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# Validation of urinary trichloroacetic acid as a biomarker of exposure to drinking water disinfection by-products

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## ABSTRACT

Disinfection by-products (DBPs) in drinking water represent a public health issue and a challenge for epidemiology to provide evidence towards the causation of various hypothesized health effects. Validation of a biomarker of exposure to DBPs is a strategy to achieve progress which has been advocated. The objective of this study was to validate urinary trichloroacetic acid (TCAA) excretion as a biomarker of exposure to DBPs in an experimental exposure cohort. A total of 52 healthy women participated in the study. Participants consumed supplied tap water for 15 d and provided urine and blood samples for TCAA measurements. The findings revealed that (1) background levels of TCAA in urine and blood were readily detectable, (2) TCAA levels in blood and urine increased with increased amounts of TCAA ingested, (3) the correlations between measurements of TCAA ingestion and urinary excretion were modest ($r = 0.66$, $p < 0.001$) based on one days' sampling and high ($r = 0.77–0.83$, $p < 0.001$) based on two to four days' sampling, (4) the correlations between measurements of TCAA ingestion and blood TCAA concentration were high ($r = 0.80$, $p < 0.001$) and (5) multiple days' urinary TCAA measures improved the prediction of TCAA ingestion through urinary TCAA excretion. TCAA can be a valid biomarker of exposure for DBPs in drinking water.

**Key words** | biomarker, disinfection by-products, drinking water, exposure assessment, trichloroacetic acid

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## ABBREVIATIONS

| | |
|---|---|
| Cr-a | creatinine-adjusted |
| DBPs | disinfection by-products |
| DCPA | 2,3-dichloropropionic acid |
| ECD | electron capture detector |
| FMU | first morning urine |
| GC | gas chromatograph |
| HAAs | haloacetic acids |
| LLME | liquid–liquid microextraction |
| PDMS | polydimethysiloxane |
| SPME | solid phase microextraction |
| TCAA | trichloroacetic acid |
| THMs | trihalomethanes |

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## INTRODUCTION

Some epidemiological studies have reported weak associations between exposure to several disinfection by-products (DBPs) and occurrence of adverse reproductive and developmental effects (Tardiff *et al.* 2006). Epidemiological research results cannot become more conclusive, in part, because of the limitations of DBP exposure assessment. A biomarker of exposure has been recognized as an important approach towards improving exposure assessment (Swan & Waller 1998). In the epidemiological studies related to exposure to DBPs in drinking water, trihalomethanes (THMs) were generally selected as surrogates of DBP exposure because THM data were routinely collected

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in many water treatment plants. Savitz *et al.* (2005, 2006) did not find support for total THMs being associated with any increased measures of pregnancy loss. In reviewing the findings of their study, which has arguably invested the most into improving exposure classification, they noted (Savitz *et al.* 2006): "While the present study could be improved, the available refinements in capturing individual exposure are limited at this time because we do not have a suitable biomarker…". It is important to find a valid suitable biomarker of exposure for epidemiological studies in the future. A valid biomarker provides both qualitative and quantitative information about external exposure.

A candidate exposure biomarker should be the most representative measure of a particular component in the continuum of the exposure event. THMs and haloacetic acids (HAAs) are the two most abundant classes of DBPs, and other DBP classes occur at substantially lower levels (Krasner *et al.* 1989; Richardson *et al.* 2007).

Because of their prevalence, either THMs or HAAs have been used as surrogates of DBP exposure. THMs, which are volatile, are rapidly absorbed following ingestion, inhalation and dermal contact. They are mainly metabolized to carbon dioxide and/or carbon monoxide in the liver, and/or rapidly exhaled (Fry *et al.* 1972; NAS 1987). Thus, urine will not be among the most promising sampling media for measuring THMs as a biomarker of exposure, leaving only exhaled breath or blood. Weisel *et al.* (1999) found only 9 out of 49 post-shower breath samples showed detectable levels of chloroform, the predominant THM. Likewise, no clear correlation of blood THM levels with THM concentrations in tap water was observed (Miles *et al.* 2002; Savitz *et al.* 2005). THMs are not suitable for use as a biomarker to measure DBP exposure over any meaningful period because of the transient presence of THMs in the body.

Trichloroacetic acid (TCAA) is one of the most common HAAs found in chlorinated drinking water and it offers greater persistence in the body than the THMs. The TCAA elimination half-lives range from 1.2 to 6 d in humans. In the earliest published study (Paykoc & Powell 1945), sodium TCAA (1.5–3.0 g) was administered by intravenous drip to six volunteers, and the elimination half-life for blood was 1.25 d (30 h). Muller *et al.* (1974) reported a blood elimination half-life of 2.1 d (50.6 h) based on an oral dose of 3 mg Na-TCAA/kg in three volunteers. More recently, Froese *et al.* (2002) and Bader *et al.* (2004) studied exposure to TCAA via drinking tap water. Ten volunteers ingested TCAA-containing tap water for 12 d at concentrations ranging from 1.8 to 29 μg/L, and the estimated urinary elimination half-lives ranged from 2.3 to 2.9 d (Froese *et al.* 2002). The estimated urinary elimination half-lives varied from 2.1 to 6.3 d in five volunteers who consumed TCAA-containing tap water (50–180 μg/L) for 2 weeks (Bader *et al.* 2004).

