used to comprehensively account for halogenated DBPs in finished drinking waters. This measurement includes known DBPs that we can measure, as well as unknown DBPs that are still unidentified. TOX measurement involves the adsorption of organic compounds onto activated carbon (AC) columns, pyrolysis of the AC in a furnace at 1000°C, and absorption of produced gases (i.e., CO₂, HCl, HBr, HI, HF) into an aqueous solution that is titrated online specifically for halides. Because of the increasing concern of halogen-specific toxicity of DBPs (I > Br >> Cl), TOX analysis has been further developed to distinguish between different halogenated species. Total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI) are three measurements that pertain to the specific organic halogen, and the sum of all of these compounds is known as TOX. The halogen-specific analysis involves adsorption of the effluent gas onto an online ion chromatography (IC) column (Kristiana et al., 2015) or absorption into an aqueous solution that can be analyzed with IC (Echigo et al., 2000; Hua and Reckhow, 2006; Kristiana et al., 2009; Smith et al., 2010), inductively coupled plasma-mass spectrometry (ICP-MS) (Yang et al., 2014), or ultra performance liquid chromatography–electrospray ionization-mass spectrometry (UPLC–ESI-MS) (Gong and Zhang, 2013; Pan and Zhang, 2013).

Human urine is a complex mixture of urea, inorganic and organic salts, and organic compounds, with a range of total dissolved solids of 28.1 to 37.1 g/kg (Putnam, 1971). A comprehensive TOX measurement was previously carried out on 51 human urine samples from human subjects that lived in two cities in Sweden (Salkinojasalonen and Jokela, 1991). Results show a positive correlation between TOX measured in drinking water from six different sampling points and TOX measured in urine samples from human subjects that lived close to each drinking water sampling point. The findings suggest that TOX is possibly a good indicator for DBP exposure from disinfected treated waters. However, the TOX values measured did not distinguish between TOCl, TOBr, and TOI. Because it has been shown in comparative studies that toxicity is halogen-specific (Attene-Ramos et al., 2010; Plewa et al., 2004b), TOCl, TOBr, and TOI in urine could provide more insight between DBP exposure and adverse health outcomes in human exposure studies. In recent literature, an analytical method was developed to measure TOI, iodide, and iodate for various sample matrices, including urine (Gong and Zhang, 2013). The analytical method was not optimized for TOCl and TOBr in human urine samples.

The aim of this research was to develop a bioanalytical method that can comprehensively capture DBP exposure from disinfected treated waters in human populations. In this study, we tested different conditions, including the volume of urine, dilution of urine, number of AC columns, and composition of the absorption solution to accurately capture TOCl, TOBr, and TOI concentrations in human urine samples. This bioanalytical method will test TOCl, TOBr, and TOI in urine as potential DBP exposure biomarkers. This method could ultimately be used to assess adverse human health effects to exposure of halogenated DBPs and other environmental contaminants in human exposure or epidemiological studies.
1. Material and methods

1.1. Reagents and solutions

4-Iodophenol (99.0%), 2,4,6-tribromophenol (99.8%), 2,4,6-trichlorophenol (98.6%), chloroacetic acid (≥ 99.0%), bromoacetic acid (≥ 99.0%), iodoacetic acid (≥ 99.5%), sodium bromide (≥ 99.0%), sodium iodide (≥ 99.5%), and sodium chloride (≥ 99.0%) were purchased from Sigma-Aldrich (Saint Louis, MO). Potassium phosphate monobasic (≥ 99.0%), ammonium chloride (≥ 99.5%), potassium nitrate (≥ 99.0%), sodium carbonate anhydrous (≥ 99.5%), hydrogen peroxide (30%) and nitric acid (70%) were purchased from Fisher Scientific (Pittsburg, PA). High-performance liquid chromatography (HPLC) grade methanol (Honeywell, B&J) was purchased from VWR (Radnor, PA).

4-Iodophenol (4-IP), 2,4,6-tribromophenol (2,4,6-TBP), and 2,4,6-trichlorophenol (2,4,6-TCP) were used to determine method recovery. Chloroacetic acid, bromoacetic acid, and iodoacetic acid were used to evaluate the efficiency of the aqueous solution to absorb halide ions. Stock solutions were prepared by dissolving each pure standard in methanol. Ten milligram per liter sub-stock solutions were prepared to spike samples.

