University of Alberta

Validating Urinary Trichloroacetic Acid as a Biomarker of Exposure for Disinfection By-Products in Drinking Water

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

Medical Sciences-Public Health Sciences

Edmonton, Alberta
Spring, 2006

Your file Votre référence
ISBN: 0-494-14071-2
Our file Notre référence
ISBN: 0-494-14071-2

ABSTRACT

Disinfection by-products (DBPs) in drinking water represent a pervasive public health exposure issue and a difficult challenge for epidemiology. Validation of a biomarker of exposure is essential for progress in resolving causation aspects of this issue. Recent studies from our research group in Adelaide, South Australia and Edmonton, Alberta confirmed trichloroacetic acid (TCAA) to be the most promising candidate as a biomarker of exposure to DBPs. The objectives of this study were to validate urinary TCAA excretion as a biomarker of exposure to DBPs in a larger cohort, examine intraindividual and interindividual variability of TCAA excretion, and explore the feasibility of using TCAA as a biomarker in the field study. A total of 52 healthy women participated in the study. Participants consumed supplied tap water for 15 days and provided urine and blood samples for TCAA measurements. The findings revealed that (1) the utility of TCAA as a surrogate of other DBP compounds depended on the nature of water treatment systems and geographical locations; (2) background levels of TCAA in urine and blood were detected; (3) TCAA levels in blood and urine increased with increased amounts of TCAA ingested; (4) there was no correlation between dichloroacetic acid (DCAA) ingestion and excretion; (5) laboratory variation was not a major contributor to overall variation; (6) interindividual reliability was relatively high; (7) intraindividual variability contributed to background noise to a certain extent (ICC>0.70); (8) the correlations between measurements of TCAA concentration in water/amount of TCAA ingestion and urinary TCAA excretion were modestly high (r: 0.55–0.77, $p<0.001$); (9) the correlations between measurements of

TCAA concentration in water/amount of TCAA ingestion and blood TCAA concentration were high (r: 0.77–0.82, $p<0.001$); (10) urinary TCAA measures or multiple days' urinary TCAA measures improved the prediction of TCAA ingestion through urinary TCAA excretion; (11) it is feasible to use TCAA as a biomarker of exposure in epidemiological studies and public health surveillance. In conclusion, TCAA can be a valid, reliable, and feasible biomarker of exposure for DBPs in drinking water.

ACKNOWLEDGEMENTS

In response to significant international concerns raised by epidemiological studies about chlorinated disinfection by-products (DBPs) causing adverse reproductive and developmental outcomes, Environmental Health Sciences (EHS) in the Department of Public Health Sciences at the University of Alberta, in collaboration with Health Surveillance of Alberta Health and Wellness, initiated a research project entitled "Validating Urinary Trichloroacetic Acid as a Biomarker of Exposure for Disinfection By-Products in Drinking Water" in 2002. The project was funded by Alberta Health and Wellness and by NSERC Strategic and Research Grants.

My supervisor and the supervisory and examining committee members provided direction and valuable advice during the entire study. The individuals from the various partners in this study are listed below. The EHS Laboratory in the Department of Public Health Sciences at the University of Alberta performed chemical analysis for selected DBP compounds in water and biological samples (Wu and Yan). The Provincial Public Health Laboratory for Microbiology provided a clean and cool room for storing tap and bottled water (Chue), tested water quality of all supplied water five days per week (Ashton), and assisted in storing blood samples for future studies (Pang). The Centre for Toxicology in Calgary performed trace water analysis in water samples (Chan and Dmitrovis). Dynacare Kasper Medical Laboratories (DKML) assisted in collecting blood samples and delivering all the samples to the Environmental Health Sciences Laboratory (Galbraith and Matheson). The City Water Treatment Center in City A in Canada delivered tap water from City A to Edmonton once per week (Fletcher and Kjartanson). The mailing center in Alberta Health and Wellness assisted in water delivery. The Information Management Branch of Alberta Health and Wellness provided a mailing list for recruitment of volunteers (Nath). The project work team carried out telephone and in-person interviews (Hu), received and stored water containers shipped from City A and from a bottled water company (Xing and Zhu), delivered water and picked up urine samples (Scobie), cleaned bottles and maintained records (Boyd), and communicated with participants (Hu). Dr. Karina A.M. Bodo provided her thesis which relates to DBPs in drinking water to me for reference. Katerina Carastathis and Gayle Simonson provided careful and thorough proofreading of my thesis. Dianne Sergy was responsible for the financial management of the study. The assistance of all volunteers is gratefully acknowledged. I would like to thank my son, Edmond Chen, for his love, understanding, and support.

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List of Abbreviations and Glossary

BCAN	bromochloroacetonitrile
BDCM	bromodichloromethane
CDBM	chlorodibromomethane
CE	capillary electrophoresis
CH	chloral hydrate (trichloroacetaldehyde)
Cr	creatinine
Cr-a	creatinine-adjusted
CV	coefficient of variation
CYP	cytochrome P450 enzymes
DBAA	dibromoacetic acid
DBAN	dibromoacetonitrile
DBCM	dibromochloromethane
DBPs	disinfection by-products
DCAA	dichloroacetic acid
DCAN	dichloroacetonitrile
DCPA	2,3-dichloropropionic acid
EHS	Environmental Health Sciences
FAIMS	high field asymmetric waveform ion mobility spectrometry
FMU	first morning urine
GOG	nonchlorinated acids glyoxylate, oxalate, and glycolate
HAAs	haloacetic acids
HANs	haloacetonitriles
HKs	haloketones
HPC	heterotrophic plate count
HPLC	high-performance liquid chromatography
ICC	intraclass correlation coefficient
LLME	liquid-liquid microextraction
LMP	last menstrual period
LOD	limits of detection
MBAA	monobromoacetic acid
MCAA	monochloroacetic acid
MDL	method detection limit
MS	mass spectrometry
MTBE	methyl *tert*-butyl ether
MTW	municipal tap water
QA/QC	quality assurance and quality control
RSD	relative standard deviation
SEM	standard error of measurement
SPME	solid-phase microextraction
PCR	polymerase chain reaction
PDMS	polydimethysiloxane
PERC	tetrachloroethylene
POU	point-of-use
TBM	tribromomethane (bromoform)

TCAA	trichloroacetic acid
TCAN	trichloroacetonitrile
TCE	trichloroethylene
TCM	trichloromethane (chloroform)
TCP	1,1,1-trichloropropanone
THAAs	total haloacetic acids
THMs	trihalomethanes
TRI	trichloroethane
TTHMs	total trihalomethanes

Analytical Sensitivity: The detection of the external exposure level by means of a biomarker.

