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RESEARCH ARTICLE

Reliability of using urinary and blood trichloroacetic acid as a biomarker of exposure to chlorinated drinking water disinfection byproducts

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Abstract

This study was designed to analyse the reliability of using urinary and blood trichloroacetic acid (TCAA) as a biomarker of exposure. A total of 46 healthy women consumed supplied TCAA-containing tap water for 15 days and provided urine and blood samples for TCAA measurements. The findings revealed that the reliability of measurements was excellent by using measures of TCAA ingestion, blood concentration and urinary excretion (intraclass correlation coefficients (ICC) > 0.75, $p < 0.001$). Volume of tap water consumption (ICC = 0.69) and creatinine-adjusted urinary concentration (ICC = 0.72) were less reliable. This indicated that the intraindividual variability was small and the interindividual reliability was high by using these measures in this cohort study. Laboratory variability did not significantly contribute to total variance (ICC > 0.95, $p < 0.001$). Other possible sources of variation such as bathing, showering, dishwashing and physical activities were unlikely to contribute significantly to total variance. For sampling strategies, 1-day blood sampling and 2-day urine sampling are sufficient to achieve reliability for an epidemiological study if a quasi-steady-state TCAA level in the body is reached. The results suggest that TCAA ingestion, TCAA loading in blood and urinary TCAA excretion are reliable measures for use as biomarkers in epidemiological studies.

Keywords: *Disinfection byproducts; biomarker; exposure; trichloroacetic acid*

Introduction

Possible health effects from chlorinated disinfection byproducts (DBPs) in drinking water have attracted research attention over the past 35 years because the use of drinking water is an essential, pervasive exposure in society whereby even a small increase in relative risk might pose a substantial population health risk (Hrudey 2009). Some epidemiological studies have reported weak associations between exposure to various DBPs and occurrence of adverse reproductive and developmental effects (Tardiff et al. 2006). Epidemiological research results remain inconclusive because of the limitations of exposure assessment. Epidemiologists have identified this problem and the need for a valid biomarker of exposure to improve the validity of population studies (Swan & Waller 1998, Savitz et al. 2006).

A candidate exposure biomarker should be the most representative measure of a particular component in the continuum of the exposure event. More than 600 DBPs have been reported in the literature (Richardson et al. 2007). The two most abundant, regulated classes of chlorinated DBPs are trihalomethanes (THMs) and haloacetic acids (HAAs) that account on average for

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66% and 27%, respectively, of the measured chlorinated DBP compounds in drinking water (USEPA 2002). Other classes of DBPs including emerging unregulated DBPs such as 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) or nitrosamines occur at substantially lower levels. Either THMs or HAAs have been used as surrogates of DBP exposure. THMs are volatile compounds and are rapidly absorbed following ingestion, inhalation and dermal contact. THMs are not suitable for use as a biomarker of exposure because of their transient presence in the body.

The two most prevalent species of HAAs are trichloroacetic acid (TCAA) and dichloroacetic acid (DCAA), which account on average for 33% and 40% of HAAs in drinking water, respectively (USEPA 2002). DCAA is extensively metabolized in the body with a short half-life of elimination (2–60 min) (Larson & Bull 1992, Schultz et al. 1999) making it an unsuitable candidate biomarker of exposure. TCAA offers greater persistence in the body than the THMs and DCAA. The TCAA elimination half-life ranges from 1.2 to 6 days in humans (Paykoc & Powell 1945, Muller et al. 1974, Froese et al. 2002, 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 (Kim et al. 1999, Weisel et al. 1999, Froese et al. 2002). Our validity analysis provides evidence of a significant correlation between TCAA ingestion and urinary excretion (r values: 0.77–0.83, $p < 0.001$), and between TCAA ingestion and loading in blood ($r = 0.80$, $p < 0.001$) (Zhang et al. 2009). These findings indicate that TCAA may be a potentially useful biomarker of exposure to chlorinated drinking water DBPs.

Although the association between TCAA ingestion and urinary excretion is evident, the effect of random error for each measurement needs to be assessed by reliability analysis to provide confidence in the accuracy for such association (McDowell 2006). The random measurement error can result from different sources of variability which consist of interindividual variation such as source, external exposure and metabolism among individuals, intraindividual variation such as sampling, laboratory errors, exposure unique to individual samples or biological factors, and others such as diet, lifestyle or personal characteristics. Such variability reveals the potential for misclassification of exposure to reduce the effective sample size and statistical power of a study, inaccurately representing the typical characteristics of an individual, and contributing to background noise in biological monitoring and epidemiological studies (Mayeux 2004).