Nanopure water (≥ 18 MΩ/cm) was used to prepare all solutions in this study. A Thermo Electron ROSS Ultra pH electrode connected to an Thermo Orion Star A211 pH Benchtop meter (Thermo Fisher Scientific, Waltham, MA) was used to measure pH. Electrode calibration was performed with commercial pH 4, 7, and 10 standards.

1.2. Collection of urine samples

Urine samples were collected from 5 unidentifiable volunteers located in the same geographical area that were not taking halogen-containing pharmaceuticals (e.g., antihistamines). Participants were required to drink at least 2 L of tap water per day for three consecutive days. Urine samples were collected as a 24-hr composite on the third day. Aliquots (200 mL) of urine from each volunteer were mixed together and stored at 4°C until used for analytical method development for a period of two months. On two separate urine collection campaigns, volunteers were required to drink tap or mineral water, respectively, and collect their urine as described previously. Urine samples were processed individually within a 24 hr period after collection for TOCl, TOBr, and TOI. Tap water resulted from disinfection of surface water with chlorine dioxide as a pre-oxidant and chloramines as the final disinfection step before distribution.

1.3. Experimental procedure and instrumentation

Urine samples were processed with a sample adsorption and combustion unit (Mitsubishi Chemical Analytech, Chigasaki, Japan; Cosa Xentaur, Yaphank, USA) as shown in Fig. 1. Organic compounds were loaded onto pre-loaded AC columns (Mitsubishi Chemical Analytech, Chigasaki, Japan) with an adsorption module (TXA-04, Fig. 1A). Each AC was then loaded onto a ceramic boat and automatically loaded into a quick furnace (AQF-2100H) using an automatic solid sampler (ASC-240S, Fig. 1B). AC was pyrolyzed inside the furnace at 1000°C, and the produced gases were bubbled into centrifuge tubes that contained 5 mL of adsorption solution using a gas absorption unit (AU-250, Fig. 1C). The furnace program for AC columns consisted of 240 sec at the end position, 200 sec at the cooling position, and 200 sec at the home position at an argon and oxygen flow rate of 30 and 600 mL/min, respectively. A slightly different furnace program was used for the adsorption solution optimization process because standards were injected directly into ceramic boats containing quartz wool. Each centrifuge tube was weighed with an analytical balance before and after each run to obtain the exact volume of each sample.

The adsorption solution was analyzed for chloride and bromide with a 1600 IC System (Dionex, Sunnyvale, CA). The IC was optimized to increase sensitivity for chloride, bromide, and iodide. A Finnigan ELEMENT XR double focusing magnetic sector field ICP-MS instrument (Thermo Electron Corporation) was used for the analysis of low iodide concentrations (<20 μg/L). A Micromist U-series nebulizer (GE, Australia; 0.2 mL/min), quartz torch, and injector (Thermo Fisher Scientific, USA) were used for sample introduction. ICP-MS samples were prepared and analyzed for iodide as described elsewhere (Yang et al., 2014), and the ICP-MS method detection limit (MDL) for iodide was 0.14 μg/L. MDL was calculated by multiplying the standard deviation of seven
replicates of 1 μg/L by the Student’s value for six degrees of freedom for 99% confidence limit.

Urine samples were diluted at different ratios and tested at different volumes of diluted urine to 1) reduce matrix effects and 2) determine conditions that will adsorb halogenated compounds (TOCl, TOBr, and TOI) onto the AC without reaching breakthrough. Three dilution factors (DFs) (10, 50, and 100) and four volumes (50, 100, 150, and 250 mL) were tested. Additionally, several aqueous solutions were tested to maximize the recovery of HCl, HBr, and HI from the effluent gas produced by the quick furnace. Each sample was adjusted to pH 2 with concentrated nitric acid, passed through two or three AC columns, and washed with 10 mL of 5000 mg/L of KNO₃ adjusted to pH 2. Extracting at a low pH is important for adsorbing HAAs, which have very low pK₅₅s. Spiked samples consisted of directly spiking urine, followed by dilution and method development.

2. Results

2.1. Absorption solution

The gas effluent produced by the quick furnace contains several combustion products of interest, including hydrogen halides (HCl, HBr, and HI). Hydrogen halides were collected by bubbling the gas effluent into an aqueous solution. The composition of the aqueous solution is important because the solution should scrub the products from the gas effluent and should be compatible with the subsequent IC and ICP-MS analysis.