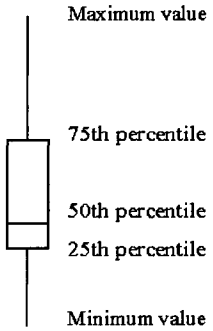
Analytical Specificity: The probability that a biomarker is an indicator of actual exposure to a specific xenobiotic in the environment.

Biomarker of Exposure: A xenobiotic and/or its metabolites, or an event in relation to the exposure of interest that can be qualitatively or quantitatively identified in a biological system or in samples such as biological fluids, tissues, and expired air.

Biological Monitoring: The direct estimation of internal doses of parent xenobiotics or their metabolites over the entire time in the human body, the quantification of macromolecular adducts, or the indirect estimation of the amounts of external doses of a xenobiotic to which humans are exposed.

Bias: A systematic error that is different in direction or size in one of the groups under study.

Boxplot: A graphical representation of dispersions and extreme scores. Represented in this graphic are minimum, maximum, and quartile scores in the form of a box with "whiskers." If any whisker is more than 1.5 times as long as the length of the box, values are marked as outliers "o" or extreme "*."



Case-Control Study in Epidemiology: Identification of subjects at the beginning of a period of follow-up based on the presence or absence of a particular disease or health condition, then identification of the exposure of interest, and comparison of a group of incident cases from a representative population. In a nested case-control study the controls may be selected by sampling the same base population which produced the cases.

Coefficient of Determination (r^2): The extent to which the variability in one measure may be accounted for through knowledge of the value of the other measure.

Coefficient of Variation: Ratio of standard deviation to mean.

Cronbach's α: The estimation of the reliability 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. The values range from 0 to 1.0. The larger the Cronbach's α, the more consistent the measurement.

Cross-Sectional Study in Epidemiology: Data collection on the average exposure and health status at one point in time or over a short period of time in a group of individuals.

Differential Exposure Error: Error where the outcome depends on the source of the error in the exposure.

Ecological Study in Epidemiology: Data collection on average exposure and health status of a population group in a geographically defined area.

Elimination Half-Time: The time required to eliminate half of the amount of the xenobiotic from the body.

Exposure Assessment: The estimation of exposure to physical, chemical, and biological factors in the general population.

First Morning Urine (FMU): Urine collection spanning the period from bedtime until the first urine void the following morning.

Intraclass Correlation Coefficient (ICC): Differences in mean estimates and the degree of correlation between the two sets of measures, which is the analysis of variance. The intraclass correlation coefficient also indicates the extent of interindividual variability relative to total variability. The total variability includes reproducibility, repeatability, and sampling variation. The values range from 0 to 1.0. The larger the intraclass correlation coefficient, the more reliable the measurement.

Intraindividual Reliability: The agreement of measurements within the same individual. Biological variation and various other sources such as sample collection, transportation, storing, and random errors inherent in laboratory analysis can affect intraindividual reliability.

Interindividual Reliability: The agreement of measurements between individuals. It is an indication of the differences between individuals but it is also affected by all the sources of error contributing to intraindividual reliability.

Internal Consistency: The measurement of the extent to which items in a test are homogeneous. It consists of the average inter-item correlation, the average item total correlation, and split-half reliability. Cronbach's α is used for continuous data and Kuder-Richardson for dichotomous data.

Internal Dose: The amount of the parent xenobiotic that is actually absorbed into the systemic circulation of the human body shortly following external exposure, during the preceding day, or from past exposure.

Inter-Observer Reliability: The measurement of the extent to which different observers provide consistent estimates of the same phenomenon.

Misclassification of Exposure: Any discrepancy between the true exposure and the measured exposure.

Nondifferential Exposure Error: Error where the outcome does not depend on the source of the error in the exposure.

Parallel-Form Reliability: Assessment of the consistency of the values of two tests or two forms of instruments constructed in the same way.

Polymerase Chain Reaction: a technique for amplifying DNA, making it easier to isolate, clone, and sequence.

Precursor: the organic compounds in untreated water that contribute to the formation of chlorinated disinfection by-products.

Product-Moment (Pearson) Correlation Coefficient: The extent to which the pairs of numbers of these two variables lie on a straight line. **r** is used for bivariate correlation (ranging from -1 to +1) and **R** is used for multivariate analysis (ranging from 0 to +1). In the literature, most authors used R for bivariate correlation. In this thesis, R is cited from the original papers for consistency.

Prospective Cohort Study in Epidemiology: The measurement of exposure status at the beginning of follow-up and during follow-up, and comparison of the occurrence or changes of all diseases or health conditions during the period of

follow-up under study between all exposed individuals during the period of exposure and non-exposed individuals.

Random Error: The distribution of measured exposure randomly around the true exposure.

Reliability: The reproducibility of a measurement.

Reliability Coefficient: The proportion of the total variance in the measurements resulting from the "true" differences between individuals, consisting of the error variance and the variance between individuals. Reliability coefficients include the product-moment (Pearson) correlation coefficient and the intraclass correlation coefficient.

Retrospective Cohort Study in Epidemiology: The measurement of exposure status at the beginning of the study, and comparison of the occurrence or changes of all diseases or health conditions at the initiation of the study between all exposed individuals during the period of exposure and an ad hoc group of non-exposed individuals or the general population.

Systematic Error: The distribution of measured exposure that is not random around the true exposure.

Test-Retest Reliability: Assessment of the consistency (stability) of a measure from the same individual (or sample) based on different occasions over time, such as interindividual and intraindividual.

Validation of a Biomarker of Exposure: Assessing the measurement of performance characteristics of a biomarker of exposure.

Validity: The agreement between the value of a measurement and its true value.

CHAPTER 1 INTRODUCTION

Chlorination is the main disinfection process for public drinking water systems. This process can result in the formation of a number of halogenated organic compounds, chlorine and/or bromine compounds that are referred to as disinfection by-products (DBPs) (Rook 1974). Natural organic matter (NOM) such as humic and fulvic materials serves as the organic precursor of DBPs and the bromide ion serves as an inorganic precursor of DBPs (Oliver and Lawrence 1979; Chang *et al.* 2001a, 2001b). The formation of DBPs is described as:

$$HOCl + NOM \Rightarrow DBPs$$

More than 250 DBPs can be identified or measured in treated water at water treatment plants, within the distribution system, and in tap water at homes and workplaces (Williams *et al.* 1997; Richardson 1998; Rodriguez *et al.* 2003; Sadiq and Rodriguez 2004). There is a wide range of variation in DBP levels in drinking water. The formation of DBPs is determined by the disinfection process, water source, pH, temperature, season, a level of chlorine residual, residence time in the distribution system, total organic carbon, bromide content, and abiotic and biotic degradation processes (Singer 1993; Hozalski *et al.* 2001; Espigares *et al.* 2003; Liang and Singer 2003; Richardson *et a.l* 2003; Serodes *et al.* 2003; Yang and Shang 2004; Symonski *et al.* 2004; Rodriguez *et al.* 2001, 2004).