TCAA has also been found to demonstrate an exposure–response relationship between ingestion of TCAA-containing water and urinary TCAA excretion. In the studies by Kim *et al.* (1999) and Weisel *et al.* (1999), the TCAA urinary excretion rate for the first morning urine (FMU) samples was found to correlate with ingestion of TCAA-containing tap water in volunteers. TCAA exposure was estimated from a 48-h recall questionnaire asking about tap water consumption and a measure of TCAA concentration in a cold, unfiltered tap water sample collected from each volunteer's home. A correlation between ingestion and urinary excretion of TCAA was also found in the volunteers who consumed tap water for 2 weeks (Froese *et al.* 2002).

TCAA in blood can also be formed via exposure to chloral hydrate, another common chlorination DBP (Richardson *et al.* 2007; Dębrowska & Nawrocki 2009). A volunteer was treated with chloral hydrate and TCAA was measured in blood with a half-life of 1.2 d (Humbert *et al.* 1994).

Background TCAA has been detected in 76% of archived urine samples with a median concentration of 3.3 μg/L in a US general population sample (Calafat *et al.* 2003). Regarding non-DBP contributions to background levels in humans, TCAA can be formed via exposure to trichloroethylene. In two studies of Monster *et al.* (1976, 1979), a total of nine volunteers were repeatedly exposed to trichloroethylene via inhalation and TCAA concentrations were measured in blood with half-lives of 2.9–4.2 d.

These findings, combined with knowledge of toxicokinetics and the sufficiently long elimination half-life of TCAA, indicate that TCAA may be a potentially useful biomarker for measuring DBP exposure by ingestion of drinking water.

This study is designed to validate urinary and blood TCAA as a potential biomarker of exposure for exposure to DBPs in drinking water in a cohort large enough to allow statistical analysis. Our study included determination of

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background level, validity analysis and reliability analysis. Validity analysis provides evidence of the relationship between TCAA ingestion and excretion. The reliability analysis, being published separately, provides an evaluation of the intraindividual and interindividual variability in the cohort using the intraclass correlation coefficient (ICC) and Cronbach's $\alpha$ in order to examine the consistency of TCAA levels within individuals over time and the agreement of different measurements for measuring TCAA excretion in urine and loading in blood (Zhang *et al*. 2009).

This part of the whole study addresses the validity of using urinary TCAA as a biomarker of exposure to DBPs in drinking water by a controlled, direct exposure experiment in a relevant human cohort. In this paper, the results of validity analysis using Pearson's correlation coefficient and regression analysis, as well as background levels, are presented. The results of our reliability analysis are presented separately (Zhang *et al*. 2009).

## MATERIALS AND METHODS

Recruitment procedures, sample collection and laboratory methodology are summarized below. This study protocol was reviewed and approved by the Health Research Ethics Board at the University of Alberta, Edmonton, Alberta, Canada.

### Recruitment

Recruitment information was posted in the newsletter of the Graduate Students' Association at the University of Alberta. Telephone interviews and person-to-person interviews were conducted. A total of 52 healthy female (at reproductive age, non-pregnant) volunteers were recruited. All volunteers lived in the City of Edmonton during the period of the study (May 2003–April 2004). During the telephone interviews, the following information was collected: demographics, sources of drinking water, volume of water consumption per day, types of drinking water and beverages, duration of shower/bath, physical activities and use of medications. During the person-to-person interviews, the volunteers signed consent forms and answered a few questions about their detailed volumes and patterns of fluid intake, and their physical activities. They received a diary booklet and instructions for water delivery, water consumption and urine/blood collection. A schedule and location for sample collection were set up.

### Exposure

Tap water from City A, which is a city population in excess of 100,000, was used to provide the TCAA exposure via authentic drinking water for this study. Bulk samples of City A tap water were shipped to Edmonton for this study. Tap water from City A was diluted with TCAA-free bottled water to achieve different TCAA concentrations for the exposure experiment. The 52 participants were randomly stratified into five sub-groups. Group 1 was a control receiving TCAA-free bottled water. Groups 2–5 were exposure groups receiving TCAA-containing water at concentrations of 12.5%, 25%, 50% and 100% of City A tap water, respectively. Participants were asked to commence the study on the first Wednesday after the completion of their menstrual cycle to preclude the likelihood of pregnancy during this experiment. Each participant ingested cold supplied tap water every day for a 15-d period.

### Sample collection

Each 1 L Nalgene bottle was labeled with each participant's identifier, date of ingestion and number order of the bottle. Each bottle was filled with tap water with designated concentrations based on each participant's assigned exposure level. A total of 3 L of water was provided to each participant per day. Extra TCAA-free bottled water was provided to some participants who often consumed more than 3 L of water per day. All bottles were stored in the refrigerators in the participants' homes. The supplied bottles were collected from each participant the following morning. The remaining volume of water in the bottle(s) was recorded to provide an objective measure of the volume of tap water consumed by each participant. Participants recorded volumes of tap water and beverage consumption, and physical activities in their diaries every day. Tap water samples from bottles were sent to the laboratory for TCAA analysis twice per week.

A urine collection kit with instructions for urine collection was prepared for each participant. The urine

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samples were collected on the 1st day before supplied tap water consumption (designated Exposure Day 0) and at the 2nd, 8th, 13th, 14th, 15th and 16th day after supplied tap water consumption (designated Exposure Day 1, 7, 12, 13, 14 and 15, respectively). The urine collection kit was delivered to each participant one day prior to scheduled urine collection. Participants were encouraged to avoid water consumption close to bedtime. They collected the entire volume of urine within 30 min after waking up in the morning and before drinking any liquid so that this sample constituted the first morning urine (FMU) sample. The urine sample bottle was immediately packed into the cooler and kept at 4°C. The urine samples were picked up within 2 h and immediately delivered to our laboratory. The volume of urine was measured and recorded. The bottle was refrigerated at 4°C prior to TCAA analysis.