Several components were added to the absorption solution, including three buffers (carbonate, phosphate, or ammonium hydroxide) and hydrogen peroxide (H₂O₂) and tested in triplicate. To assess only the efficiency of the absorption solution to scrub all three halides of interest (and not the AC adsorption), a 10 μL standard mix of 100 mg/L as X⁻ that contained chloroacetic acid, bromoacetic acid, and iodoacetic acid was directly injected into a ceramic boat containing quartz wool for combustion in the furnace. Ammonium hydroxide (0.1%) was used for ICP-MS analysis and was initially tested as an absorbing solution. However, ammonium hydroxide significantly interfered with the IC analysis (data not shown), so it was not tested further. A buffer solution was found to be necessary for HI absorption (Fig. 2), in addition to H₂O₂. The average recovery of HI with carbonate and phosphate was between 53% and 76%, respectively. Phosphate buffer showed the highest average recovery for HI (73%–76%). However, HCl recovery was higher for 0.1–0.5 mmol/L carbonate solutions (88%–92%) compared to 0.01–0.1 mmol/L phosphate solutions (73%–83%). HBr recovery across all conditions did not differ significantly with average recovery between 84% and 94%. Because TOI concentrations are about 2 orders of magnitude lower than TOCl in urine, the solution that maximized AOI adsorption was chosen for this study (0.01 mmol/L phosphate + 0.003% H₂O₂).

2.2. IC method optimization

The IC system was optimized to increase Cl⁻, Br⁻, and I⁻ sensitivity. The system was tested for different sample loop volumes, two suppressors, and a carbonate removal device (CRD). Table 1 shows the peak areas for a 20 μg/L Cl⁻, Br⁻, and I⁻ standard for each IC configuration that was tested. The initial system configuration (IC-1) consisted of a 250 μL sample loop, and an AERS 500 (Dionex, Sunnyvale, CA) suppressor. Under these conditions Cl⁻, Br⁻, and I⁻ detection

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**Table 1**: IC system configurations and chloride, bromide, and iodide sensitivity for a 20 μg/L standard solution.

<table>
<thead>
<tr>
<th>IC setup</th>
<th>Description</th>
<th>Chloride (μS * min)</th>
<th>Bromide (μS * min)</th>
<th>Iodide (μS * min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC-1</td>
<td>250 μL loop, AERS 500 carbonate</td>
<td>0.0324*</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>IC-2</td>
<td>250 μL loop, AERS 500 carbonate</td>
<td>0.0171</td>
<td>0.0109</td>
<td>0.0048</td>
</tr>
<tr>
<td>IC-3</td>
<td>500 μL loop, AERS 500 carbonate</td>
<td>0.0343</td>
<td>0.0168</td>
<td>0.0078</td>
</tr>
<tr>
<td>IC-4</td>
<td>500 μL loop, AERS 500 carbonate, carbonate removal device (CRD)</td>
<td>0.0083</td>
<td>0.0040</td>
<td>0.0016</td>
</tr>
</tbody>
</table>

IC: ion chromatography.

* 10 μg/L chloride, bromide, and iodide standard.
limits were 1, 30, and 60 μg/L, respectively. A new AERS 500 carbonate suppressor was installed that was more sensitive for the compounds of interest (IC-2). Detection limits were lowered to 1, 5, and 10 μg/L for Cl⁻, Br⁻, and I⁻ respectively. A 500 μL sample loop was then used and decreased Br⁻ detection limits to 1 μg/L (IC-3). An increased sample volume also increased the noise and did not improve Cl⁻ and I⁻ detection limits, so a higher sample loop volume was not tested further. Finally, a CRD was installed after the suppressor to remove the carbonate from the eluent before it reached the detector, in order to reduce the background signal (IC-4). Results showed a decrease in sensitivity for all three halides. A possible explanation is that halides were also being removed with the CRD.