The two most abundant classes of DBPs are trihalomethanes (THMs), which are volatile, and haloacetic acids (HAAs), which are semi-volatile. Other DBPs occur at low levels (Krasner *et al.* 1989). Chloroform and bromodichloromethane are the first and second principal THM species. Total THMs (TTHMs) are now routinely measured at water treatment facilities. Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) are the principal HAA species.

DBPs in drinking water represent a pervasive public health exposure issue and a difficult challenge for epidemiology. In the past decades, weak and inconclusive associations between exposure to DBPs and incidence of bladder cancer and adverse reproductive and developmental outcomes have been reported (Boorman *et al.* 1999; Bove *et al.* 2002; Nieuwenhuijsen *et al.* 2000; IPCS 2000; Graves *et al.* 2000; IARC 2004). Reproductive and developmental outcomes included increased or decreased fertility, low birth weight, preterm delivery, intrauterine growth retardation, congenital anomalies/birth defects, central nervous system anomalies, spontaneous abortion or miscarriage, stillbirth or fetal death, and neonatal death (Aschengrau *et al.* 1989, 1993; Aggazzotti *et al.* 2004; Bove *et al.* 1995, 1996; Cedergren *et al.* 2002; Deane *et al.* 1992; Dodds *et al.* 1999; Dodds *et al.* 2004; Dodds and King 2001; Fenster *et al.* 1992; Fenster *et al.* 2003; Gallagher *et al.* 1998; Graves *et al.* 2000; Hert-Piccitto *et al.* 1989; Jaakkola *et al.* 2001; Kallen and Robert 2000; Kanitz *et al.* 1996; King *et al.* 2000; King *et al.* 2005; Klotz and Pyrch 1999; Kram *et al.* 1992; Magnus *et al.* 1999; Savits *et al.* 1995; Savitz *et al.* 2005; Shaw *et al.* 1990, 2003; Toledano *et al.* 2005; Tuthill *et al.* 1982; Waller *et al.* 1998; Windham *et al.* 1992, 003; Wrensch *et al.* 1992; Wright *et al.* 2003;Yang *et al.* 2000a, 2000b, 2004). Causal mechanisms between exposure to DBPs and these outcomes and any confounding

factors were not identified. Thus, epidemiological evidence must be refined to define the appropriate risk management of this issue.

A major limitation of all of these studies has been the limited basis for exposure classification (Arbuckle *et al.* 2002). Indirect measurement of exposure was employed in these studies. In most of the better studies, THMs were selected as surrogates of DBP exposure. The THMs were rapidly absorbed following inhalation, dermal contact, and ingestion (minor route). They were mainly metabolized to carbon dioxide and/or carbon monoxide in the liver, and/or rapidly exhaled (Fry *et al.* 1972; NAS 1987). Two types of exposure indices have been used:

- *Exposure Index I*: estimation of exposure by using surrogates (water sources) or quantification of the concentrations of THMs in water treatment systems in a given population. This approach provides a basis for analysis of exposures in a given population rather than for each individual.

- *Exposure Index II*: estimation of exposure by using surrogates or quantification of the concentrations of THMs in water treatment systems, and integration of the information with time-activities and individuals' characteristics. This approach offers speed, economy, and convenience. Inaccurate estimates from this approach arise from conservative assumptions for modeling or inadequate monitoring.

These exposure measurement methods frequently lead to misclassification of exposure. Exposure misclassification is a major obstacle to obtaining accurate rates of association between adverse health outcomes and exposure, and is usually expected to cause attenuation in health risk estimates if it is random and non-differential. On the other hand, if exposure classification can be substantially improved and resulting risk estimates do not increase, there may be grounds to question whether the observed associations are causal.

In order to improve the current exposure assessment of epidemiological studies pertaining to DBP exposure in drinking water, development of a useful biomarker of exposure is critical. A candidate exposure biomarker is the most representative of a particular component in the continuum exposure event. This biomarker provides insight into qualitative and quantitative information about external exposure (Dor *et al.* 1999). Research exploring a useful biomarker of exposure such as urinary TCAA for DBP exposure was conducted in recent years (Kim *et al.* 1999; Weisel *et al.* 1999; Bader *et al.* 2004; Froese *et al.* 2002; Calafat *et al.* 2003).

The identity of TCAA is described in Appendix I. TCAA was shown to be readily absorbed into the blood following ingestion of water (Muller *et al.* 1974; Curry *et al.* 1991; Larson and Bull 1992) and bound to plasma proteins (Paykoc and Powell 1945; Marshall and Owens 1954; Sellers and Koch-Weser 1971; Muller *et al.* 1972; Monster *et al.* 1976). The protein-bound TCAA cannot be filtered through the kidney. TCAA can

also bind with conjugates such as glucuronides (Fisher *et al.* 1991). TCAA was metabolized into CO_2, DCAA, and GOG (nonchlorinated acids glyoxylate, oxalate, and glycolate) (Green and Prout 1985; Larson and Bull 1992). TCAA can be excreted in urine, feces, and bile (Green and Prout 1985; Larson and Bull 1992; Schultz *et al.* 1999). The half-life of urinary elimination after direct TCAA ingestion ranged from 30 hours to 6 days in humans (Paykoc and Powell 1945; Muller *et al.* 1974; Monster *et al.* 1976 and 1979; Humbert *et al.* 1994; Fisher *et al.* 1998; Froese *et al.* 2002; Bader *et al.* 2004).

TCAA was measured in urine samples in the general population and demonstrated an exposure-biomarker relationship between ingestion of TCAA-containing water and urinary TCAA excretion (Kim *et al.* 1999; Weisel *et al.* 1999; Froese *et al.* 2002; Calafat *et al.* 2003; Bader *et al.* 2004). TCAA was identified as a promising biomarker of exposure to DBPs in drinking water in two pilot studies (Bader *et al.* 2004; Froese *et al.* 2002). These findings, combined with knowledge of toxicokinetics and its sufficiently longer elimination half-life, suggest that TCAA may be a potentially useful biomarker for measuring DBP exposure in drinking water.

This study is designed to validate urinary TCAA as a potential biomarker of exposure for exposure to DBPs in drinking water in a large cohort. The hypotheses for this study are

1) cumulative urinary TCAA excretion is directly proportional to drinking water ingestion;
2) measured urinary TCAA predicts TCAA ingestion and exposure to a range of other DBPs in drinking water; and
3) urinary TCAA levels differ among individuals depending on external exposure dose, variation in volumes of water consumption, exposure to other sources, and other factors yet to be identified.

The objectives of the study include:

1) validating urinary TCAA as a useful biomarker of exposure,
2) assessing intraindividual and interindividual variability,
3) evaluating the correlation between TCAA ingestion and excretion,
4) establishing background TCAA levels in biological samples in a given study population,
5) identifying potential confounding factors, and
6) examining the feasibility by using TCAA biomarker under field conditions.