The characterization of interindividual and intraindividual variability and assessment of the reliability before conducting an epidemiological study are important for understanding the utility of a biomarker as a means to reduce exposure misclassification.

The aim of our reliability analysis is to reveal the reproducibility of each TCAA measurement used in biological monitoring. In our two previous human exposure pilot trials, intraindividual and interindividual variability of TCAA ingestion and excretion were observed in small cohorts (Froese et al. 2002, Bader et al. 2004). The current reliability study evaluated the intraindividual and interindividual variability in a substantially larger cohort with experimental management of exposure in order to establish the reliability of various measurements and examine whether TCAA levels in the body are sufficiently consistent within individuals over time to allow TCAA to serve as a biomarker. A group of healthy women of reproductive age was selected for the study because of various observed epidemiological associations between DBP exposure and adverse reproductive and developmental effects (Tardiff et al. 2006).

To perform reliability analysis for continuous data, intraclass correlation coefficients (ICC) between individuals were calculated for multiple measurements of TCAA levels in various samples obtained at consistent times for each individual. The ICC is an analysis of variance (ANOVA) and estimates the expected correlation between two randomly chosen measures, that is, reproducibility (Shrout & Fleiss 1979). Cronbach's coefficient α was calculated to estimate the internal consistency obtained by combining a given number of separate measures into a single composite, which is the proportion of the observed variance due to true differences among individuals in the sample (Cronbach 1951). Information about some possible sources of variation such as physical activities, solvent use and bathing/showering/dishwashing time was collected and examined for each case. These were not statistically analysed for all factors because of the small number or narrow range of values reported for some factors.

Materials and methods

Recruitment procedures, sample collection, and laboratory methodology are summarized below. The 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 (of reproductive age, non-pregnant) volunteers were

recruited. All volunteers lived in the City of Edmonton during the period of the study. 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, a city with a population above100 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 a range of TCAA concentrations for the exposure experiment. Fifty-two participants were randomly stratified into five subgroups. Six participants in group 1 were a control group receiving TCAA-free bottled water. Forty-six participants in groups 2–5 received TCAA-containing water at concentrations of 12.5%, 25%, 50% and 100% of City A tap water, respectively, in TCAA-free bottled water. 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 the supplied cold tap water every day for a 15-day period.

Sample collection

Each 1 l Nalgene bottle was labelled with each participant's identifier, date of ingestion and number order of the bottle. Each bottle was filled with tap water with designated TCAA 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 water bottles were stored in the participants' refrigerators. 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 our laboratory for TCAA analysis twice per week.

A urine collection kit with instructions for urine collection was prepared for each participant. The urine samples were collected on the first day before supplied tap water consumption (designated exposure day 0) and on the 2nd, 8th, 13th, 14th, 15th and 16th day after supplied tap water consumption (designated exposure days 1, 7, 12, 13, 14 and 15). The urine collection kit was delivered to each participant 1 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 provided 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 urine 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 on exposure days 0, 7, 13 and 14. One whole blood sample was collected on each exposure day in a private medical laboratory (DKML) in Edmonton 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. 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 microlitres 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 *tert*-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 fibre. The sample components were absorbed from the headspace by the PDMS fibre 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 fibre 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^{-1}. The column temperature programme was 40°C (0 min) to 70°C at 10°C min^{-1} holding for 4 min, and then to 205°C at 15°C min^{-1} holding for 3 min. The detector temperature was 260°C.

The estimated detection limit was 0.2 μg l^{-1} for TCAA in this study. A total of 1460 water, urine and blood samples and 108 blank samples were analysed. The laboratory QA/QC protocols to assess analytical performance called for duplicate analysis for each sample and triplicate analysis for one out of every eight water samples and one out of every five urine or blood samples. Quadruplicate analysis was performed randomly. TCAA recovery was assessed based on analysis of a fortified sample matrix. Recovery of TCAA in the water samples ranged from 70% to 126% except for one sample (out of nine) from City A (61%). Recovery of TCAA in the urine samples ranged from 77% to 108%. Recovery of TCAA in the blood samples ranged from 70% to 130% except for one sample (out of 76) with a low value (51%) and five samples with high values (137–149%).

Statistical analysis

The data from 46 out of 52 participants who were classified as exposure groups and provided urine samples in the last four consecutive exposure days and 25 participants in exposure groups who donated blood samples on two consecutive exposure days were included in the reliability analysis. The data from six participants in the control group were not included in the analysis because they consumed TCAA-free bottled water and did not experience repeated TCAA-exposure measures. Considering a half-life of 30 h to 6 days for TCAA in the human body, the data collected from urine samples at the exposure days 12, 13, 14 and 15 and blood samples at exposure days 13 and 14 were used for the analysis to allow measurements at a quasi-steady state (i.e. not substantially varying over time).

