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Formation and risk assessment of trihalomethanes through different tea brewing habits

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ABSTRACT

Trihalomethanes (THMs) are suspected carcinogens and reproductive toxicants commonly found in chlorinated drinking water. This study investigates the formation of THMs and their associated risks during different tea brewing habits. Three main categories of tea (black, oolong, and green) under various brewing conditions and drinking water sources were tested. Tea samples prepared in ordinary thermos flask formed significant levels of total THM (TTHM). The highest TTHM formation came from black tea made with tap water, plausibly due to higher concentrations of reactive THM precursors. Compared with tap water, when the background solution is bottled water or distilled water, less TTHM was observed in prepared tea infusions. The results also revealed that unlike the traditional teapot-based tea serving habit, the removal of THMs is significantly reduced when tea infusion is stored in enclosed containers. Risk assessment analysis based on the survey among tea shop costumers also revealed that cancer risks induced by ingestion of THMs through drinking tea infusions prepared in thermos flask exceeded the tolerable level. Data obtained in this research demonstrated that drinking tea infusions directly from enclosed containers can be a significant source of exposure to THMs.

1. Introduction

The protection of communities' drinking water supply has been a priority for many years. Accordingly, the disinfection of drinking water was a major accomplishment in public health protection since chlorine was introduced as a disinfectant in the 20th century (Nieuwenhuijsen et al., 2009). It significantly improved the hygienic requirements of water quality by eliminating waterborne bacterial pathogens and consequent transmission of waterborne diseases (Nieuwenhuijsen et al., 2000).

Besides the numerous advantages of chlorination, one side effect of drinking chlorinated water is disinfection byproducts (DBPs) formation. Reactions between natural organic matter (NOM) and chlorine form different types of carcinogenic DBPs in drinking water (Chowdhury et al., 2011) where trihalomethanes (THMs)—the most prevalent group of chlorination by-products (Villanueva et al., 2006)—have been recognized as a potential carcinogenic by-product and hazardous to human health (Krasner et al., 2001; Rodriguez et al., 2003).

Exposure to compounds in residential drinking water may occur by direct ingestion of prepared beverages or food, bathing, washing, showering, swimming, and humidifiers (Weisel et al., 1999).

The concentrations of DBPs depend on both the raw water characteristics and the condition of distribution system (Villanueva et al., 2006). Moreover, it also depends on how water is processed shortly before it is consumed (e.g., through boiling or filtering tap water) (Krasner and Wright, 2005; Levesque et al., 2006).

With global production of about three million tons per year, tea infusions are among the most consumed beverages around the world (Wang et al., 1997). Tea is produced from the leaves of Camellia sinensis and is classified into three types based on the extent of fermentation: unfermented green tea, semi-fermented oolong tea, and fully-fermented black tea, the latter of which accounts for roughly 80% of the total world tea production (Flaten, 2002).

Being rich in organic matter, tea leaves can potentially serve as DBP precursors (Bond et al., 2016a) as it is evident that the organic matter present in tea leaves is similar to humic substances found in drinking water. Tea infusions contain approximately 500 components, belonging mainly to eight categories: polyphenols, caffeine, amino acid, pigments, vitamins, esters polysaccharides, minerals, and aromatic substances (Yuan et al., 2013).

A tea infusion's composition also depends on temperature (Khokhar and Magnusdottir, 2002), brewing period (Xie et al., 1998), leaf–water ratio (Astill et al., 2001), and water composition (Mossion et al., 2008).

Most of the relevant studies measured the effect of boiling water on THM formation for one to 5 min. In Taiwan and many other countries, boiled water for hot drinks (such as tea or coffee) is prepared in an

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electric kettle or an instant boiling water unit. Most domestic electric kettles keep water at a rolling boil for less than 1 min (typically less than 15 s), while the instant boiling water units can keep water at ~100 °C for longer periods (minutes to hours).

In spite of the large consumption of tea beverages, the formation of DBPs during the tea making process has received little attention regarding the reactions between the residual chlorine present in background water and the organic precursors in tea infusions.

Among the few relevant studies, Wu et al. (1998) reported that instant tea generated similar amounts of THMs as aquatic humic substances when chlorinated under potential formation conditions. Bond et al. (2016b), investigated the generation of selected DBPs following chlorination of tea and coffee samples. They showed that aqueous chlorine at initial concentrations of 1–19 mg/L was reduced by 5–19% upon boiling in a kettle, indicating that the majority of chlorine remained available for reaction with organic matter in tea.

Despite the importance of tea drinking exposure route, the authors are not aware of any relevant studies regarding the health risk assessment associated with tea drinking exposure pathway. The present work aims at investigating the formation of THMs and their associated health risks during different tea drinking habits. Three main categories of tea (black, oolong, and green) were tested using various brewing conditions and drinking water sources. The possible cancer risk and hazard index of THMs were also examined according to tea drinking habits.

2. Materials and methods

2.1. Tea making procedure

Tea samples were obtained from a local supermarket in Taipei, Taiwan and placed in a cotton bag with a desired mass concentration for different solid/boiling-water ratios (w/v, dry weight basis). Four different drinking water sources were used to prepare infusions, including distilled water (A), tap water (B), water from a public dispenser (C), and bottled mineral water (D).