A conceptual framework of validation processes is illustrated in Figure 1-1. Validation processes include an understanding of the purpose of study, biological relevance, toxicokinetics, temporal and spatial variability, persistence, laboratory methodology and types of specimens, determination of background level, evaluation of reliability, validity of relevant statistical analysis, selection of an appropriate biomarker, and distinguishing confounder factors and their effect on the marker.

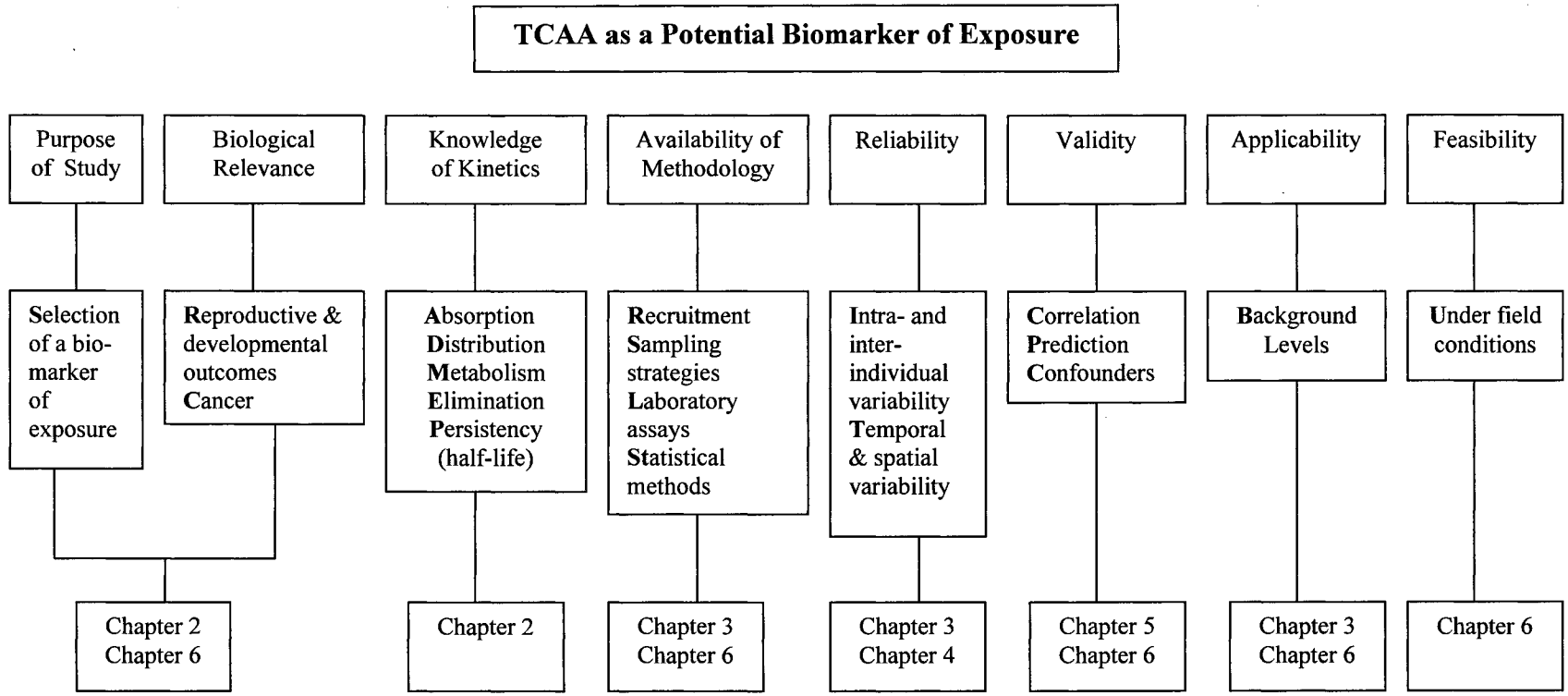


Figure 1-1 Conceptual Framework of Validation Processes for a Biomarker of Exposure

Chapter 2 offers a review of the characteristics of DBPs in drinking water and of the correlations between THMs and HAAs. Exposure assessment indices from reproductive and developmental outcome studied related to DBP exposure are grouped into two types. Exposure measurement error and its effects are discussed. An overview of the biomarker of exposure and its strengths and limitations for application to epidemiological studies is presented. The processes of validation of a biomarker of exposure using a case of TCAA are discussed. The information and knowledge about biomarkers of exposure and characteristics of TCAA in drinking water and biological systems derived from this review have provided a basis for the experimental cohort design, sample collection, laboratory analysis, data analysis, and data interpretation in this validation study.

In Chapter 3, the details of study design, recruitment process, sample collection, laboratory method and QA/QC, and statistical methods are described. The outlines of recruitment and sampling strategies are illustrated in Figure 1-2. The results discussed in this chapter include general information about participants, TCAA exposure doses in various exposure groups, temporal and spatial variation in tap water, background TCAA levels in tap water, urine, and blood, TCAA levels in urine and blood after exposure gradients to TCAA and with time changes of exposure, and DCAA levels in water and biological samples.

Reliability analysis is one of the major components in validation processes for a biomarker of exposure. The study in Chapter 4 was carried out to evaluate the intraindividual and interindividual variability in a substantially larger cohort in order to establish the reliability of various measurements and to examine whether TCAA levels in the body are sufficiently consistent within individuals over time to allow TCAA to serve as a biomarker. Reliability analysis was performed by using the coefficient of variation (CV) for analytical variation, the intraclass correlation coefficient (ICC) for intraindividual, and interindividual variability and Cronbach's α for internal consistency of repeated sampling. The findings provided a basis for validity analysis and improvement of sampling strategies.

Chapter 5 presents the results of validity analysis using Pearson's correlation coefficient and regression analysis. The findings reveal evidence of the relationship between TCAA ingestion and excretion and indicate that urinary TCAA could be a valid biomarker of exposure for DBPs in drinking water.

In Chapter 6, the feasibility of the application of TCAA as a biomarker of exposure is summarized. The selection of TCAA as a biomarker of exposure to DBPs and the candidate populations in a study required an understanding of the purposes of a study. Sampling strategies involved determination of sample size, selection of types of samples, timing of sampling, sample transportation and storage, and cost-effectiveness. The TCAA biomarker could be applied to the general population but confounding factors and ethical issues must be considered in any study using TCAA as a biomarker of exposure.