Provision of a blood sample was optional for each participant. Blood samples were collected from volunteers at Exposure Day 0, 7, 13 and 14. Whole blood samples were collected in a private medical laboratory and delivered to our laboratory within 24 h for TCAA analysis.

## Laboratory analysis

A method for TCAA analysis was developed by collaborators in our laboratory (Wu *et al.* 2002). This method was developed as an adaptation of existing methods to provide the additional analytical sensitivity required for this study. Delinsky *et al.* (2005) have reviewed the available methods. All reagents used in our study are provided by Wu *et al.* (2002). Water (0.1 mL), urine (0.1 mL) or blood (25–50 μL) were combined with 0.1 M acetate buffer (0.2 mL, pH 5.2) and vortex-mixed in a 1.5 mL polypropylene microcentrifuge tube. Ten μL of 2,3-dichloropropionic acid (DCPA) was added as an internal standard. The solution was acidified with 25 μL of 50% sulfuric acid. TCAA was extracted from the mixture with 0.6 mL methyl t-butyl ether. After extraction, the organic layer was placed in a 2 mL autosampler gas chromatograph (GC) vial and evaporated just to dryness under a gentle stream of $N_2$ (99.999% pure). Sodium sulfate (0.10 g), methanol (10 μL) and sulfuric acid (10 μL) were added to the dried residue in the vial and the vial was sealed with a Teflon-lined crimp cap. The solution was vortex-mixed and the TCAA was derivatized at 80°C for 20 min. After derivatization, the sample was cooled to room temperature. Solid phase microextraction (SPME) was performed with a 100 μm thickness polydimethysiloxane (PDMS)-coated fiber. The sample components were absorbed from the headspace by the PDMS fiber for 10 min at room temperature (25°C).

Analyses were performed on a Varian CP 3800 (Varian Inc. Walnut Creek, CA, USA) capillary GC with a $^{63}$Ni electron capture detector (ECD) and 8200/SPME autosampler. The PDMS fiber was desorbed for 2 min in splitless mode at 200°C. A DB-1 MS fused silica capillary column (20 m × 0.18 mm I.D) with 0.4 μm film thickness was used, with helium as the carrier gas at a flow rate of 0.8 mL/min. The column temperature program was 40°C (0 min) to 70°C at 10°C /min holding for 4 min, and then to 205°C at 15°C/min holding for 3 min. The detector temperature was 260°C.

The estimated detection limit was 0.2 μg/L for TCAA in this study. A total of 1460 water, urine and blood samples and 108 blank samples were analyzed. Duplicate analysis was performed for each sample. Triplicate analysis was performed for one out of every eight water samples and one out of every five urine or blood samples. Quadruplicate analysis was performed for some samples. TCAA recovery was performed based on analysis of a fortified sample matrix. Recovery of TCAA in the water samples ranged from 70–126%, except for one sample from City A (61%). Recovery of TCAA in the urine samples ranged from 77–108%. Recovery of TCAA in the blood samples ranged from 70–130%, except for one sample with a low value (51%) and five samples with high values (137–149%).

## Statistical analysis

Data from 52 participants who provided urine samples and 31 participants who donated blood samples were included in the validity analysis. The data collected from urine samples at Exposure Day 12, 13, 14 and 15 or blood samples at Exposure Day 13 and 14 were used for statistical analysis to allow measurements at a steady state. TCAA concentrations in tap water, urine and blood samples were measured directly by laboratory analysis. Creatinine-adjusted (Cr-a) TCAA concentrations in urine were calculated based on urinary creatinine levels measured

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from a Bayer ADVIA 1650. The amount of TCAA ingestion was calculated based on the measured volume of tap water intake per day and the measured TCAA concentration in tap water. The amount of TCAA excretion over 24 h was estimated based on the total volume of 24-h urination extrapolated from the FMU volume and measured TCAA concentrations in urine.

Higher TCAA levels were observed in blood or urine when exposure was continuous for 15 d. With continuous exposure for three, five and seven half-lives, the blood concentrations of a xenobiotic can reach approximately 90%, 97% and 99% of the steady-state concentration, respectively. In this study, assuming a median urinary excretion half-life of 4 d, TCAA levels in blood and urine should reflect 65% of the steady-state condition after the 6th day of exposure and almost 90% of the steady-state condition after the 12th day of exposure. Thus, continuous exposure after the 12th day was considered sufficient for stable measurement. The reliability of urinary TCAA measurements at Exposure Day 12, 13, 14 and 15 was high (Zhang *et al.* 2009). This is the basis for using the data from the last four days of urine collection for validity analysis.

Because of the large range of concentrations of TCAA in tap water (0–121 μg/L) that was intentionally provided, and the restriction of the range of the volume of tap water intake (up to 3 L per day per individual), the observed relationship between TCAA ingestion and urinary excretion could be small. In this case, the four-days' (Exposure Day 12, 13, 14 and 15) data in each item were combined into a single variable to perform correlation analysis or partial correlation analysis. For the analysis of two or more exposure days, the data from each exposure day were added into a regression model.

The distribution of TCAA concentrations in tap water was approximately normal. Arithmetic means were used for this set of data. The distribution of TCAA excretion and ingestion measures in the human body was skewed, but it is approximately a log-normal distribution. Geometric means were used for these skewed data. The geometric mean is the average of the logarithmic values of a data set and the better measure of central tendency.