A standardized procedure for the preparation of tea infusions was applied. An electric kettle (600 W; capacity: 1.425 L, material: coated steel) was filled with the appropriate amount of each drinking water source (except for water dispenser, where hot water was directly obtained from the machine) and heated to boiling temperature. Water samples were collected directly from the kettles and quickly poured into typical thermostat flasks (volume of 500 ml), which already contained tea leaves. Vacuum flasks or vacuum bottles, also known as the thermos, keep their contents hotter or colder than the environment's temperature. The thermostat flask was placed on a table in the laboratory, and tea infusions were sampled after 1, 2, 5, 10, 15, and 30 min of brewing. After each steeping reaction (without agitation), sodium thiosulfate was added to quench further THMs formation. Finally, five ml of the liquid sample were withdrawn for THMs analysis by GC/ECD. All tests were performed in duplicate. The so-called blank water followed the same procedure without tea leaves. THMs data were derived from just the four water sources without chlorine addition.

2.2. Tea container experiment

Many people drink their hot beverages (tea/coffee) directly from the flask (without pouring into a cup) which prevents the volatile compounds from being vaporized into the atmosphere. To assess the effect of tea making containers on TTHM concentration, preparing tea in a traditional Chinese teapot was also examined in this study where tea samples were prepaid at steeping time of 10 min with tap water as background solution and tea/water ratio of 1:50.

2.3. THMs analysis and calibration

The THM extraction process is based on dispersive liquid-liquid microextraction (DLLME), a simple and rapid pre-concentration and microextraction method that was developed by Kozani et al. (2007) with a slight modification. It is an environmentally friendly sample preparation method with highly promising target analytical potential.

A five ml sample was placed in a 10 ml screw cap glass test tube with a conical bottom and spiked with 1.2-dibromopropane as the internal standard. Acetone (0.50 mL), as a disperser solvent containing 20.0 μL carbon disulfide (extraction solvent), was rapidly injected into the sample solution using a 0.50-mL syringe (gas-tight; Hamilton, USA). A cloudy water-acetone carbon disulfide mixture was formed in the test tube. The mixture was gently shaken and then centrifuged for 1 min at 6000 rpm. The dispersed fine particles of the extraction phase settled to the bottom of the conical test tube, approximately one μL of which was injected into the GC.

A solution of the four THMs (TCM, BDCM, DBCM, and TBM) in methanol (2000 mg/L each) was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Gas chromatographic separation and determination of the THMs was performed using a GC system (AGILENT 6850, USA) with a split/splitless injector and an electron capture detector (ECD). Compounds were separated on a 25 m × 0.32 mm capillary column coated with a 1.20 μm film of CP-Sil 13CB (86% methyl, 14% phenyl siloxane). The oven temperature was maintained at 30 °C for 5 min and then programmed at 10 °C min − 1 to 140 °C, where it was held for 2 min. The ECD temperature was maintained at 300 °C. Ultrapure nitrogen (99.9999%, Air Products), used as a make-up gas for ECD, was passed through a molecular sieve trap and an oxygen trap (CRS, USA) at a flow of 30 mL/min. To confirm the results, each experiment was performed in duplicate, approximately 25% of the GC-ECD analyses were repeated, and blank water samples were collected and analyzed. Almost all coefficients of variation were < 10%.

2.4. Analytical methods

The pH and electrical conductivity (EC) of water sources were measured using the multiparameter digital meter (HACH, USA). Dissolved organic carbon (DOC) was measured using a TOC analyzer (Aurora model 1030w/1088) after filtration through filter paper with a pore size of 0.45 μm. Elemental analyses of the tea samples were carried out using a Perkin-Elmer 2400 Elemental Analyzer in duplicate.

Chlorine demand tests were carried out on each 1:100 (vol/vol) diluted tea samples prepared in chlorine-free water with an applied chlorine dose of 20 mg/L at pH 7 ± 0.2, and 25 ± 2 °C. Chlorine demand was calculated by subtracting the residual measured at the end of the experiment from the applied chlorine dose after the contact time of 24 h. The free chlorine was measured by the *N*,*N*-diethyl-*p*-phenylenediamine – ferrous ammonium sulphate (DPD-FAS) titration method (APHA, 2005).

Before beginning the laboratory experiments, all containers and glassware were cleaned with de-ionized water and heated at 450 °C for at least 4 h to ensure that no chlorine demand was previously present.

2.5. Exposure and risk assessment

A descriptive, cross-sectional survey carried out in an urban society in Taipei, Taiwan. Subjects (n = 200) were selected through a multistage cluster random sampling method. Of the total 12 districts in Taipei, four were randomly selected. Ten tea shops within each area were randomly chosen and were characterized as the second cluster. Five tea shop customers with hot tea drinking habit were randomly selected in each tea store. A questionnaire was designed to collect the demographic information and participants' tea drinking habit. The descriptive statistics used to express the distribution frequencies and mean comparison analysis using one-way ANOVA and post-HOC Tukey test at $\alpha < 5\%$ by SPSS 16 software (SPSS Inc.: Chicago).