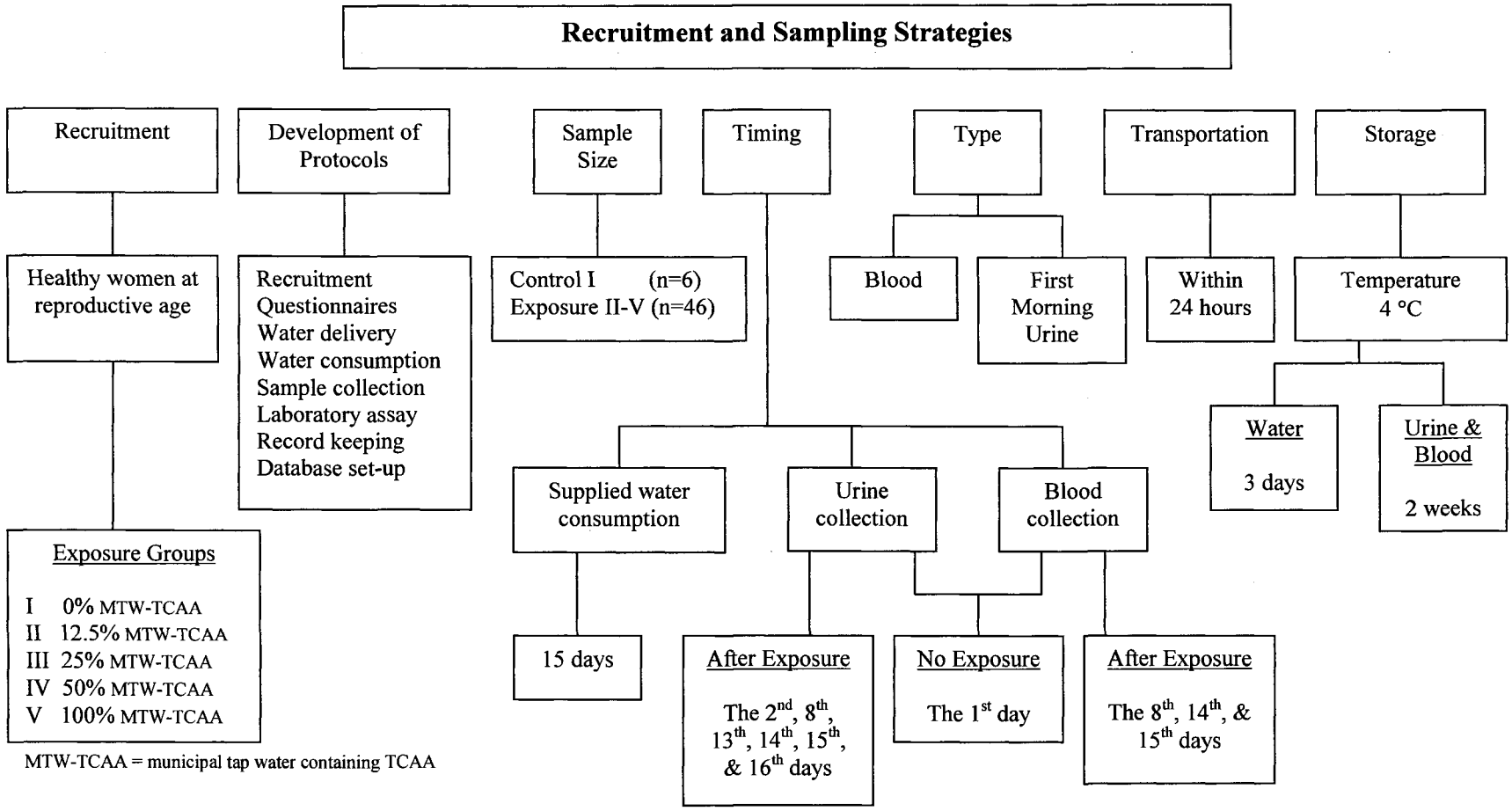


Figure 1-2 Framework of Recruitment and Sampling Strategies

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CHAPTER 2 LITERATURE REVIEW

2.1 Characteristics of Disinfection By-Products

2.1.1 DBP Levels in Drinking Water

Disinfection by-products (DBPs) are a set of chlorine and/or bromine compounds that are formed during chlorination for disinfecting water (Rook 1974). Natural organic matter such as humic and fulvic materials serves as the organic precursor of DBPs and the bromide ion serves as an inorganic precursor (Oliver and Lawrence 1979; Chang *et al.* 2001a, 2001b). There is a wide range of variation in DBP levels in drinking water. The formation of DBPs is determined by the disinfection process, water source, pH, temperature, season, the levels of chlorine residual, residence time in the distribution system, total organic carbon, bromide content, and abiotic and biotic degradation processes (Singer 1993; Hozalski *et al.* 2001; Espigares *et al.* 2003; Liang and Singer 2003; Richardson *et al.* 2003; Serodes *et al.* 2003; Yang and Shang 2004; Symonski *et al.* 2004; Rodriguez *et al.* 2001, 2004). Drinking water from surface water sources contains higher levels of DBPs as compared to that from groundwater sources (Arora *et al.* 1997). The content of humic substances influences the formation of DBPs in surface water (Nikolaou *et al.* 2004a, 2004b).

More than 500 DBPs can be identified or measured in treated water at water treatment plants, within the distribution system, and in tap water at homes and workplaces (Williams *et al.* 1997; Richardson 1998; USEPA 2002; Rodriguez *et al.* 2003; Sadiq and Rodriguez 2004). The limited DBPs have been studied for adverse health effects. The major DBPs and their measured concentrations reported in US drinking water are listed in Table 2-1. The two most abundant classes of DBPs are trihalomethanes (THMs) and haloacetic acids (HAAs). Other DBPs occur at low levels (Krasner *et al.* 1989; USEPA 2002).

The THMs are the most prominent volatile compounds. Chloroform and bromodichloromethane are the first and second principal species. Total THMs (TTHMs) are now routinely measured at water treatment facilities. TTHMs were measured in drinking water from water treatment plants and distribution systems in eight provinces of Canada between 1994 and 2000 (Health Canada 2004). The mean THM concentrations ranged from 10 to 66 μg/L depending on the season and on the treatment technique (Health Canada 1995, 2004; Williams *et al.* 1997; Rodriguez *et al.* 2003).

The mean TTHM concentrations in household tap water samples from eastern Ontario and Nova Scotia in Canada ranged from 51 to 63 μg/L (King *et al.* 2004). The mean chloroform concentration ranged from 43 to 51 μg/L. The Canadian

Drinking Water Guideline for THMs is 100 μg/L (Health Canada 1996). The World Health Organization Drinking Water Guidelines allow 200 μg/L TCM, 60 μg/L BDCM, 100 μg/L DBCM, and 100 μg/L TBM (WHO 1996, 2004).