A logarithmic transformation of the values was performed. The data under detection limits were reported as zero, which became missing values with log transformation. In order to include missing values in the statistical analysis to avoid the effect of an artificially restricted exposure range, a value of 0.0001 was added to all data before log transformation. Pearson's correlation analysis and linear regression model were performed on the log-transformed data. For analysis of the correlation between the volume of tap water intake and urinary TCAA excretion, original (non-transformed) data were used. Because the effect from the large range of concentrations of TCAA in tap water was controlled in this data analysis, partial correlation analysis was performed. The SPSS 14.0 software package was used for performing correlation analysis.

The extent of the agreement between unadjusted and Cr-a urinary TCAA concentrations was analyzed by using the Bland–Altman method (Bland & Altman 1986). The Bland–Altman plot was used to display a scatter graph of the differences between two readings plotted against the average of two readings. The limits of agreement are defined as the mean difference plus or minus 1.96 times the standard deviation of the difference. The narrower the range of the limits of agreement is, the better the agreement is. If the mean of the difference of two readings is close to zero, this indicates that two readings are slightly different.

## RESULTS AND DISCUSSION

### Selection of participants

A total of 52 women of reproductive age (mean = 27 years old, ranging from 19–41) participated in the study. The criteria for recruitment were determined in order to control some important factors associated with TCAA exposure in this human trial. Because of the concern about reproductive and developmental outcomes associated with TCAA exposure in animal studies (Smith *et al.* 1989; Epstein *et al.* 1992; Linder *et al.* 1997; Johnson *et al.* 1998), recruiting women of reproductive age is highly relevant for this study. Pregnant women were not selected in this study to avoid their exposure to any unknown risk factors. Pregnant women experience physiological, biochemical and anatomical changes (Koos & Moore 2003). Healthy women who did not use any medication were selected so

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that some confounders derived from other exposure sources and alteration of health conditions could be controlled. For example, chloral hydrate can be used as medicine. A proportion of chloral hydrate can be converted to TCAA in the human body (Marshall & Owens 1954; Sellers *et al.* 1972; Allen & Fisher 1993; Humbert *et al.* 1994). Some health conditions such as kidney malfunction can influence the excretion rates of TCAA in urine.

## Background levels

Participants who lived in Edmonton and consumed tap water daily were selected to ensure that the test protocol was as consistent as possible with their normal behavior. Drinking water in Edmonton is drawn from a river within the city limits and treated initially with free chlorine before conventional coagulation–filtration water treatment. The chlorine is converted to chloramine before treated water enters the distribution system. Chloramine is a weaker oxidant and forms a more stable chlorine residual. The use of chloramines reduces the formation of TCAA (Singer 1993). Drinking water in City A originates from a lake located far from the city limits. After the water is drawn from the lake, the water is treated with chlorine at three points along a 160 km aqueduct to City A. The water is stored in a large open reservoir near City A and is re-chlorinated before being pumped without filtration into the distribution system. The lake source disinfected by chlorination at several points along the water supply system results in a relatively high level of DBPs in the tap water of City A.

Because of differences in the source water quality and treatment processes, lower concentrations of TCAA were observed in the tap water of Edmonton compared to City A (Table 1). Participants living in Edmonton were exposed to a relatively low level of TCAA via drinking water ingestion. Because low levels of TCAA in tap water and possible other minor TCAA sources contribute to TCAA levels in the human body, background levels of TCAA in the urine and blood samples were examined (Table 2). The detected background TCAA concentrations in urine and blood in participants reflected the low, but non-zero, exposure to TCAA in Edmonton tap water. The geometric means were 1.4 μg/L for urinary TCAA concentration and 9.6 μg/L for blood TCAA concentration. The median background concentration of urinary TCAA was 3.5 μg/L (2.2 μg/g Cr) in our cohort. In a US background survey, the median concentration of urinary TCAA in archived urine samples (1988–1994) was 3.3 μg/L (3.2 μg/g Cr) in a general population (Calafat *et al.* 2003).

## Exposure status

Participants in the five groups had a large range of exposure levels. The mean TCAA concentrations for 15-d tap water consumption in four exposure groups ranged from 9.5 to 80 μg/L and the mean amount of TCAA ingested ranged from 25 to 174 μg/d (Figure 1).

The time of sampling is important for assessing the relationship between TCAA ingestion and urinary excretion. The time of appearance, persistence and disappearance of urinary TCAA was related to the time of external exposure or the fluctuation in time of the internal dose in blood. TCAA has a substantially longer blood or urinary elimination half-life than THMs and dichloroacetic acid. This elimination half-life reflects the affinity of TCAA for plasma protein and the efficiency of excretion and metabolic processes of elimination (Paykoc & Powell 1945; Marshall & Owens 1954; Sellers & Koch-Weser 1971; Muller *et al.* 1972; Monster *et al.* 1976). The urinary TCAA concentration was relatively stable after the 7th day of tap water consumption (Figure 2). In this study, the urinary TCAA levels were statistically significantly higher after

**Table 1** | Concentrations of TCAA (μg/L) in tap water samples

| Location | Median | Mean | SD | SE | Min. | Max. | 95% CI for mean | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Lower | Upper |
| City A ($n = 59$) | 79 | 80 | 20 | 2.6 | 45 | 130 | 75 | 86 |
| Edmonton ($n = 28$) | 6.9 | 6.7 | 2.4 | 0.5 | 2.1 | 12 | 8.0 | 8.9 |

SD = Standard Deviation, SE = Standard Error.