For a variability analysis of the laboratory assay, the coefficient of variation (CV%) was calculated as:

$$\mathrm{CV}\,\% = \frac{SD}{Mean} \times 100$$

where SD is the standard deviation and mean is an average of TCAA measures in multiple samples on the same day for an individual. CVs up to 20% are acceptable for laboratory analysis of biomarkers in epidemiological studies (Tworoger & Hankinson 2006).

Reproducibility of measurements of TCAA ingestion and excretion between individuals in different daily samples was evaluated by the ICC. The ICC is expressed as:

$$\mathrm{ICC} = \frac{CV_b}{CV_b + CV_w}$$

where CV_b is the coefficient of variation between individuals and CV_w is the coefficient of variation within individuals. The possible values of ICC range from 0 (no reproducibility) to 1.0 (perfect reproducibility). The larger the ICC, the more reliable the measurement. The adequacy of reliability of coefficients should be interpreted in terms of the purpose of the measurement. The measurements used for clinical studies require higher reliability than those used in population studies (McDowell 2006). Lower standards of reliability of measurement can be tolerated in a study with a large sample size compared with one having a small sample size. Recommended values vary from statistic to statistic. For example, an ICC of 0.4 was considered for sufficient reproducibility in a biomarker to justify its use in an epidemiological study and an ICC of 0.75 or greater is considered as excellent reproducibility (Rosner 2006). Some authors (Adibi et al. 2008, Teitelbaum et al. 2008) adopted the cut-offs of the ICC proposed by Rosner in reliability analysis for their biomarkers of exposure studies. To be consistent with these studies, we used a guideline proposed by Rosner (2006) for interpreting ICC values in our analysis: < 0.4 is poor, 0.4–0.74 is good and ≥0.75 is excellent.

In order to determine the internal consistency of the repeated measures, Cronbach's coefficient α is calculated as:

$$\alpha = \frac{ICC \cdot n}{(1 + [n-1] \cdot ICC)}$$

where ICC is the average correlation between any two measures and *n* is the number of measures pooled. The α values were calculated manually based on the above equation. The values range from 0 to 1.0. The larger the α, the more reliable the measurement. An α of 0.8 was considered to be consistent with adequate reliability (McDowell 2006), the level we adopted to indicate an acceptable level of reliability.

ICCs and Cronbach's coefficient α were calculated by using the random-effects one-way ANOVA model (SPSS15.0 software package).

Box plots were used to display a relationship between the temporal measured exposure and amount of urinary excretion. A box plot is a summary plot that plots graph data as a box representing statistical values.

The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. The dots outside the box indicate outlying values.

Results and discussion

Sample collection and laboratory analysis

Laboratory analytical variation in replicate urine samples is presented in Table 1. ICCs for duplicate, triplicate and quadruplicate samples ranged from 0.952 to 0.977 ($p < 0.001$).

CVs less than 20% were found in 91% of urine samples. ICCs for laboratory CVs under 20% ranged from 0.955 to 0.998 ($p < 0.001$). A CV is used to assess specific sources of variation such as intra- and interassay variation. The ICC is used to measure the reproducibility of the assay. The results indicated that a laboratory CV of 20% was acceptable in our study because of the substantial interindividual variability (ICC > 0.955).

Technical variability is a function of instrumentation and reagents as well as the possibility of human error in sampling, labelling, preparation and analysis (Stites 1991). In our study, the procedures for sample collection and laboratory analysis were carefully selected, including the types of samples, timing of collection, amounts of samples, duration of sample storage, types of laboratory analysis, processing of the sample and QA/QC for laboratory analysis. Assay variability from using the analytical method developed by Wu et al. (2002) in our laboratory was assessed in a pilot study before starting the primary study. In the pilot study, variation for analysing TCAA in tap water and biological samples was also evaluated for using different types of containers for samples (glass or plastic), different storage temperature (room or 4°C), and time of delivery (2 h, 4 h and 6 h). Consistency of these procedures reduced laboratory variability.

The highest CVs were found in four urine samples and the ICC was 0.819 with 95% confidence interval (CI) 0.002–0.987. This larger variation resulted from the measured urinary TCAA concentrations in two samples with CVs of 47% and 61%, respectively. These measured values were close to the detection limit of 0.2 μg TCAA l^{-1}. Overall, our findings suggested that laboratory variability was not an important contributor to total variability in our study.