The HAAs are semi-volatile compounds. Dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) are the principal species. The concentrations of HAAs in Canadian water systems (water treatment plants and distribution systems) ranged from 10 to 100 μg/L (Health Canada 1995). The mean concentrations of total HAAs (THAAs) in residential water samples from eastern Ontario and Nova Scotia ranged from 44 to 47 μg/L (King *et al.* 2004). TCAA was detected at concentrations ranging from 4.1 to 48.9 μg/L in Canadian drinking water (Health Canada 1995).

Table 2-1 Major Disinfection By-products in Drinking Water

Group	Compound	Concentration (μg/L)*
Trihalomethanes (THMs)		**58**
	Chloroform (Trichloromethane, TCM)	14
	Bromodichloromethane (BDCM)	14
	Dibromochloromethane (DBCM)	25
	Bromoform (Tribromomethane, TBM)	5
Haloacetic Acids (HAAs)		**24**
	Monochloroacetic acid (MCAA)	<2
	Dichloroacetic acid (DCAA)	9.5
	Trichloroacetic acid (TCAA)	7.9
	Monobromoacetic acid (MBAA)	<1
	Dibromoacetic acid (DBAA)	6.6
Haloacetonitriles (HANs)		
	Dichloroacetonitrile (DCAN)	2
	Trichloroacetonitrile (TCAN)	<0.1
	Dibromoacetonitrile (DBAN)	1
	Bromochloroacetonitrile (BCAN)	2
Haloketones (HKs)		
	1,1-Dichloropropanone	0.3
	1,1,1-Trichloropropanone	1
Haloacetaldehydes	Chloral hydrate (Trichloroacetaldehyde, CH)	4
Halonitromethanes	Chloropicrin (Trichloronitromethane)	<0.1

* Source: USEPA 2002. The concentrations represent the measured DBP compounds in finished drinking water in one water treatment plant in the US in October 2000. The water treatment process included coagulation and filtration. Chlorine was applied to the raw, settled, and filtrated waters. Ammonia was added to the finished water to form chloramines.

2.1.2 Correlations between THMs and HAAs in Drinking Water

The median concentrations of total HAAs were approximately equal to the median concentrations of total THMs in US treated drinking water systems according to data from the USEPA's Information Collection Rule (Roberts *et al.* 2002). In a study from North Carolina, the mean and median DCAA and TCAA concentrations were approximately equal to the corresponding chloroform concentrations (Singer *et al.* 1995). Because of data availability, TTHMs have often been used as surrogates for the overall formation of halogenated DBPs in drinking water.

There was a strong correlation ($R=0.96$, $n=140$) between total THMs and the sum of 19 individual halogenated DBPs in US drinking water (Krasner *et al.* 1989). In another US study, TTHMs were moderately correlated with HAAs ($R=0.667$, $p<0.001$, $n=40$) (Hinckley *et al.* 2005). In a UK study, good correlations between TTHMs and chloroform ($R=0.98$, $p<0.01$, $n=1494$), and TTHMs and BDCM ($R=0.62$, $p<0.01$, $n=1494$) were observed (Keegan *et al.* 2001). In another UK study, correlations between TTHMs and THAAs in the water supply from three water companies varied from no correlation ($R=0.10$, $p>0.01$, $n=27$) to a high correlation ($R=0.87$, $p<0.01$, $n=37$) (Malliarou *et al.* 2005).

When treatment conditions were relatively uniform and the water had a low concentration of bromide, a good correlation ($R=0.907$, $n=93$) of THM formation with HAA formation was observed in North Carolina drinking water (Singer *et al.* 1995). In a study from Spain, a good correlation between TTHMs and THAAs ($R=0.815$, $p<0.0005$, $n=18$) was reported (Villanueva *et al.* 2003). Some specific HAAs were correlated with specific THMs. For example, TCAA was correlated fairly with chloroform ($R=0.66$, $p=0.003$, $n=18$) in Spanish drinking water (Villanueva *et al.* 2003).

In Nova Scotia, TTHMs for 140 household water samples were fairly or highly correlated with chloroform ($R=0.97$), BDCM ($R=0.63$), THAAs ($R=0.74$), DCAA ($R=0.70$) and TCAA ($R=0.65$) in tap water (King *et al.* 2004). In eastern Ontario, TTHMs for 214 household water samples were fairly or highly correlated with chloroform ($R=0.96$), THAAs ($R=0.52$), and TCAA ($R=0.56$) in tap water (King *et al.* 2004). TTHMs were weakly correlated with BDCM ($R=0.26$) and DCAA ($R=0.39$).

Strong correlations between DCAA plus TCAA and TCM plus BDCM (R: 0.92–0.97, $p<0.01$, $n=32$–36) were observed in drinking water from water treatment plants and distribution sites in Calgary, Canada (Rizak *et al.* 2000). Conventional treatment is used in these water treatment plants. In the same study, water monitoring was performed for the City of Winnipeg. The correlation between THAAs and TTHMs in one reservoir site was fair ($R=0.72$, $p<0.01$, $n=26$). Poor correlations were found within the distribution system (R: 0.26–0.52, $p>0.01$, $n=18$-36). The poorer correlation likely resulted from loss of volatile THMs in the

open storage reservoir in this city and also from the possible biodegradation of HAAs in the distribution system (Chen and Weisel 1998; McRae *et al.* 2004).

The TTHMs are correlated well with chloroform, but are not a good surrogate of exposure to other specific THMs or specific DBPs, especially brominated species (Keegan *et al.* 2001; King *et al.* 2004). Water treatment processes and geographic areas contributed to the differences of specific DBP levels found in water treatment plants, distribution systems, and tap water.

2.2 Characteristics of Exposure Assessment

2.2.1 Type of Exposure Indices

There are two basic approaches for exposure assessment: direct measurement and indirect measurement. Direct measurement refers to the measure of either the exposure dose, which is the total mass of a xenobiotic at point of contact in environmental media, or the internal dose, which is the total mass of a xenobiotic absorbed by the human body (Hrudey *et al.* 1996a). This approach includes personal exposure monitoring and biological monitoring. Biological monitoring uses the mean of biomarkers of exposure to estimate past or current exposure. Biomarkers of exposure can be measured in an individual or in a given population. Direct measurement provides a relatively accurate measurement of past and current exposure in an individual. This approach is commonly used for occupational exposure. For environmental epidemiologic studies, this approach provides a basic method for measuring exposure as a continuous variable to identify a causal agent related to adverse health outcomes (NRC 1991). The use of direct measurement has not been reported in epidemiological studies of adverse reproductive and developmental outcomes related to exposure to DBPs in drinking water. Research exploring a useful biomarker of exposure such as urinary TCAA for DBPs exposure has been conducted in recent years (Kim *et al.* 1999; Weisel *et al.* 1999; Bader *et al.* 2001, 2004; Froese *et al.* 2002; Calafat *et al.* 2003).