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**Table 2** | Background levels of TCAA in urine and blood samples

| Parameter | Geo. mean | Median | Arith. mean | SD | SE | Min. | Max. | 95% CI for mean Lower | Upper |
|---|---|---|---|---|---|---|---|---|---|
| *Urine (n = 52)* | | | | | | | | | |
| Concentration (μg/L) | 1.4 | 3.5 | 6.6 | 10 | 1.4 | ND | 52 | 3.8 | 9.4 |
| Cr-a (μg/g Cr) | 1.1 | 2.2 | 5.3 | 10 | 1.4 | ND | 56 | 2.5 | 8.0 |
| Amount (μg/d) | 1.3 | 2.6 | 6.2 | 10 | 1.4 | ND | 57 | 3.4 | 9.0 |
| *Blood (n = 35)* | | | | | | | | | |
| Concentration (μg/L) | 9.6 | 8.7 | 13 | 12 | 1.9 | 2.3 | 54 | 9.2 | 17 |

SD = Standard Deviation, SE = Standard Error, Geo = Geometric, Arith = Arithmetic.

Exposure Day 7 compared with Exposure Day 0 and Exposure Day 1 ($p < 0.001$). There were no statistically significant differences among the TCAA levels at Exposure Day 7, 12, 13, 14 and 15 ($p > 0.05$). The increased trend was clearly observed in Exposure Groups 3–5. This finding is consistent with the reported TCAA elimination half-life in the literature. TCAA has adequate persistence in blood and urine to allow the measurement of current and recent TCAA exposure via drinking water.

After the 12th day of supplied tap water consumption, TCAA levels in blood and urine samples increased significantly with increased exposure levels ($p < 0.05$) in 96% participants from Groups 2–5 as compared to the control group (Figure 3). The mean values for Exposure Day 12–15 in the control group were 1.5 μg/L for urinary TCAA concentrations, 2.0 μg/g Cr for Cr-a urinary TCAA, 1.4 μg/d for the amount of TCAA excreted and 6.1 μg/L for blood TCAA concentration. The mean values of TCAA in the four exposure groups ranged from 7.5 to 39 μg/L for urinary concentrations, 7.8 to 34 μg/g Cr for Cr-a concentration, 11 to 39 μg/d for amount of urinary excretion and 26 to 80 μg/L for blood concentration. The results indicated that the increased TCAA levels in blood and urine resulted from the ingestion of TCAA in the supplied tap water.

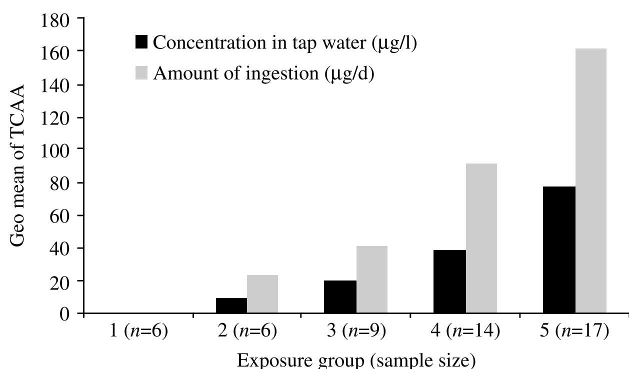


**Figure 1** | TCAA concentrations in tap water and ingested amount of TCAA during a 15-day period exposure in five groups.

## Correlation

The correlation coefficients ($r$) between TCAA ingestion and urinary excretion are summarized in Table 3. Linear regression lines between log-transformed TCAA ingestion and log-transformed urinary TCAA excretion in a single-day exposure are shown in Figures 4 and 5. The positive correlation between TCAA concentration in supplied tap water and urinary TCAA concentration in a single-day exposure was observed with an $r$ value of 0.66 ($p < 0.001$). The positive correlation between the amount of TCAA

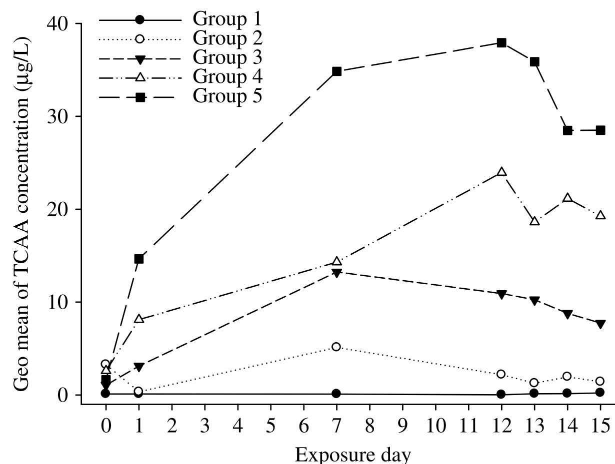


**Figure 2** | Urinary TCAA concentration vs. exposure days in five groups.

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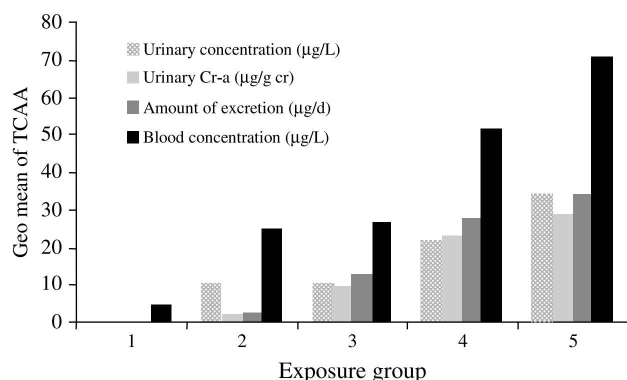


**Figure 3** | Levels of TCAA loading in blood and urinary excretion between Exposure Day 12 and 15 in five groups.

ingestion and the amount of TCAA excretion in a single-day exposure was also observed with an $r$ value of 0.66 ($p < 0.001$). Compared to a single-day measure between ingestion and excretion, the $r$ values were higher when the urinary measures were combined for a two-day or three-day exposure ($r$: 0.77–0.78) or a four-day exposure