The final optimized IC system (IC-3) included a 500 μL sample loop, an IonPac AS9-HC carbonate eluent column, and AERS-500 carbonate suppressor at a flow rate of 1.5 mL/min. Two stock solution mixes of 10 and 100 mg/L as X⁻ (chloride, bromide, and iodide) were used to prepare a calibration curve (1, 5, 10, 20, 30, 50, 75, 100, 200, 300, 500 μg/L) with a coefficient of determination ≥ 0.99 in the IC system. Halide peaks were well separated, as shown in Fig. 3. Detection limits for chloride, bromide, and iodide were 1, 1, and 10 μg/L, respectively. However, over time it was observed that the iodide detection limit changed from 10 to 20 μg/L. A new guard column was enough to bring the iodide detection limit back to 10 μg/L. Calibration curves were prepared every month and checked before each run with two freshly prepared calibration points checks (20 and 75 μg/L). Processed samples repeatedly had concentrations of iodide lower than the IC MDL (20 μg/L). For this reason, all processed samples were analyzed for iodide with an ICP-MS (MDL = 0.14 μg/L).

2.3. Breakthrough tests

Several sample extraction conditions were tested to reduce matrix effects and efficiently extract organic halogens from urine. Sample dilution is commonly used to reduce matrix effects and has been previously applied for TOI low-level quantification in urine (Gong and Zhang, 2013). Three DFs were tested: 10, 50, and 100. For each DF, varying volumes (50, 100, 150, and 250 mL) of the diluted urine sample were passed through three ACs in series. Organic halogens (TOCl, TOBr, and TOI) of each diluted urine sample were adsorbed onto ACs without breakthrough through. Each AC was pyrolyzed individually with the quick furnace, and gases produced from each AC were collected into individual containers (C#1, C#2, C#3). Processed samples were immediately analyzed for chloride, bromide, and iodide concentrations.

TOCl and TOBr adsorption onto each AC are shown in Fig. 4. For 50 mL of diluted urine with a DF of 50, TOCl and TOBr were adsorbed within the first two ACs with no breakthrough onto the third column. When 100 mL was processed, TOBr was adsorbed within the first two ACs, while TOCl broke through. One hundred and fifty millimeters of diluted urine showed breakthrough for both TOCl and TOBr. A similar trend was observed for 50 mL of diluted urine samples with a DF of 10. To obtain TOCl and TOBr within the first two ACs with a sample of DF 100, about 150 to 250 mL of sample had to be processed (data not shown). Additionally, a DF of 100 had no apparent advantages over a DF of 50 and had two major drawbacks: 3 to 5× more time to process each sample and a significant relative standard deviation (RSD) increase. The optimized extraction conditions for TOCl and TOBr analysis were a DF of 50 and 50 mL sample size.

TOI concentration in urine samples was considerably lower than TOCl and TOBr. While the same optimized extraction conditions were applied for all three measurements, the analysis of the absorption solution was analyzed with IC (for chloride and bromide) and ICP-MS (for iodide). With a lower iodide detection limit from ICP-MS, it permitted the measurement of TOI in urine. Fig. 5 (left) shows the iodide measurement per AC from nanopure water, urine, and urine spiked with 50 and 100 μg/L of 4-IP as I⁻. Results suggest that organic iodine is adsorbed primarily within the first AC. Also, because of the low-level iodide detection, virgin AC and nanopure water were found to also contain trace levels of iodide that can be detected in the control. These results highlight the importance of processing a blank control with the same water used for urine dilution and AC packages.

Fig. 3 - Ion chromatography (IC) chromatogram for chloride, bromide, and iodide standards with setup IC-3.
opened on the day of sample processing. In this study, a control blank was processed with all urine samples on the same day.

2.4. Recovery of standards

Breakthrough experiments showed that a DF of 50 and a sample volume of 50 mL were sufficient to adsorb TOCl, TOBr, and TOI with two ACs without achieving breakthrough. Furthermore, the method was tested to determine the recovery of spiked standards from urine using two or three AC columns. Levels of $200 \mu g/L$ of 2,4,6-TCP as $Cl^-$, $50 \mu g/L$ of 2,4,6-TBP as $Br^-$ and $10 \mu g/L$ of 4-IP as $I^-$ were directly spiked into urine before dilution and pH adjustment. The concentrations of each standard were similar in order of magnitude to what was measured in urine in this study. Measurements where done in triplicate.