Variation in tap water

TCAA levels varied substantially in City A water ranging from 45 to 130 μg l^{-1} over 1 year (Figure 1). The variations may result from water quality factors such as total organic carbon, bromide, pH, temperature, ammonia, carbonate alkalinity and treatment conditions such as disinfectant dose, contact time and removal of natural organic matter (Liang & Singer 2003). In City A, the TCAA levels in tap water were higher during the cold water months. In two Canadian National Surveys (Health Canada 1995, 1996), the mean TCAA levels increased from the plant to the mid-point of the distribution system but were similar in winter and summer within the distribution system. Seasonal variation in tap water could add measurement errors resulting from only collecting one or a few samples within a short time. The results suggested that frequently collecting tap water samples across different seasons to measure TCAA concentrations in various types of samples would characterize the variability resulting from external exposures in individuals. In our study, we measured TCAA levels regularly for the tap water from City A, so this source of variability was known.

Table 1. Laboratory replicate variation in urine samples.

	Sample Size	Percentage of total sample	ICC	95% CI Lower	95% CI Upper	*p*-Value
Replicates in analysis						
Duplicate	274	76	0.977	0.971	0.982	< 0.001
Triplicate	61	17	0.952	0.928	0.969	< 0.001
Quadruplicate	24	7	0.956	0.921	0.979	< 0.001
Calculated CV range for replicates						
< 5%	116	32	0.998	0.997	0.999	< 0.001
6–10%	69	19	0.985	0.977	0.991	< 0.001
11–15%	66	18	0.969	0.950	0.981	< 0.001
16–20%	77	21	0.955	0.930	0.971	< 0.001
21–30%	28	8	0.865	0.730	0.935	< 0.001
> 30%	4	1	0.819	0.002	0.987	0.025

ICC, intraclass correlation coefficient; CI, confidence interval; CV, coefficient of variation.

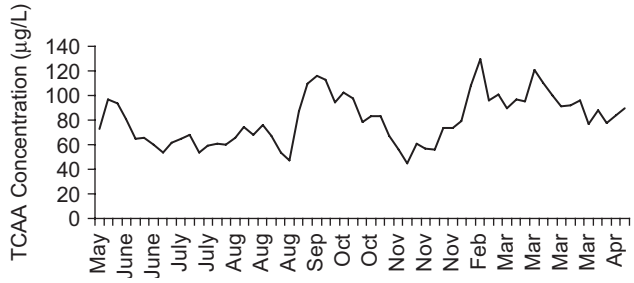

Figure 1. Seasonal variation of trichloroacetic acid (TCAA) concentrations in tap water from City A (each interval on *x*-axis is one measure per week).

Ingestion

The ICCs of TCAA ingestion in all exposure groups are summarized in Table 2. The ICCs of three TCAA ingestion

Table 2. Variation in repeated trichloroacetic acid (TCAA) ingestion measures.

Measurement	Sample size	No. of repeated measures[a]	ICC	95% CI Lower	95% CI Upper	*p*-Value
TCAA concentration in tap water ($\mu g\ l^{-1}$)	46	2	0.967	0.952	0.979	<0.001
Volume of tap water intake (l daily)	46	2	0.690	0.598	0.783	<0.001
Amount of TCAA ingestion (μg daily)	46	2	0.893	0.850	0.931	<0.001

[a]Repeated measures in any 2 days within the 15 days of tap water consumption.
ICC, intraclass correlation coefficient; CI, confidence interval.

measures (concentration in tap water, volume of tap water intake and amount of TCAA ingestion) within any 2 days of the 15-day water consumption among all individuals ranged from 0.690 to 0.967 ($p<0.001$). Reproducibility using TCAA concentration in tap water and amount of TCAA ingestion in our study was excellent (ICC > 0.75) for our study.

The results indicated that 97% of the variance of measurement of TCAA concentration in tap water and 89% of the variation of amount of TCAA ingestion were due to interindividual variability. These two measurements were stable in our study. Although weekly variation of measured TCAA was observed, it did not significantly affect reproducibility of measurement of TCAA concentration in tap water. Measured concentrations of TCAA in tap water supplied to participants were relatively consistent because tap water was shipped every week and participants consumed supplied water with two or three different concentrations during the 15 days. A high ICC of measurement of TCAA concentration in tap water reflects this controlled factor in our experimental design. In a field study, interindividual reliability of measured tap water TCAA concentrations will not be as high as found in our study because TCAA concentrations in water treatment systems may vary greatly depending on the nature of water at different sites and in different seasons (Health Canada 1996).