**Table 3** | The correlation ($r$ values) between TCAA ingestion and urinary excretion

| Exposure Day* | 1 Day | 2 Days | 3 Days | 4 Days |
|---|---|---|---|---|
| *Concentration in tap water (µg/L) vs.* | | | | |
| Urinary concentration (µg/L) | 0.66 | 0.78 | 0.78 | 0.81 |
| Urinary Cr-a. (µg/g Cr) | 0.65 | 0.78 | 0.78 | 0.83 |
| Amount of excretion (µg/d) | 0.66 | 0.77 | 0.77 | 0.77 |
| *Amount of ingestion (µg/d) vs.* | | | | |
| Urinary concentration (µg/L) | 0.65 | 0.77 | 0.77 | 0.80 |
| Urinary Cr-a. (µg/g Cr) | 0.64 | 0.77 | 0.77 | 0.82 |
| Amount of excretion (µg/d) | 0.66 | 0.77 | 0.77 | 0.77 |
| *Volume of tap water intake (L/d)† vs.* | | | | |
| Urinary concentration (µg/L) | 0.13 | | | |
| Urinary Cr-a. (µg/g Cr) | 0.27 | | | |
| Amount of excretion (µg/d) | 0.27 | | | |
| *Concentration in blood (µg/L) vs.* | | | | |
| Concentration in tap water (µg/L) | 0.80 | | | |
| Urinary concentration (µg/L) | 0.64 | | | |

*Regression model: enter method for predictors. For urine samples: 1 Day = a single variable composed of measures at Day 12, 13, 14 and 15; 2 Days = Day 12 plus Day 13; 3 Days = Day 12 plus Day 13 plus Day 14; and 4 Days = Day 12 plus Day 13 plus Day 14 plus Day 15. For blood samples: 1 Day = a single variable composed of measures at Day 13 and 14; 2 Days = Day 13 plus Day 14.

†Partial correlation analysis, a controlled variable was TCAA concentration in tap water. $p < 0.001$ for all the above values.

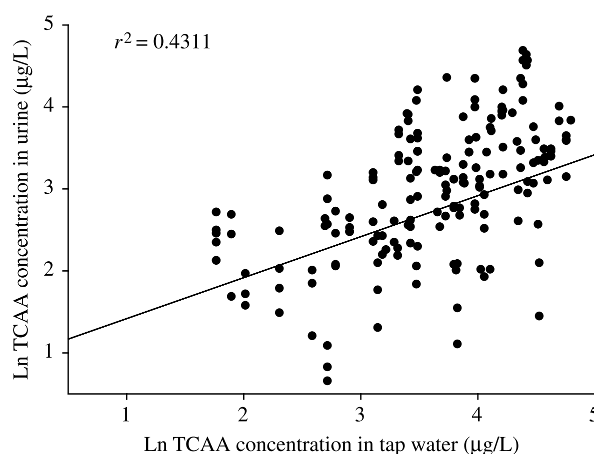


**Figure 4** | Correlation between log-transformed TCAA concentrations in supplied tap water and log-transformed TCAA concentrations in urine in a single Exposure Day.

($r$: 0.77–0.83) (Table 3). The prediction of TCAA exposure and urinary excretion was improved by using four-day combined urinary TCAA measures. The difference in $r$ values between two-day and four-day combined measures was small. For a practical, large-scale sampling strategy, collecting two-day urine samples is likely to be adequate.

The correlations ($r$ values) between urinary TCAA concentrations and Cr-a concentrations or between urinary TCAA concentrations and the amount of TCAA excreted ranged from 0.82–0.99 from one day of exposure to another. The results suggested that all three measurements could be considered to be similar for measuring urinary TCAA excretion.

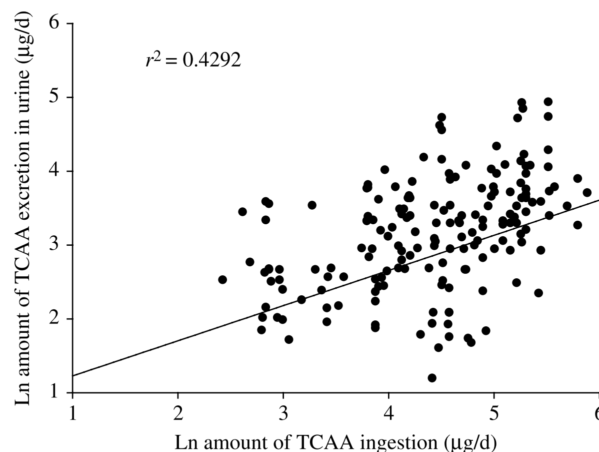


**Figure 5** | Correlation between log-transformed amount of TCAA ingestion and log-transformed amount of urinary excretion of TCAA in a single Exposure Day.

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The urinary TCAA concentrations are affected by the rate of urine production. The traditional practice for reducing this variation is to correct for creatinine excretion. Creatinine is excreted by glomerular filtration at a relatively constant rate over time (Rosenberg *et al.* 1989). By using the Bland–Altman analysis, the agreement of using unadjusted and Cr-a urinary TCAA concentrations as measures of urinary TCAA excretion was good (mean difference was 0.05 with 95%CI: −0.02, 0.13; 95% limits of agreement: −1.03 and 1.14) (Figure 6). The result suggested that adjustment of creatinine to correct the excretion of urinary TCAA did not improve the results of the validity analysis for this study group, and either urinary TCAA concentrations or Cr-a concentrations can be interchangeable measures of urinary TCAA excretion for this study group. Some studies have reported that the correction of the excretion of some compounds for urinary creatinine improved biological monitoring to a limited extent (Edwards *et al.* 1969; Bailey & De Wardener 1970; Curtis & Fogel 1970; Greenblatt *et al.* 1976; Wilson & Crews 1995).