Indirect measurement refers to the measurement of external dose through information derived from estimation of exposure by using surrogates or quantification of the concentrations of a xenobiotic in a microenvironment. Indirect measurements can be classified into two indices:

- *Exposure Index I*: estimation of exposure by using surrogates or quantification of the concentrations of a xenobiotic in a microenvironment in a given population. Surrogates of exposure are usually used for initial screening in some epidemiological studies. The microenvironment represents the location where exposures occur, such as the drinking water, food, and air of a given population in the studies. This approach provides a

basis for analysis of exposures for a given population rather than for each individual. The general nature of this exposure index is prone to misclassification of individual exposure.

- *Exposure Index II*: estimation of exposure by using surrogates or quantification of the concentrations of a xenobiotic in a microenvironment, and integration of the information with time-activities and individuals' characteristics. The concentrations of a xenobiotic can be measured by site monitoring or estimated by modeling. Information on individuals can be obtained from questionnaires and interviews. This approach offers speed, economy, and convenience. Inaccurate estimates from this approach arise from conservative assumptions for modeling or from inadequate monitoring.

The types of exposure indices in the epidemiological studies of adverse reproductive and developmental outcomes related to exposure to DBPs in drinking water are summarized in Table 2-2. Two types of measurements are used in Exposure Index I:

- *Type a* is a surrogate based on water sources that includes the information on surface water vs. groundwater, chlorination vs. non-chlorination, color measurement of chlorinated water, and different disinfection processes in combination with maternal residential information. This exposure measurement was used for ecological studies, retrospective cohorts, and case-control studies (Tuthill *et al.* 1982; Aschengrau *et al.* 1989, 1993; Kanitz *et al.* 1996; Magnus *et al.* 1999; Yang *et al.* 2000a, 2000b, 2004; Kallen and Robert 2000; Cedergren *et al.* 2002; Jaakkola *et al.* 2001).

- *Type b* is a quantification of TTHMs, specific THMs, or THAAs in the distribution system in combination with maternal residential information. The concentrations of DBPs were usually obtained from routinely collected data from water treatment plants. This exposure measurement was used for a cross-sectional survey, retrospective cohorts, and case-control studies (Kram *et al.* 1992; Bove *et al.* 1995, 1996; Gallagher *et al.* 1998; Klotz and Pyrch 1999; Dodds *et al.* 1999; King *et al.* 2000; Dodds and King 2001; Wright *et al.* 2003; Toledano *et al.* 2005).

Neither of two types of indices provides any information on spatial and temporal variability in the concentrations of DBPs within the water supply systems, intraindividual and interindividual source variability, and personal exposure.

Two types of measurements are also used in Exposure Index II:

- *Type a* is a surrogate based on the information about water sources in combination with individual water-use behavior. This exposure measurement has been used for retrospective cohorts, prospective cohort,

and case-control studies (Hert-Piccitto *et al.* 1989; Shaw *et al.* 1990; Deane *et al.* 1992; Windham *et al.* 1992; Fenster *et al.* 1992; Wrensch *et al.* 1992; Shaw *et al.* 1998). Although water-use behavior in an individual is assessed, this type of measurement does not provide any information on spatial and temporal variability in the concentrations of DBPs within water supply systems, and on intraindividual and interindividual source variability.

- *Type b* is a quantification of TTHMs, specific THMs, or THAAs in the distribution system in combination with the information on individual water-use behavior. This exposure measurement has been used for prospective cohort and case-control studies (Savits *et al.* 1995; Waller *et al.* 1998; Windham *et al.* 2003; Fenster *et al.* 2003; Dodds *et al.* 2004; Aggazzotti *et al.* 2004; King *et al.* 2005; Savitz *et al.* 2005). In this type of exposure assessment, in most studies, an individual's water-use patterns and activities are collected for semi-quantitative analysis. The amount of ingestion of THMs by individuals was calculated for exposure assessment in three studies (Savitz *et al.* 1995 and Windham *et al.* 2003; Savitz *et al.* 2005). Exposure assessment was refined in a recent prospective cohort study (Savitz *et al.* 2005). Three different exposure windows for pregnant women were defined: 4 weeks after the last menstrual period (LMP), 4–8 weeks following the LMP, and 9–20 weeks post-LMP. Volumes of cold and hot water consumption were derived from daily self-report records. Factors identified included drinking cold vs. hot water and the presence of point-of-use (POU) filtration devices. Information on tap water consumption at home vs. in the workplace was collected. The ingested amount of measured DBPs was calculated based on DBP concentrations in tap water in the treatment system serving the participants' home, volume of daily water consumption, adjustment of factors resulting from drinking cold vs. hot water, and use of POU filtration devices. The exposure amount due to showering and bathing was calculated.

2.2.2 Water-Use Behaviors

Water is a vehicle for the transfer of DBPs from the environment to the human body. Variability of water-use behaviors during a critical exposure period can significantly affect an individual's exposure to DBPs from drinking water. Variation of TCAA levels in the human body can result from variations in volume and frequency of fluid intake. Variation of DBP exposure also depends on the water source, e.g., tap vs. bottled, surface vs. ground, and hot vs. cold water tap. Concentrations of most DBPs are higher in tap, surface, and cold water. People can be exposed to more DBPs in swimming pools and hot tubs, or while

Table 2-2 Types of Exposure Indices in Epidemiological Studies

Type	Exposure Data	Reference
Exposure Index Ia *Water Source – Population Data*		
Chlorinated vs. disinfection with chlorine dioxin	Exposure based on maternal residence	Tuthill *et al.* 1982 (Ecological Survey)
(1) Surface water vs. groundwater (2) Chlorinated vs. non-chlorinated	Exposure based on maternal residence from medical records and routinely collected data on inorganic and organic chemicals in public water supplies	Aschengrau *et al.* 1989, 1993 (Case-Control)
Disinfection with chlorine dioxide/sodium hypochlorite vs. non-disinfection	Exposure based on maternal residence from medical records	Kanitz *et al.* 1996 (Retrospective Cohort)
Chlorinated vs. non-chlorinated by measurement of mean color	Exposure based on a 1994 national waterworks registry	Magnus *et al.* 1999 (Retrospective Cohort)
Chlorinated vs. non-chlorinated	Exposure based on maternal residence from medical records	Yang *et al.* 2000a, 2000b, 2004 (Retrospective Cohort)
Disinfection with chlorine dioxide/sodium hypochlorite vs. non-disinfection	Exposure based on maternal residence derived from a Medical Birth Registry	Kallen and Robert 2000 (Retrospective Cohort)
(1) Surface water vs. groundwater (2) Disinfection with chlorine dioxide vs. non-disinfection (3) THMs and nitrate concentrations	Exposure based on maternal residence from a National Health Registry	Cedergren *et al.* 2002 (Retrospective Cohort)
Chlorinated vs. non-chlorinated by measurement of mean color	Exposure based on maternal residence from a National Medical Birth Registry and Water work Registry	Jaakkola *et al.* 2001 (Retrospective Cohort)