TOCl as $Cl^-$ was measured for two solutions: a urine control and spiked urine sample using two or three AC columns. TOCl was determined to be 741 and 931 $\mu g/L$ as $Cl^-$, respectively, as shown in Fig. 6. The standard recovery was 99%. However, when only two AC columns were used, the TOCl for the urine control and spiked urine were 460 and 544 $\mu g/L$, respectively. The standard spike recovery was lower: 82%. The results suggest that TOCl is breaking through with two AC columns but is being recovered with three AC columns. Even though breakthrough tests suggested that two ACs are enough to adsorb all the TOCl for the composite urine sample of five volunteers, it might be possible that a third AC will be needed to compensate for the variability within human subjects with

Fig. 4 – Breakthrough analysis of organic chlorine (left) and organic bromine (right) adsorbed to three activated carbon columns in series from urine. Shown results are chloride and bromide concentrations measured from the absorption solution that represents the recovered organic chlorine and organic bromine as the result from the pyrolysis of each individual activated carbon.

Fig. 5 – Breakthrough analysis of organic iodine adsorbed on three activated carbon columns in series from 50 mL of nanopure water, 50 mL of diluted urine (1:50), and 50 mL of diluted urine (1:50) spiked with 50 $\mu g/L$ and 100 $\mu g/L$ 4-iodophenol (4-IP) as $I^-$ (left). Shown result is the iodide concentration measured from the absorption solution that represents the recovered organic iodine as the result from the pyrolysis of each individual activated carbon. Total organic iodine (TOI) measurement with three activated carbons in urine and urine spiked with 10 $\mu g/L$ 4-IP as $I^-$ (right). TOI measurement is calculated by measuring iodide from one single absorption solution that recovered organic iodine from the pyrolysis of three consecutive activated carbons and adjusting for dilution and concentration factors.
a slightly higher TOCl (represented with a standard spike). Therefore, recovery test results showed that three AC columns are necessary to capture most organic chlorine compounds and provide a better TOCl value in urine.

Similarly, TOBr was also analyzed for a urine control and a spiked urine sample processed with two and three ACs. Results in Fig. 6 show a slightly better result when using two versus three ACs. For samples processed with two AC columns, the TOBr values were 71 and 109 μg/L for urine and spiked urine, respectively. When using three AC columns, the TOBr for the urine control and spiked urine was 80 and 115 μg/L, respectively. Spike recoveries for samples processed with two and three AC columns were 90% and 88%, respectively. However, because TOCl breaks through when using two ACs and TOBr has similar recoveries in both extraction conditions, only three ACs were tested for spike recovery of TOI. Fig. 5 shows that the average spike recovery was 78% with an average concentration of 14.9 and 19.5 μg/L for the urine control and the spiked urine, respectively. Spike recoveries for all three TOX measurements ranged between 78% and 99% and were mostly recovered with this method.

2.6. Quantification of TOCl, TOBr, and TOI in urine samples

Tap water and urine from five volunteers that consumed tap water were processed individually in triplicate to determine TOCl, TOBr, and TOI (Fig. 7). Fifty millimeters of tap water was processed with three ACs and analyzed with the same procedure as diluted urine samples. Urine results obtained with IC and ICP-MS were divided by a concentration factor (from 50 to ~10 mL) and then multiplied by DF of 50. Nanopure and tap water results were adjusted by the concentration factor.

TOCl concentrations of five human samples were 715, 1040, 5250, 1230, and 1010 μg/L as Cl⁻, respectively. Urine concentrations are 8–66 times higher than 79.7 μg/L as Cl⁻ measured in drinking water, suggesting that TOCl concentrates in urine from environmental exposure to organic chlorine compounds. Sample #3 had a magnitude of order higher compared to the other four samples. Because this phenomenon was not observed with TOBr or TOI, it might be possible that the volunteer of sample #3 had an increased exposure to an organic chlorine compound from either a particular environmental source or from medication. This highlights the need for detailed questionnaires relating to diet and medication in future human exposure studies.

TOBr concentrations in urine were considerably lower than TOCl, with values of 63.0, 84.3, 121, 83.7, and 59.6 μg/L as Br⁻. Tap water TOBr concentration of 29.1 μg/L as Br⁻ was 2–4 times lower than those found in urine. The ratios of urine-to-tap water TOBr were more consistent than TOCl and TOI. The ratios varied between 2.0 and 2.9, with the exception of Sample #3, which had a slightly higher ratio of 4.1. It might be possible that other environmental exposure routes other than drinking water might affect TOCl and TOI.