In our study, reproducibility by measuring volume of tap water intake was lower compared with the other two measures, but it was still a good measure (ICC = 0.69). Thirty-one per cent variation for the volume of tap water consumption could be attributed to intraindividual variability. Variation in the water intake rate has been observed in healthy adults in other studies. Sources of variation of water intake rates often arises from differences in climate, physical activities, lifestyle and culture, but little of the variance is explained by anthropometric factors such as age, weight, height and body mass index. In one study, water intake ranges observed in men were 1.4–7.7 l per day and in women were 1.2–4.6 l per day (Raman et al. 2004). Only 4–8% of the variability of water intake was explained by physiological variables such as age, weight and height among individuals. Individual behaviour was likely to contribute to the large interindividual variability. Significant ethnic variation in water intake was observed (Williams et al. 2001). Water turnover was found not to be related to energy intake and physical activities in women compared with men (Westerterp et. al. 2005).

Loading in blood

The ICC of blood TCAA concentration measurement was 0.895 in this study (Table 3). The result indicates that the intraindividual variability only accounted for 10% when using the blood measurement. Variation of metabolic rate of TCAA in the human body could be a source of interindividual variability. The blood TCAA concentration was related to current TCAA exposure. TCAA is readily absorbed in humans (Muller et al. 1974). After absorption, TCAA is highly bound to plasma proteins in blood (Paykoc & Powell 1945, Sellers & Koch-Weser 1971). The bound fraction is relatively constant, with a mean of 82% over a 3.7-order of increase in TCAA concentrations (Lumpkin et al. 2003). For continued exposure to TCAA in drinking water over 2 weeks, the blood TCAA reflects the equilibrium between daily intake and excretion.

The bound TCAA cannot be filtered through the kidney. TCAA in serum or urine reflects the free TCAA that can be eliminated from the body. TCAA can also conjugate as glucuronides (Fisher et al. 1991). Free TCAA in blood is rapidly eliminated by glucuronidation (Nomiyama & Nomiyama 1979). Theoretically, the analysis of TCAA in blood is more reliable because of its sufficiently long elimination half-life and less variability within individuals compared with the measurement of urinary TCAA. The blood level of TCAA is an important exposure index with a high specificity. However, use of blood samples for a larger cohort within an epidemiological study will be logistically challenging because of the invasive sampling required.

Urinary excretion

The ICCs of measurements of TCAA excretion in all exposure groups are summarized in Table 3. The ICCs of three TCAA excretion measures within any 2 days of urine collection within the last 4 days of the experimental exposure among all individuals ranged from 0.729 to 0.766. Reproducibility was good to excellent for these three urinary TCAA measurements.

Interindividual variability is influenced by the sample size and study power. The ICCs by using urinary TCAA

Table 3. Variation in repeated blood and urinary trichloroacetic acid (TCAA) excretion measures.

Measurement	Sample size	No. of repeated measures[a]	ICC	95% CI Lower	95% CI Upper	*p*-Value
Based on types of measurement						
Blood TCAA concentration ($\mu g\,l^{-1}$)	25	2	0.895	0.779	0.952	<0.001
Urinary TCAA concentration ($\mu g\,l^{-1}$)	46	2	0.745	0.639	0.834	<0.001
Cr-adj. concentration ($\mu g\,g^{-1}$ Cr)	46	2	0.729	0.615	0.821	<0.001
Amount of TCAA excretion (μg daily)	46	2	0.766	0.666	0.849	<0.001
Based on exposure groups for urinary TCAA concentration ($\mu g\,l^{-1}$)						
12.5%	6	2	0.674	0.303	0.935	0.002
25%	9	2	0.520	0.195	0.833	<0.001
50%	14	2	0.348	0.092	0.658	0.002
100%	17	2	0.753	0.572	0.887	<0.001

[a]Repeated measures in any 2 days at exposure day 12, 13, 14 and 15.
ICC, intraclass correlation coefficient; CI, confidence interval.

concentration measurement for each exposure group are presented in Table 3. The ICCs ranged from 0.35 to 0.75. The ICCs were lower in each subexposure group than all exposure groups combined. This effect was due to small sample size in each exposure group. This illustrated that restricting the range of exposure lowered the reliability of individual measurements. The finding suggests that the effect of interindividual variability could be reduced by increasing the sample size.

Reproducibility was higher when using TCAA ingestion and blood TCAA measurements than TCAA excretion measurements. This may be due to different source errors in measurements of TCAA ingestion, loading in blood and excretion.