To estimate the daily exposure of an individual, USEPA suggests the Lifetime Average Daily Dose (LADD) as the exposure metric. The following equation is a similar representation of daily exposure for the ingestion route modified from USEPA (1992) and Chrostowski (1994):

$$CDI = \frac{C \times DI}{BW} \quad (1)$$

where *CDI* is the chronic daily intake (mg/kg/d), *C* is the drinking water contaminant concentration (mg/L), *DI* is the average daily intake rate of drinking water (l/d), and *BW* is body weight in (kg). Using Eq. (1), the deterministic exposure assessment was used to estimate individual exposures to total THM (TTHM). The cancer risk associated with ingestion exposure was calculated using the following equation (Patrick, 1994):

$$R = CDI \times SF \quad (2)$$

where *R* is the excess probability of developing cancer over a lifetime as a result of exposure to a contaminant (or carcinogenic risk), and *SF* is the slope factor of the contaminant $(mg/kg/d)^{-1}$.

To estimate noncarcinogenic risk, the hazard quotient (HQ) is calculated using the following equation (USEPA, 1999):

$$HQ = \frac{CDI}{RfD} \quad (3)$$

where *RfD* is the reference dose (mg/kg/d). *SF* and *RfD* values employed in this study were obtained from the USEPA (IRIS, 2005).

3. Results and discussion

3.1. Chemical composition and chlorine demand of tea samples

Tea leaves, like all other plant matter, contain carbohydrates, protein, and lipids and are full of genetic material, enzymes, and secondary metabolites. Moreover, tea leaves are known by their high content of methylxanthins and polyphenols, especially catechins (flavanols) (Ho and Zhu, 2000).

To date, hundreds of volatiles have been characterized in black tea, with fewer numbers in oolong and green tea due to the lesser degree of fermentation during tea production process. In addition, tea aroma is also influenced by the consumer's drinking habits, such as brew temperature and steeping time period.

The elemental composition, as well as ratios of C/N and C/H of tea infusions, are provided in Table 1. Changes in the elemental contents indicated different chemical compositions of the tea infusions. As an indicator of the aromaticity structure, the lower ratio of C/H in green tea can be attributed to high levels of aliphatic carbon and low levels of unsaturated carbon (Steelink, 1985). Black tea has higher levels of nitrogen content, suggesting that it contains a higher proportion of nitrogen-containing moieties (Song et al., 2008). It has been hypothesized that nitrogen groups and activated aromatic carbons serve as active sites for reaction with chlorine, producing chlorinated byproducts (Reckhow et al., 1990).

The chlorine demand of tea samples was in the range of 8.6–11.4 mg/L and 11.9–16.1 mg/L for 2 and 10% mass concentrations of tea samples under experimental conditions. Black tea showed a higher chlorine demand (Fig. 1), C/H ratio, and nitrogen content (Table 1.) suggesting that samples with higher aromatic organics and nitrogen content will consume more chlorine during disinfection. Moreover, samples with higher concentration are found to have a higher chlorine demand possibly due to greater proportions of hydrophilic neutral and hydrophobic acid fractions (Yee et al., 2006).

Table 1
Main element contents and ratios of the tea infusions.

Sample	C%	N%	H%	C/N	C/H
Black tea	40.85	5.35	4.74	7.63	8.61
Green tea	41.63	3.09	5.23	13.47	7.95
Oolong tea	43.65	3.18	5.15	13.72	8.47

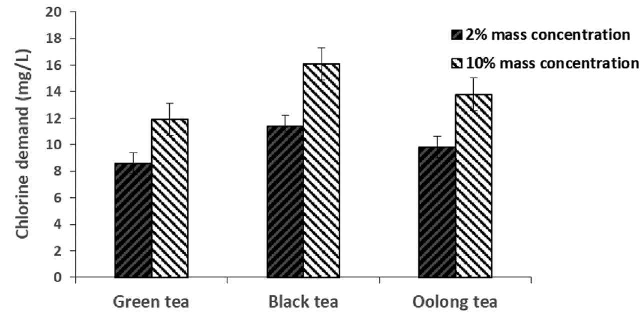

Fig. 1. Chlorine demand of tea samples at different mass concentrations with applied chlorine dose of 20 mg/L at pH 7 ± 0.2, and 25 ± 2 °C after 24 h reaction time.

3.2. Effect of background water on THM formation

A total number of 84 samples were randomly collected from a residential area in Taipei, Taiwan, and the mean concentration of water quality parameters was reported for each type of water. Additional samples were randomly taken to verify the sufficient number of samples in which the differences between additional and allocated samples in each stratum were not significant (*P*-value ≥ 0.1). Table 2 shows the quality parameters of different water types used in this study as background solutions.

The regular drinking water pH range mentioned in WHO (2011) and NDWQS (2004) guidelines is between 6.5 and 8.5. The pH values of all the drinking water samples (except distilled water) are found to be in the range between 7.6 and 8.0.