Urinary Cr-a concentration is commonly used for spot urine samples like the FMU samples. Many factors such as age, gender, race, health conditions and sample collection time can influence its validity (Barr *et al.* 2005). The correction coefficients between ingestion of TCAA and urinary TCAA concentration or ingestion of TCAA and Cr-a concentration were similar. Two of the factors may be taken into account for this similarity: (1) participants were healthy women at reproductive age who were a more homogenous group in terms of gender, age and health conditions and (2) collection time for the FMU samples was controlled (in the morning only).

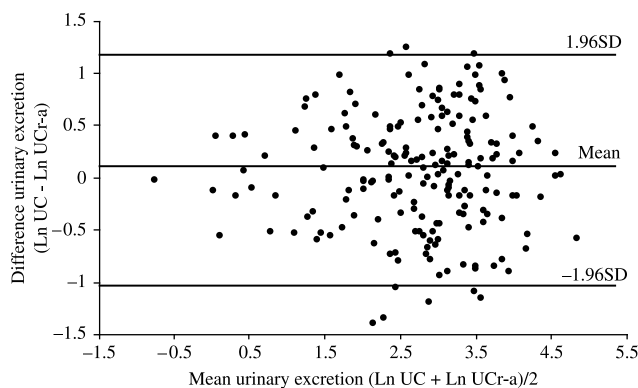


**Figure 6** | Bland–Altman analysis: agreement between log-transformed urinary and Cr-a TCAA concentrations. UC = urinary TCAA concentration (μg/L), UCr = urinary Cr-a concentration (μg/g Cr).

The relationship of the volume of tap water intake to urinary TCAA excretion was examined by using partial correlation analysis. After controlling for variability of the concentrations of TCAA in tap water, the volume of tap water intake was weakly correlated with urinary TCAA excretion, particularly with Cr-a urinary TCAA concentrations and amount of TCAA excretion ($r = 0.27$, $p < 0.001$) (Table 3). The results indicated that an increase of the volume of tap water intake increased urinary TCAA excretion in individuals, but the effect was very small compared to the primary effect of the concentration of TCAA in tap water ($r = 0.66$).

The relationship between TCAA concentration in tap water and urinary TCAA excretion was investigated in New Jersey by Kim *et al.* (1999) and Weisel *et al.* (1999). The characteristics of the reports of their studies and the current study are compared (Table 4). The advantages of the current study are that (1) participants were assigned to a wide range of exposure levels to avoid the effect of restriction of the range of exposure during statistical analysis and to improve the ability to detect correlation between ingestion and excretion; (2) the volume of tap water intake was measured directly every day rather than estimated through a 48-h recall questionnaire; (3) repeated water, urine and blood samples from an individual were collected during the 15-d period of the study rather than a single sample, which allowed for the assessment of the reliability of measures and (4) the skewed distribution of original data was corrected using a log-normal distribution.

The discrepancy between the results from the studies of Kim *et al.* and Weisel *et al.* and the results from the current study was the correlation between TCAA concentration in tap water and urinary TCAA excretion. In our current study, good correlations were observed between TCAA ingested from supplied tap water containing known concentrations of TCAA and urinary TCAA excretion. The TCAA concentrations in supplied tap water were relatively controlled. On average, tap water intake accounted for 83% of total fluid intake. Thus, we infer that the major source of TCAA excretion came from the TCAA-containing tap water that we supplied. These controlled factors and a wide range of exposure among individuals may contribute to the good

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**Table 4** | Characteristics of TCAA biomarker studies in two locations

| Feature | Kim et al. (1999) and Weisel et al. (1999) | Current study |
|---|---|---|
| Objective | The relationship between TCAA ingestion and urinary excretion | The same |
| Study design | One-time survey (48 h) | Experimental cohort (15 d) |
| Location | New Jersey, USA | Alberta, Canada |
| Gender | Female | Female |
| Age (yr) | 18–43 | 19–41 |
| Sample size | 25–42 | 52 |
| *Tap water sample* | | |
| Source | Residential drinking water (cold, hot, bottled, filtered or unfiltered) | Supplied cold tap water with known TCAA conc. |
| Sampling per person | A single cold unfiltered water from the kitchen or bathroom tap during a home visit | Cold supplied water per day for 15 d |
| Volume measured | Estimated using 48-h recall questionnaire | Direct measurement per day |
| TCAA conc. (μg/L) (a single day) | Range: 0.25–120 (skewed to the low end of the range) | 0–121 |
| | Mean: 18 | 41 |
| | Median: 5.7 | 33 |
| Amount ingested | Waterconc × (VolCold + 0.61VolHot) (μg/48 h) | WaterConc × Vol (μg/24 h) |
| *Urine sample* | | |
| Type | First Morning Urine | First Morning Urine |
| Sampling per person | One sample during a home visit | 7 samples during 15 d |
| Measurement | Urinary excretion rate | Urinary concentration |
| | | Amount of urinary excretion |
| *Blood sample* | No | 4 samples during 15 d from 35 volunteers |
| Laboratory analysis | LLE-GC-ECG | LLME-SPME-GC-ECG |
| Data analysis | Regression analysis | Log-transformed data |
| | | Correlation and regression analysis |
| Findings | No relationship between TCAA conc. in tap water and urinary TCAA excretion rate | Correlation between TCAA conc. in tap water and urinary TCAA excretion ($p < 0.001$) |
| | Linear relationship between amount of TCAA ingested and urinary TCAA excretion rate ($r^2 = 0.575$, $p < 0.0001$) | (2) Correlation between amount TCAA ingested and urinary TCAA excretion ($p < 0.001$) |
| | | (3) Log-linear relationship between TCAA ingestion and urinary excretion or blood |
| | | ($r^2 = 0.43$ or 0.69, $p < 0.001$) |