Fluctuations of biological factors can affect considerably the reliability of using urinary TCAA excretion measurements. The rates of absorption, metabolism and excretion of TCAA vary from individual to individual and are influenced by age, sex and physical conditions. The effect of metabolism is different among individuals. These differences will contribute to different levels of TCAA in body fluids between individuals for the same exposure.

In our study, about 25% variation for urinary TCAA concentrations could be attributed to intraindividual variability (ICC = 0.75). Because urine samples were used for monitoring, the most influential factors are time of collection and urinary excretion volume (output) (Rosenberg et al. 1989). The variability of urine output can result from variation of fluid intake or loss and temperature and humidity in the environment. The standardization of sample collection time will reduce the effects of diurnal variation and the effects of preceding meals (Aitio 2002). The FMU is a traditional practice for this purpose. The individual was asked to void urine prior to going to bed at night and the FMU sample is collected immediately after getting up in the morning. This provides a more constant interval between an exposure and sample collection as well as increasing the concentration of target compounds in urine.

Urinary TCAA concentrations are affected by the rate of urine production. The traditional practice for reducing this variation is to correct by measuring creatinine excretion. The determination of urinary creatinine is recommended to normalize overdiluted or overconcentrated urine samples. Creatinine is excreted by glomerular filtration at a relatively constant rate of 1.0–1.6 g daily over time (Rosenberg et al. 1989). Urinary creatinine concentrations can fluctuate widely throughout the day. The factors affecting the excretion of creatinine in urine include gender, age, the muscularity of the individual, physical activity, urine flow, time of day, diet, pregnancy and health conditions. The creatinine concentration is inversely related to fluid intake. Analysis performed on very dilute ($<0.3\,g\,l^{-1}$) or concentrated ($>3\,g\,l^{-1}$) urine samples must be interpreted with caution (Rosenberg et al. 1989). When extremes of creatinine variability are observed, creatinine correction is not valid. In our study, the ICC of Cr-adj. TCAA concentration (0.73) was similar to that of the unadjusted urinary TCAA concentration (0.75). The correction of urinary TCAA excretion for urinary creatinine did not significantly improve the results of reproducibility.

Interindividual variability of urinary TCAA excretion can be affected by kinetic process among individuals. Kinetic variation was not assessed in our study. Information on biological variation can be viewed from the literature. TCAA is metabolized into CO_2, dichloroacetic acid and GOG (nonchlorinated acids glyoxylate, oxalate and glycolate) and excreted in urine, faeces and bile (Green & Prout 1985, Larson & Bull 1992). In human experiments, 23–50% of the administrated doses via oral were recovered in urine (Muller et al. 1974; Humbert et al. 1994).

Other sources of variation

Some data on sources of variability related to exposure to TCAA in drinking water were collected in our study. These data were used to explore possible sources of variation rather than for analysing confounding factors as done in a validity study.

Interindividual variability of TCAA ingestion variation can arise from other exposures such as the source of water consumed (e.g. tap, filtered or bottled, and used for preparing food), non-ingestion sources such as

showering, bathing, swimming or dishwashing in TCAA-containing water, exposure to chlorinated dry-cleaning solvents or using chlorinated bleach and other solvents.

In our study, ingestion source of tap water consumption was well controlled. A questionnaire was administered to each individual to evaluate non-ingestion TCAA sources. Out of 46 participants in all exposure groups, 40 participants reported time for bathing, showering, dishwashing and swimming by using water in the City of Edmonton during the 15-day period of study. For the last 4 days of urinary collection, mean time for bathing, showering and dishwashing was 17 min per day (Table 4). No correlation was observed between urinary TCAA concentrations and length of time for bathing, showering or dishwashing ($p > 0.05$). Two major possible reasons may be considered for finding no relationship. First, the sample size was restricted to four measures per individual. Second, HAAs (including TCAA) are semi-volatile compounds that will not be inhaled nor easily penetrated through skin. The permeability of the skin to HAAs is very low so the daily exposure dose resulting from the above activities was insignificant for HAAs (Xu et al. 2002, Xu & Weisel 2003). For example, the proportion of absorbed doses of TCAA from daily bathing via dermal contact was only about 0.005–0.5% of the daily ingestion doses of TCAA (Cleek & Bunge 1993, Xu et al. 2002). Additional sources of exposure to TCAA via bathing, showering and dishwashing were unlikely to contribute significantly to interindividual variability of urinary TCAA excretion in our study.