The highest concentration of THMs was found in tap water, whereas distilled water showed the minimum level. Higher levels of THMs in tap water might be due to higher concentrations of precursor organic materials in the raw water, particularly during the warmer period (LeBel et al., 1997). All the THMs levels in the tested water samples were below the maximum contaminant level (MCL) for THMs in drinking water at 80 μg/L set by USEPA (2006).

THMs levels can increase as the chlorinated water moves from the water treatment plant throughout the distribution system due to the presence of chlorine residual. The extreme weather conditions may also significantly affect the concentration of DBPs in drinking water (particularly from public water dispensers). The presence of THMs in water dispensers could be due to either higher organic loading in the source water or a deficient filtration system treatment used in public water dispensers. Fountains with lack of proper maintenance, such as overused or out-of-date filters, can cause secondary contaminations particularly in the wet seasons with heavy rainfalls and higher organic matter loading (Fakour et al., 2016). While TOC concentration is slightly higher in water dispenser compared with tap water, lower THMs were detected in water coming from public dispenser. This can be explained by the fact that beside TOC levels, the residual chlorine is also another important parameter that greatly affect DBPs levels. Activated carbon filters applied in some public water dispensers, are able to remove chlorine residual and consequently reduce the DBPs in provided water.

THMs should not be found in bottled water as many bottled water companies use various forms of water treatment to disinfect their water supplies, such as ozone or ultraviolet light. Although the TTHM concentration in bottled water was the lowest among other water sources, it was still detectable in bottled water samples. The industry standard, Bottled Water Code of Practice, published by the International Bottled Water Association (IBWA, 2008), sets a limit for TTHM at 10 ppb.

For chemical contaminants, the regulations for bottled water are somehow weak. While city tap water must be tested at least quarterly

Table 2
Quality parameters of different water background solutions.

water source	pH	EC (μS/cm)	TOC (mg/L)	Free residual chlorine (mg/L Cl_2)	TTHM (μg/L)
Distilled water	6.1 ± 0.2	1.1 ± 0.47	0.05 ± 0.003	ND	2.2 ± 0.51
Tap water	7.6 ± 0.2	83.4 ± 14	2.10 ± 0.130	0.95 ± 0.09	25.1 ± 3.1
Water dispenser	7.8 ± 0.3	65.7 ± 11	2.40 ± 0.210	0.78 ± 0.04	18.9 ± 2.9
Bottled water	8.0 ± 0.4	181.2 ± 21	0.67 ± 0.020	0.71 ± 0.08	10.3 ± 2.4

for the presence of organic chemicals (such as industrial chemicals, pesticides, and THMs), bottlers only need to test once a year under the FDA's rules. This inspection procedure may cause serious health problems as the levels of these contaminants sometimes vary considerably depending on when they are tested (Olson, 1999).

Ahmad and Bajahlan (2009) also showed the presence of THMs in bottled water of an industrial city of Yanbu (Saudi Arabia) in the range of 0–18.07 μg/L.

3.3. Effect of tea type on THMs formation

The changes in TTHM concentration after brewing different tea types under various water backgrounds is shown in Table 3. The tea infusions showed increased levels of TTHM. The concentrations of THMs, when tap water was used as the background water source, were the highest and Black tea infusions prepaid in tap water and water dispenser were above the MCL standards for THMs in drinking water (80 μg/L) (USEPA, 2006). The highest THMs formation was observed in black tea prepared with tap water (88.3 ± 4.4 μg/L) and concentrations of TTHM in different water type backgrounds were as follows: tap water > water dispenser > bottle water > distilled water.

Compared with tap water, when the background solution is bottled water or distilled water, less TTHM was observed in prepared tea infusions. Lower TOC and free residual chlorine in distilled and bottled water resulted in less TTHM levels as THM formation is decreased by reducing the amount of organic matter available to react with chlorine. It is thus recommended to use distilled water, bottled water, or household water treatment devices during cooking activities to minimize the organic matter and free residual chlorine concentration in applied water.

As shown in Tables 2 and 3, TTHM slightly increased from 2.2 μg/L in blank distilled water to 4.8 μg/L in black tea infusions prepared with same water while no chlorine was added to the background water. This finding might be attributed to either tea leaves contamination by DBPs. To prove this, the potential discharge of THMs in blank distilled water and tea infusions prepared in distilled water as a function of time and temperature of water has been shown in Fig. 2. THMs concentration decreased at 90 °C temperature in blank sample (without tea leaves) through volatilization of chlorinated compounds. However, due to enclosed container, volatilization of THMs was greatly reduced. The THMs did not significantly change at lower temperature. This is not surprising as lower temperature reduces the kinetics of the hydrolysis reactions and volatilization of THMs into the air (Zhang et al., 2015). The THMs release rate into the atmosphere is then much slower at lower temperature.

Table 3
Mean concentration ± SD of the TTHM (μg/L) after different tea making procedure with various water background sources (Tea/water ratio of 1:50 (w/v, dry weight basis); brewing time of 10 min in enclosed thermostat flasks).