2.5. Final optimized method

The final optimized method consists of a urine dilution with a ratio of 1:50 (urine:water) and adjusted to pH 2. Diluted urine (50 mL) is then passed through three AC columns, followed by a rinsing step with 10 mL of KNO₃ solution. Each AC is pyrolyzed individually with a quick furnace and gases are collected with an aqueous solution containing 0.01 mM phosphate and 0.003% hydrogen peroxide. This solution was then analyzed for chloride and bromide with IC and iodide with ICP-MS.
values. However, TOBr seems to provide more consistent values compared to drinking water.

TOI concentrations in urine were also considerably much lower than TOCl and TOBr with values of 16.9, 27.9, 19.2, 22.4, and 18.5 μg/L as I⁻. TOI concentrations in tap water, 0.96 ± 0.1 μg/L as I⁻, was 17–29 times lower than those found in urine. TOI values were in general agreement with another study that measured TOI in a pooled urine sample from 10 volunteers and reported a value of 15.2 μg/L as I⁻ (Gong and Zhang, 2013). Gong and Zhang (2013) also measured TOI concentrations in tap water samples that treated surface waters as their source. Levels of 1.3, 1.6, 1.4 and 5.4 μg/L as I⁻ were reported in these tap waters, which are also similar to TOI levels measured in tap water our study.

Urine results from volunteers who consumed tap water were compared to a control experiment where volunteers consumed mineral water (Fig. 8). Results show that there is a significant difference between TOCl, TOBr, and TOI measured in tap water (79.7, 29.1, and 0.96 μg/L as X⁻) and mineral water (12.4, 1.12, and 0.16 μg/L as X⁻). The average of five urine samples from the control experiment was 1090, 88, and 10.3 μg/L as X⁻ for TOCl, TOBr, and TOI, respectively. TOCl and TOI results were lower compared to tap water urine samples, with an average of 1850 and 21.0 μg/L, respectively as X⁻. The average TOBr for tap water measured in urine was 82 μg/L as Br⁻, similar to the average TOBr in mineral water. Therefore, an extensive study with more samples is needed to determine whether there is a statistical difference in urinary TOBr between participants who drink tap and mineral water.

This finding was somewhat surprising, given the relatively high levels of brominated DBPs in tap water, and it indicates significant exposures to other sources of organic bromine besides DBPs. Possibilities include brominated flame retardants (Schauer et al., 2006; Hoffman et al., 2014, 2015; Ho et al., 2015; Butt et al., 2016; Feng et al., 2016), natural brominated compounds in seafood (Da Silva et al., 2005, 2007), and brominated azo dyes that were recently discovered as a major source of organic bromine exposure in the environment.
(Peng et al., 2016). In recent studies, Butt et al. reported a maximum of 225 ng/L for the flame retardant metabolite 2,3,4,5-tetrabromobenzonic acid in urine samples from the U.S. (Butt et al., 2016). In urine samples from China, Feng et al. reported mean levels of 0.7–3.5 μg/g creatinine for 2-bromo-, 4-bromo-, and 2,4-bromophenol, which are metabolites of polybrominated diphenylether flame retardants (Feng et al., 2016). In addition to these known sources of exposure, there are also likely other brominated compounds not yet found. Finally, for future human exposure studies of TOCl, TOBr, and TOI, variations in individuals’ urinary volumetric flow rate could be normalized using creatinine concentrations (Hoffman et al., 2014).

3. Conclusions

A new and sensitive method that simultaneously quantifies halogen-specific adsorbable organic compounds (TOCl, TOBr, and TOI) in urine has been developed. This novel method could be used to quantify TOCl, TOBr, and TOI in human urine as potential biomarkers for environmental exposure to halogen-containing contaminants, such as DBPs. Five urine samples from volunteers who consumed tap water or mineral water were processed and analyzed. Results showed slightly higher concentrations of TOCl and TOI in urine from volunteers who consumed tap water compared to mineral water. In a future study, a larger sample population of individuals who consume tap water from different cities that disinfect with free chlorine and chloramines with a detailed questionnaire of diet and lifestyle (to account for other sources that might influence TOCl, TOBr and TOI) will be conducted to determine statistical differences between groups.

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