Time on daily physical activities was recorded by participants during the study period. In the last 4 days of urinary collection, 39 out of 46 participants reported physical activities like jogging, walking or biking. Mean time of physical activities was 36 min per day (Table 4). No correlation between physical activities and volume of tap water consumption or physical activities and urinary TCAA concentrations was observed ($p > 0.05$). Physical activities may affect the volume of water consumption. In our study, the volume of tap water consumption was correlated to urinary TCAA excretion (r: 0.13–0.27, $p < 0.01$), but not to a convincing extent (Zhang et al. 2009). Physical activities were unlikely to contribute significantly to interindividual variability of urinary TCAA excretion in our study.

TCAA was detected in swimming pool water (WHO 2004). In our study, four participants swam during the study period. One participant swam about 2–3 h per day during the last 4 exposure days, but the urinary TCAA levels declined for this participant. In another case, the urinary TCAA levels increased slightly after 1 week of the study. In two cases, the urinary TCAA levels remained stable. Thus, no relationship between swimming and urinary TCAA levels emerged ($p > 0.05$) for a very small sample size. Additional sources of exposure to TCAA via swimming pool water were unlikely to contribute significantly to interindividual variability in our study.

Some solvents such as trichloroethylene (TCE), tetrachloroethylene (PERC), trichloroethanol, trichloroethane (TRI) and tetrachloroethane can be metabolized to TCAA (WHO 2004). These solvents are used for metal degreasing and as cold cleaning agents, dry-cleaning solvents, printing, printing ink and some consumer products such as typewriter correction fluid, paint remover, adhesive, stain remover and rug-cleaning fluid. The use of household cleaning solutions and products containing solvents was recorded by only three participants in our study. The recorded products did not specifically identify solvents like TCE, PERC and TRI. Specific sources of exposure to TCAA via using above solvents were not likely significant contributors to inter-individual variability in our study.

Exposure to several compounds may compete for the same biotransformation sites or be metabolized to TCAA. Chloral hydrate (CH) is rapidly metabolized in the liver and other tissues to trichloroethanol, trichloroethanol-glucuronide and TCAA. An average of 35% (a range of 5–47%) of the initial dose of CH was converted to TCAA (Marshall & Owens 1954, Sellers et al. 1972; Humbert et al. 1994). The half-life of TCAA after ingestion of chloral hydrate ranges from 3 to 5 days (Breimer et al. 1974; Muller et al. 1974). In our study, CH concentrations were

Table 4. Possible sources of variation.

Measurement	Sample size	Mean (SD)	Range[a]	95% CI Lower	95% CI Upper	Pearson correlation analysis[b] (p-value)
Bathing, showering, dishwashing (min daily)	40	17(18)	none–145	14	19	Urinary TCAA ($p > 0.05$)
Physical activity (min daily)	39	36(57)	none– 360	27	43	Urinary TCAA ($p > 0.05$)Volume of tap water consumption ($p > 0.05$)
Swimming	4					Urinary TCAA($p > 0.05$)[c]
Use of solvents	3					No quantitative analysis
Ingestion of chloral hydrate from tap water	46					No quantitative analysis

[a]'none', no physical activities recorded in one of the last 4 days of urinary collection; [b]four measures in the last 4 days of urinary collection for urinary trichloroacetic acid (TCAA) concentrations, 15 measures during the 15 days of exposure for volume of tap water consumption; [c]quantitative analysis was conducted in one participant who swam within the last 4 days of urinary excretion.

measured in tap water. The ratio of CH to TCAA was 1:20. The blood levels of CH were not measured in our study. A small increase in urinary TCAA excretion is expected as a result of ingestion of CH in tap water, but quantitative data for this possible increase was not obtained.

Multiple samples

After the 7th day of consumption of TCAA containing tap water, a quasi-steady-state TCAA level in blood was reached (Figure 2). Cronbach's α estimate for blood TCAA concentration was 0.90. This means that a single-day blood sampling is sufficient to achieve good reliability in a study.

After the 7th day of consumption of TCAA-containing tap water, a quasi-steady-state TCAA level in urine was reached (Figure 3). The data from the last 4 days of exposure were used to estimate Cronbach's α. Cronbach's α estimates for four different ingestion and excretion measurements in different pooled sampling days are illustrated in Figure 4. The Cronbach's α estimates from 1 day to 4 days of sampling ranged from 0.69 to 0.90 for volume of tap water consumption, from 0.75 to 0.92 for urinary TCAA concentration, from 0.73 to 0.92 for Cr-adj. TCAA concentration and 0.77 to 0.93 for the amount of TCAA excretion. The Cronbach's α estimates were greater than 0.80 for a composite measure including 2–4 days of sampling. The results indicated an increased reliability with repeated measures. However, multiple days of sampling are unlikely to be cost-effective for most purposes. Two-days sampling is sufficient for measuring volume of tap water consumption and TCAA in urine in an individual if a quasi-steady-state TCAA level in the body is reached and the exposure range across individuals is large.