Water type				
Tea type	Distilled water	Tap water	Water dispenser	Bottled water
Black tea	4.8 ± 0.15	88.3 ± 4.4	82.5 ± 4.1	29.5 ± 2.9
Green tea	2.8 ± 0.06	63.7 ± 3.5	56.7 ± 2.2	14.8 ± 1.9
Oolong tea	2.3 ± 0.05	78.6 ± 2.9	79.4 ± 3.1	16.6 ± 3.7

Unlike the blank sample, the THMs removal followed a different pattern in sample containing black tea leaves brewed in distilled water. More obvious in higher temperature, THMs were initially reduced in first 2 min of reaction followed by a significant increase up to about 10 min. The THMs levels were then decreased up to 60 min of reaction time. Although the new THM induced by tea leaves is at very low concentrations and unlikely cause a health problem, this finding might be attributed to the contamination of tea leaves by THMs after harvesting or THMs accumulation through plantation, growing, and tea making process. When tea leaves are in contact with hot water, it induces a chemical reaction: by the effect of heat the water dissolves many different compounds which remain suspended in water. The volatile components may then emit into the air if there would be enough space above the water surface. Due to the enclosed container of the current experiment, THMs volatilization into the atmosphere is less than open system (Zhang et al., 2015).

THM formation in tea infusions may occur in similar pathways with NOM since tea leaves share several components of NOM, including phenolic compounds and other non-humic substances. It is well known that phenolic compounds, one of the reactive functional groups of the NOM for THMs formation in natural waters (Singer, 1999), are present in large amounts in teas leaves (Harbowy et al., 1997). Although the exact identity of organic materials of tea leaves are uncertain, higher THMs formation in black tea is probably due to more complex condensed polyphenols (a result of oxidation in the fermentation process of black tea) and more phenolic OH groups (Harbowy et al., 1997), compared to less fermented teas. In fact, the polyphenols of black tea endure additional chemical transformations than the green tea polyphenols, resulting in higher levels of complexity, which may potentially provide active sites for the formation of THMs compounds (Harbowy et al., 1997).

Boiling the drinking water for making hot beverages is widely applied all around the world. It has been shown that boiling may considerably reduce the DBP levels in consumed water (Krasner and Wright, 2005; Li and Sun, 2001) if the heating period is sufficiently long (e.g., 5 min) (Krasner and Wright, 2005). However, a typical kettle boils for only about 12 s, reducing the level of chlorine by 5–19% and consequently leaving the majority of chlorine free to react with organic matter resulted in THM formation (Bond et al., 2016b). Moreover, water storage strategy after boiling is also another important parameter which has been mainly overlooked in relevant studies. The few studies that estimated the concentrations of DBPs generated from reactions between aqueous chlorine and organic matters in daily beverages, prepared their samples in cups and open containers. In the study by Bond et al. (2016b), chloroform concentrations in tea prepared with water containing 1 mg/L chlorine were ≤4 μg/L after 20 min contact time, which is much lower than the current study. The discrepancy can be explained by either the longer contact time used in the earlier study that allows most of the chloroform release into the air, or different container applied for representing real-life DBP formation through tea serving process. Compared to Bond et al. (2016b) study that tea and coffee samples were made in disposable cups, thermos flask was used to prepare different tea samples in the current study. While making tea in a cup and open container will allow the THMs to evaporate into the air, THM volatilization into the atmosphere will significantly reduce when tea samples are prepared in thermos flask or enclosed container (Zhang

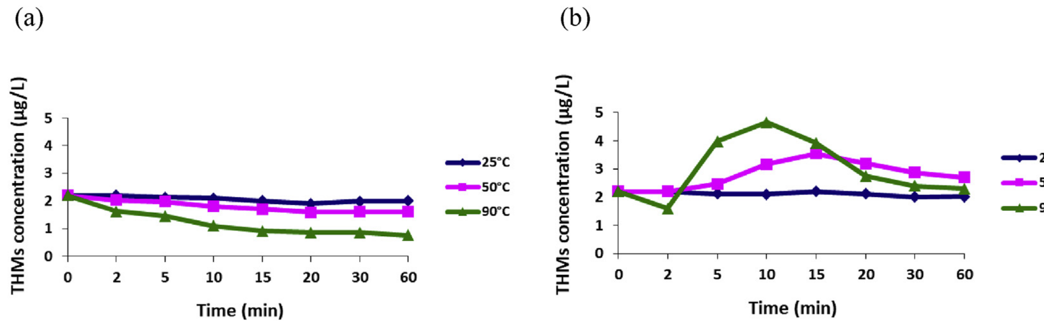

Fig. 2. THMs measurement in (a) blank distilled water (with no tea leaves) and (b) tea infusions prepared in distilled water as a function of time and temperature prepared in enclosed thermos without agitation.

et al., 2015).