correlation between TCAA concentrations in tap water and urinary TCAA excretion. The lack of an observed relationship between TCAA concentrations in tap water and urinary excretion from the Kim *et al.* and Weisel *et al.* studies may result from the narrow range of exposure from TCAA concentrations in tap water measured in a single day's sample, which were found to be skewed to low TCAA concentrations.

From a laboratory perspective, validity is the ability of an assay to detect the presence or absence of a designated

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biomarker in the specified biological medium. The validation processes include well-characterized accuracy and precision, detection limits (analytical sensitivity), analytical specificity and reliability (Sampson *et al.* 1994; Saah & Hoover 1997; Schulte & Perera 1997). In four TCAA biomarker studies, urinary TCAA excretion was sensitive to TCAA ingestion in tap water and TCAA was specifically measured in urine (Kim *et al.* 1999; Weisel *et al.* 1999; Froese *et al.* 2002; Bader *et al.* 2004). In our current study, a LLME–SPME combined with GC-ECD method was used to analyze TCAA in samples of tap water, urine and blood with only 50–100 μl of sample volume, with speed and acceptable precision (Wu *et al.* 2002).

The use of urinary TCAA as a biomarker of exposure has an advantage in sample collection since urine sample collection is non-invasive. The FMU samples are ideal for detecting TCAA which is, at that time, most concentrated in the urine and displays less variation from weighted-average concentrations (Que Hee 1993; Kissel *et al.* 2005).

### Ingestion and loading in blood

The correlation between blood TCAA concentration and TCAA concentration in tap water was high ($r = 0.80$, $p < 0.001$) (Table 3). The correlation between blood TCAA concentration and urinary TCAA excretion was modest ($r = 0.64$, $p < 0.001$). The results indicated that the source of TCAA in the blood was ingestion of TCAA-containing tap water, and that TCAA elimination in the urine was related to the blood TCAA concentration, but urine excretion variability produced a modest correlation between urinary TCAA and blood TCAA.

TCAA in whole blood refers to the total TCAA burden in the body. After absorption, a large proportion of TCAA in the blood is bound to plasma proteins at a relatively constant rate and saturation of binding has been observed in human plasma. (Sellers & Koch-Weser 1971; Muller *et al.* 1972; Lumpkin *et al.* 2003). The binding capacity was higher in humans than in rats and mice (Lumpkin *et al.* 2003). The higher binding capacity of human plasma for TCAA is related to the presence of a larger number of binding sites and higher levels of albumin. Free TCAA in blood is rapidly eliminated by glucuronidation and filtration through the kidney (Nomiyama & Nomiyama 1979; Fisher *et al.* 1991). The proposed pathway of TCAA metabolism is reductive dechlorination (Larson & Bull 1992). A one-electron reduction and homolytic cleavage catalyzed by cytochrome P450 produces the dichloroacetyl radical. The free radical abstracts a hydrogen atom to yield dichloroacetic acid. The microsomal enzyme-mediated dehalogenation process can yield $CO_2$, glyoxylate, oxalate and glycolate. About 1.4–3% TCAA was eliminated via feces in rats and mice (Larson & Bull 1992). About 50–65% of unchanged TCAA was excreted in the urine. Twenty-three percent to 50% of the ingested doses were recovered in urine in humans (Muller *et al.* 1974; Humbert *et al.* 1994).

In our current study, the ratio of TCAA concentration in urine to that in whole blood was 54% and the ratio of the amount of TCAA excretion to ingestion was 35%. The recovery ratios were consistent with those reported in the literature. The small proportion of TCAA recovered in urine from the ingested dose could result from plasma protein binding and metabolism of TCAA in the human body.

The blood concentration of TCAA is related to current exposure with high analytical specificity, and we believe TCAA can be used as an important exposure index of drinking water ingestion of disinfection by-products in an epidemiological study. The limitation for using blood TCAA as a biomarker of exposure in a larger cohort survey is the invasive sampling procedure.

## CONCLUSIONS

There were modest (based on one-day sampling) to high (based on two- to four-days sampling) correlations between TCAA concentrations in supplied tap water and urinary TCAA excretion, and between the amount of TCAA ingestion and urinary TCAA excretion. There was a weak correlation between the volume of tap water intake and urinary TCAA excretion. The major source of TCAA excretion came from supplied TCAA-containing tap water. TCAA concentration in tap water and the amount of TCAA ingested can be good surrogates for TCAA exposure from the ingestion of drinking water. Urinary TCAA, measured as concentration, Cr-a concentration or amount, is a valid biomarker of exposure to TCAA in drinking water.

There were relatively high correlations between blood TCAA concentrations and TCAA ingestion or excretion.

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The source of TCAA in the blood was mainly ingested TCAA-containing tap water. Urinary TCAA excretion correlated with the blood TCAA concentration. The blood concentration is the best biomarker of exposure to TCAA in drinking water but it requires invasive sampling and may not be the most practical biomarker for field use.

## ETHICS APPROVAL

This study protocol was reviewed and approved by the Health Research Ethics Board at the University of Alberta, Edmonton, Alberta, Canada.

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