In conclusion, in a controlled human exposure experiment with urinary blood TCAA as a biomarker of exposure, interindividual reliability was excellent (ICC > 0.75) using TCAA ingestion and urine/blood measurements. Intraindividual variability contributed to some background noise (< 25%). Variation of laboratory analysis contributed only slightly to intraindividual variability (ICC > 0.95 in replicates). No correlation to urinary TCAA excretion of non-ingestion TCAA sources (bathing, showering, dishwashing, swimming and solvent contact) and physical activities related were observed. These possible sources were unlikely to contribute significantly to interindividual variability.

One-day blood sampling or 2-day urine sampling strategies are sufficient to achieve reliability in a study if a quasi-steady-state TCAA level in the body is reached and the exposure range among participants is large. The finding of a high interindividual reliability suggested

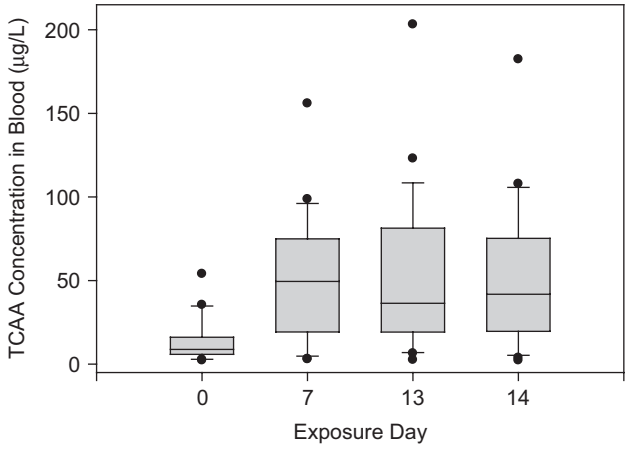

Figure 2. Trichloroacetic acid (TCAA) concentrations in blood on different exposure days.

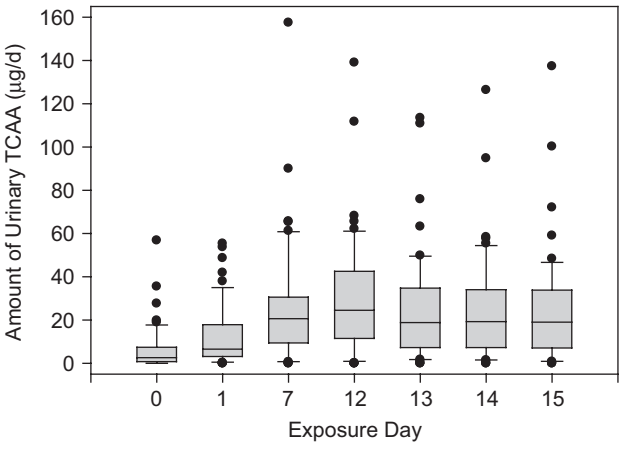

Figure 3. Amount of urinary trichloroacetic acid (TCAA) excretion in urine on different exposure days.

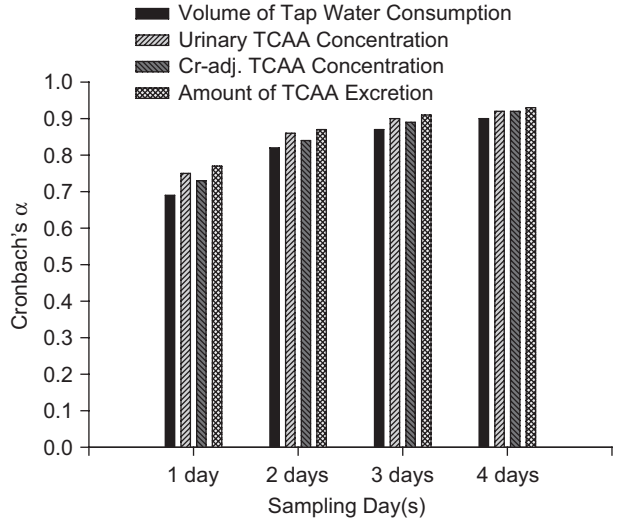

Figure 4. Cronbach's α for different sampling days (1 day = any day of sampling day, 2 days = combined any two of sampling days, 3 days = combined any three days, and 4 days = combined all last four sampling days). TCAA, trichloroacetic acid.

that the measurements of TCAA ingestion, TCAA loading in blood and urinary TCAA excretion are reliable for use in epidemiological studies.

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