Although it is unusual to boil drinking water for hours, keeping boiled water for longer periods is also quite common in many parts of the world. Moreover, electrical boiler units are also installed in many public buildings (Zhang et al., 2015). The heating method applied in water dispensers involves a heating element and storing water in a hot tank (similar to the commonly used water heaters used in residential homes). Tap water usually contains THMs precursors, chlorine residuals, and already formed THMs. Although THMs are volatile compounds, the volatilization of THMs into the atmosphere favored by agitation conditions, such as continuously boiling for at least several minutes. Therefore, the THM concentration remains quite high in the consumed boiled water, especially when the hot water is stored in an enclosed container. In such an enclosed system, THM volatilization into the atmosphere is significantly reduced, while, on the other hand, THMs can be further formed (Zhang et al., 2015). In a study by Zhang et al. (2015), hydrolysis of THMs relevant to the storage of hot boiled drinking water in enclosed containers was investigated by varying the water quality parameters. The results revealed that the removal of THMs is significantly reduced by consuming the boiled water stored in enclosed containers.

3.4. Effect of steeping time on THM formation

The effects of brewing time on the TTHM concentration of various tea types with tap water as the background solution is illustrated in Fig. 3.

THM formation was insensitive to brewing time in the range of 5–30 min. Reactions forming TTHM were rapid and completed in less than or equal to 3 min in almost all tea samples with slightly faster TTHM formation in black tea infusion.

Wu et al. (1998) proposed that the reaction time for free chlorine and tea is less than 1 min, associated with chlorine's reactivity and the organic compounds present in tea. Given such fast kinetics, the common steeping time (usually between 3 and 10 min) for tea making process is not expected to affect THM concentrations. Huang and Batterman (2009) also found that chlorine-food reaction is fast but does not depend on a reaction (steeping) time in the instant tea making process.

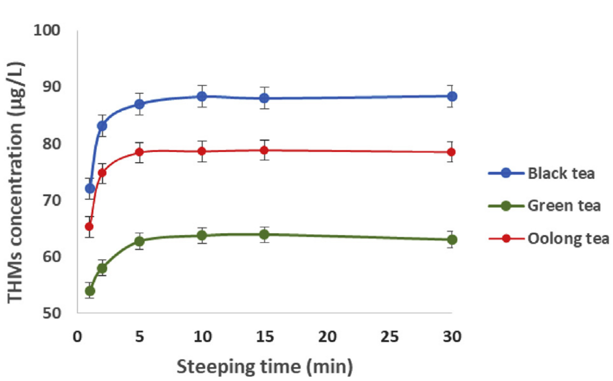

Fig. 3. Effect of steeping time on TTHM formation in different tea infusions (background solution: tap water, leaf/water ratio: 1:50 (w/v, dry weight basis)).

3.5. Effect of tea concentration on THMs formation

Tea concentrations were inversely proportional to TTHM concentrations, for example, ten times higher tea concentration, reduced 25–40% of TTHM levels in different tea samples (Fig. 4).

Therefore, THM formation is not limited by organic matter, even in very weak tea solutions with the less organic material. This is supported by chlorine demand experiments (Fig. 1) that shows higher tea concentration enhances the chlorine demand and subsequently leave less free chlorine available to form THMs (Huang and Batterman, 2009). Wu et al. (1998) also found that tea had a higher chlorine demand relative to humic substances.

3.6. THM species distribution

The THM species distribution pattern was not significantly different in various tea samples (P-value > 0.15). Fig. 5 represents the general distribution pattern of THM species in different tea infusions after 10 min of reaction, where $CHCl_3$ was the dominant THM species accounting for an average of ~82% of the total THMs in tea samples. This is probably due to the formation of chloroform from phenolic moieties, which are known as the reactive DBP precursors found in herbs and tea leaves (Huang et al., 2009).

3.7. Effect of tea brewing containers on THMs formation

Traditional Chinese teapot with a tilted lid was used to examine the effect of tea container on TTHM levels (Fig. 6).

The TTHM levels were in the range of 40–59 µ/L in different tea

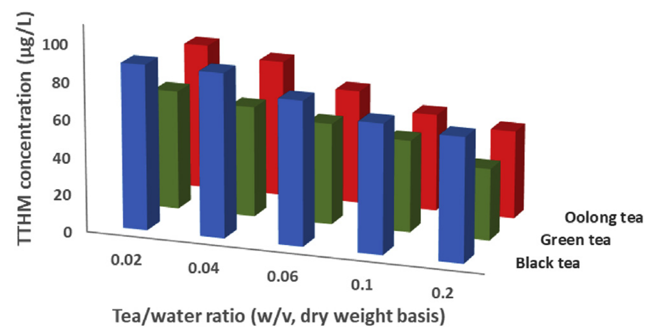

Fig. 4. Effect of tea leaf concentration on TTHM formation in different tea infusions (steeping time: 10 min, background solution: tap water).

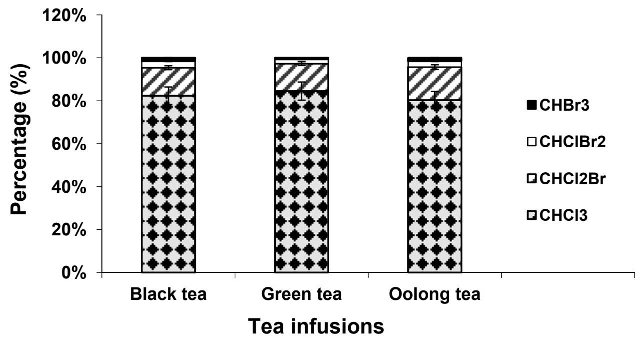

Fig. 5. THM species distribution in different tea infusions (steeping time 10 min, background solution: tap water).

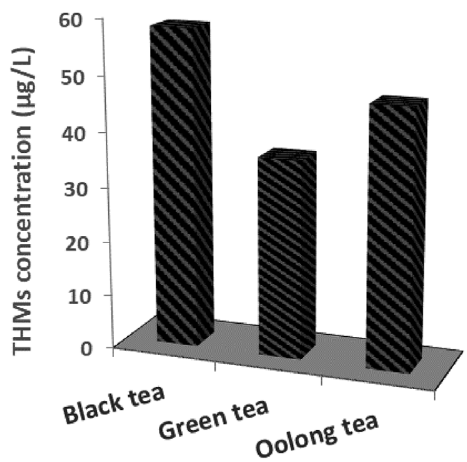

Fig. 6. TTHM concentrations in different tea infusions using traditional Chinese teapot (steeping time: 10 min, background solution: tap water, tea/water ratio: 1:50).

infusions, which is significantly (P-value < 0.05) lower than brewing tea in a thermos container by 34%, 43%, and 40% in black tea, green tea, and oolong tea, respectively.

When using a traditional teapot, all the TTHM levels are below the MCL standards for THMs in drinking water (80 µg/L) (USEPA, 2006). The main difference between making tea in traditional teapot and vacuum flask is the container's condition. Using teapot, many people prefer to keep the lid off so they can observe from above the leaf opening and determine the right time to pour. Furthermore, in the traditional method of Chinese tea-serving, the teapot is the most important element in this process. In tea serving process, it has been recommended to tilt the lid slightly open on the teapot. It is believed that this tilting allows the heat in the teapot to escape and not "cook" the leaves, but will enable them to retain their aroma at the same time.

Consequently, making tea in an open container will allow the THMs to partially evaporate into the air. Batterman et al. (2000) also proved that THM losses would be significant if water is boiled or stored in a stirred and opened container.

3.8. Health risk assessment

3.8.1. Daily tea intake

Of the 200 participants, 54% were males, and 46% were females. The mean ± SD age of the participants was 23.17 ± 5.12 (ranged from 18 to 36), and the mean body weights of the respective groups were 56.8 ± 7.05 and 65.6 ± 9.70 kg for female and male subjects, respectively. The most preferred tea type among the male group was oolong tea > green tea > black tea, and in the female group, green tea was the priority followed by oolong and black tea.

While most of the participants drank about 300 ml tea in a day, subjects with maximum tea consumption (> 1000 ml/day) formed 1.7% of all participants. Besides, participants who weighed more than 55 kg significantly have higher average tea intakes than those who weighed < 55 kg.

The questionnaire collection procedure was carried out from September 2016 to February 2017, which covered both summer and winter conditions. Neither peak high nor low-temperature days were included in the campaign. Therefore, the calculated statistics can be used as estimations of annual average values for the studied population.

Although the maximum and minimum of tea consumption were found among both genders, the numbers of high consumers in the female group were less than that of males (0.6% vs. 1%). Moreover, comparison of the mean consumption data showed that male participants are significantly higher tea consumers than females (P-value < 0.05).

It is noted that the following results may not be representative of the general public as the health risk assessment is limited to the tea shop customers in the specific range of age.

3.8.2. Ingestion exposure assessment

Although human exposure to THMs and other DBPs can occur through multiple exposure routes, it has been estimated that 50–70% of exposure to THMs comes from ingestion of tap water and beverages made from tap water (Chowdhury et al., 2010). Therefore, among the different routes of exposure, only the ingestion route was taken into consideration in this study. The exposure assessment was done based on a deterministic approach, in which the exposure was estimated individually for each subject using his or her body weight and daily intake rate of tea infusion instead of using the USEPA daily intake rate of water default value of 2 L/day and adult weight of 70 kg. This was done to avoid underestimation or overestimation of population risk.

Cancer risk (CR) values greater than one in a million (10^{-6}) are considered unacceptable by the USEPA. However, this level may change based on national standards or environmental policies and could be as high as 10^{-4} (Health Canada, 1998). HQs > 1 indicate a potential for an adverse effect or a need for further study.

Chloroform was considered for the CR assessment and HQ of THMs in oral ingestion for male and female subjects through drinking different types of tea and water background (Table 4). As shown in Table 4, while tea infusions prepared with tap water showed the highest risks among different water backgrounds, the estimated risks were much lower in tea infusions prepared by distilled or bottled water samples due to less organic matter and free residual chlorine. It is therefore suggested to use distilled or bottled water instead of tap water to make hot daily beverages when it is supposed to store in an enclosed container.

Among different types of tea, black tea was associated with the highest risks in both groups, and female participants were generally subjected to higher risks than males. The lifetime CR for $CHCl_3$ was higher than the risk level of 10^{-6}, defined by the USEPA.

The CR value was also higher in females than males. Among the three tea types and water backgrounds, the highest lifetime CR was found in black tea prepared in tap water in both genders. This indicates that people with a hot-tea drinking habit of at least 300 ml per day may have the potential for cancer risk through ingestion of tea infusion made from tap water and stored in an enclosed container.

The HQ of $CHCl_3$ through ingestion route ranges from 0.001 to 0.04. Females were generally found to show higher HQ than males. In this study, the HQ was lower than 1 for the model THMs species through the tea drinking pathway route of exposure, which complies with acceptable toxicity.

To the best of our knowledge, there is no relevant study on THM exposure assessment through ingestion pathways by daily beverages. The results of this study may thus provide useful information for future assessments of health risks associated with THMs exposure through

Table 4
Risks assessment from ingestion exposure to chloroform in different types of tea and water background.

Water type	Subjects	Risk assessment	Black tea	Green tea	Oolong tea
			Tea type		
Distilled water	Male	CR[a]	1.3×10^{-7}	7.8×10^{-8}	6.4×10^{-8}
		HQ	0.002	0.001	0.001
	Female	CR	1.5×10^{-7}	9.0×10^{-8}	7.4×10^{-8}
		HQ	0.002	0.001	0.001
Tap water	Male	CR	2.4×10^{-6}	1.7×10^{-6}	2.1×10^{-6}
		HQ	0.04	0.02	0.03
	Female	CR	2.8×10^{-6}	2.0×10^{-6}	2.5×10^{-6}
		HQ	0.04	0.03	0.04
Water dispenser	Male	CR	2.3×10^{-6}	1.5×10^{-6}	2.2×10^{-6}
		HQ	0.03	0.02	0.03
	Female	CR	2.6×10^{-6}	1.8×10^{-6}	2.5×10^{-6}
		HQ	0.04	0.02	0.04
Bottled water	Male	CR	8.2×10^{-7}	4.1×10^{-7}	4.6×10^{-7}
		HQ	0.01	0.006	0.007
	Female	CR	9.5×10^{-7}	4.7×10^{-7}	5.3×10^{-7}
		HQ	0.01	0.007	0.008

[a] Cancer risks were calculated based on the THMs concentrations for different water backgrounds shown in Table 3.

daily drinking habits particularly when hot beverages are prepared in enclosed containers. Since tea and coffee are the most consumed beverages in the world, and over 80% of tap water consumption is estimated to be associated with the preparation of tea, coffee, and other hot drinks (ITC, 2011), it is necessary to consider these new routes of exposure pathways in the regulation of THM concentrations in drinking water standards.

Moreover, further research is needed to comprehensively evaluate the net effect of tea preparation on DBP concentrations. Other possible routes of exposure should be also considered in future studies. For instance, inhalation exposure may significantly contribute to the THMs exposure if water keeps boiling in an open container for longer periods. In some countries (especially traditional Asian communities), it is very common to keep water boiling for hours in “samovar” which is a heated metal container traditionally used to heat and boil water. Lin and Huang (2000), showed that inhalation is an important pathway for THM exposure from drinking water through cooking activities including boiling. A cross-sectional study by Charisiadis et al. (2014) also showed that household cleaning activities significantly increased urinary THM levels, indicating that non-ingestion routes of THM exposures during performance of routine household activities may significantly contribute to the total THM exposures.

A more extensive range of water quality parameters (e.g., pH and bromide concentration) should also be considered in future research. Moreover, since the results of exposure assessment in the current study are limited to tea shop customers in a specific range of age with a particular way of tea making habit (thermos flask), an integrated health risk assessment considering different parameters should be performed to be representative of the general public.

Finally, as a counterpoint to the above, the health benefits of drinking tea should not be overlooked. The same features that make phenolic compounds of tea beverages effective THM precursors also explain the antioxidant properties of tea samples that are associated with positive effects and known health benefits.

4. Conclusion

The generation of THMs following preparation of tea samples and their associated health risk were investigated. The results revealed that THMs are formed by reactions between free chlorine and the organic matter present in tea samples. THMs formation decreased by increasing organic matter concentration but was insensitive to brewing time. Chloroform was found to be the major THM species, and higher levels of THMs was obtained from black tea prepared in tap water, plausibly due to the formation of more complex polyphenols through the fermentation process. Brewing tea in commonly used thermostat flasks would greatly reduce THM volatilization into the atmosphere, whereas making tea in a traditional teapot showed significantly lower THMs. The health risk assessment also revealed that tea samples prepared in enclosed containers were associated with the risk level higher than tolerable risk defined by the USEPA, and cancer risk by ingestion exposure was higher in females than males. Overall, this study demonstrates the potential for significant THMs formation through exposure pathways of drinking tea when it is prepared with water containing free chlorine and consumed in an enclosed container. This should be taken into account in drinking water standards and exposure estimates. However, potential and documented health benefits of drinking tea should not be ignored while studying exposure assessment and toxicity estimates.

Disclosure statement

The authors declare they have no actual or potential competing financial interests.

Figure captions:

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