(Continued)

Type	Exposure Data	Reference
Exposure Index Ib. *Quantification of DBPs – Population Data*		
TTHM concentration in a water survey	Exposure based on maternal residence at time of birth	Kramer *et al.* 1992 (Case-Control)
Estimated monthly TTHM concentration based on quarterly monitoring data from four samples in distribution system	Exposure based on maternal residence	Bove *et al.* 1995, 1996 (Retrospective Cohort)
(1) Estimated monthly TTHM concentration based on quarterly monitoring data in the distribution system (2) Hydraulical model	Exposure based on maternal residence at time of birth	Gallagher *et al.* 1998 (Retrospective Cohort)
(1) Estimated TTHM and specific THMs concentrations based on quarterly monitoring data in the distribution system (2) Estimated concentrations of specific THMs, HANs and HAAs from tap water samples	Exposure based on maternal residence	Klotz and Pyrch 1999 (Case-Control)
Estimated TTHM conc. based on quarterly monitoring data in the distribution system	Exposure based on maternal residence at delivery	Dodds *et al.* 1999 (Retrospective Cohort)
Estimated TTHM conc. based on quarterly monitoring data in the distribution system	Exposure based on maternal residence at delivery	King *et al.* 2000 (Retrospective Cohort)
Estimated specific THMs conc. based on quarterly monitoring data in the distribution system	Exposure based on maternal residence served by a municipal water supply	Dodds and King 2001 (Retrospective Cohort)
Estimated specific THMs conc. based on quarterly monitoring data in the distribution system or annual monitoring data	Exposure based on maternal residence	Wright *et al.* 2003, 2004 (Cross-Section)

(Continued)

Type	Exposure Data	Reference
Modeled estimates of quarterly TTHM concentration in water zones, categorized as low, medium and high groups	Exposure based on maternal residence at delivery	Toledano *et al.* 2005 (Retrospective Cohort)
Exposure Index IIa *Water Sources – Personal Data*		
Tap water vs. bottled water	Exposure based on interview (1) Type of water (tap, bottled, hot, cold) (2) Beverage consumption (3) Drink at home or workplace	Hertz-Picciotto *et al.* 1989 (Nested Case-Control)
Tap water vs. bottled water	Exposure based on interview (1) Type of water (tap, bottled) (2) Glasses of cold tap water consumption (3) Showering (4) Drink at home or workplace	Shaw *et al.* 1990 (Case-Control)
Tap water vs. bottled water	Exposure based on interview (1) Glasses of cold tap water consumption (2) Beverage consumption (3) Drink at home or workplace	Deane *et al.* 1992 (Retrospective Cohort)
Tap water vs. bottled water	Exposure based on interview (1) Glasses of cold tap water consumption (2) Beverage consumption (3) Drink at home or workplace	Windham *et al.* 1992 (Case-Control)
Tap water vs. bottled water	Exposure based on interview (1) Glasses of cold tap water consumption (2) Drink at home or workplace	Fenster *et al.* 1992 (Case-Control)
Tap water vs. bottled water	Exposure based on interview: (1) Glasses of cold tap water consumption (2) Drink at home or workplace	Wrensch *et al.* 1992 (Case-Control)
Tap water vs. bottled water	Exposure based on interview (1) Type of water (tap, bottled) (2) Glasses of cold tap water consumption (3) Drink at home or workplace	Shaw *et al.* 1998 (Prospective Cohort)

(Continued)

Type	Exposure Data	Reference
Exposure Index IIb *Quantification of DBPs – Personal Data*		
(1) Source: treated water, groundwater, bottled water (2) Amount of water consumption (3) Source x amount of water consumption per day (4) Estimated TTHM conc. based on quarterly monitoring data in the distribution system (5) Amount of THM ingestion: glasses per day x TTHM concentration	Exposure based on an interview (1) Primary source of drinking water at home (2) Glasses of tap water per day	Savitz *et al.* 1995 (Case-Control)
Estimated THM concentrations by averaging all monitoring data in water utilities during the first trimester + glasses of cold tap water consumption	Exposure based on interview (1) Number of glasses of water consumed (2) Water treatment (filter) (3) Showering	Waller *et al.* 1998, 2001 (Prospective Cohort)
(1) Estimated THM concentrations by averaging all monitoring data in water utilities during the first trimester (2) Amount of ingestion: THM conc. x no. of glasses of cold tap water consumption	Exposure based on interview (1) Number of glasses of water consumed at home (2) Type of water (cold, hot, bottled) (3) Showering	Windham *et al.* 2003 (Prospective Cohort)
(1) Estimated specific THM conc. based on quarterly monitoring data in the distribution system (2) Number of glasses of water consumed	Exposure based on interview (1) Number of glasses of cold tap water consumed at home (2) Beverage consumption	Fenster *et al.* 2003 (Prospective Cohort)
(1) Estimated TTHM conc. based on quarterly monitoring data in the distribution system (2) Number of glasses of water consumed	Exposure based on interview (1) Number of glasses of cold tap water consumed at home (2) Beverage consumption	Shaw *et al.* 2003 (Case-Control)

(Continued)

Type	Exposure Data	Reference
(1) Estimated THM concentration by measuring THMs in household tap water sample collected after one year later of the pregnancy (2) TTHM conc. + no. of cold tap water drink per day (3) TTHM conc. + time of showering/bathing	Exposure based on interview (1) Consumption of beverages made with tap water (2) Types of tap water (filter, hot, cold) (3) Bottled water consumption (4) Duration of showering and bathing	Dodds *et al.* 2004 (Case-Control)
Measured THM concentration in household tap water samples	Exposure based on questionnaires (1) Type of water supplies (public vs. private) (2) Water treatments (3) Type of water (bottles, tap) (4) Consumption of beverages (5) Activities: cooking, swimming, showering, bathing	Aggazzotti *et al.* 2004 (Case-Control)
Measured HAA concentration in household tap water samples	Exposure based on interview (1) Water consumption (2) Water-use behaviors	King *et al.* 2005 (Case-Control)
Measured 4 THMs, 9 HAAs, and total organic halide (TOX) in distribution systems in three sites and tap water in the treatment system serving the participants' home	Exposure based on specific time interval the entire pregnancy period (1) Tap water sampling weekly or every 2 weeks (2) Concentrations of measured DBPs (3) Self-report no. of glasses cold and hot water per day (measured in ounces) (4) Use of POU filtration devices (5) Amount of measured DBP ingestion (6) Measured DBP exposure for showering and bathing alone (7) Integrated DBP ingestion plus showering/bathing	Savitz *et al.* 2005 (Prospective Cohort)

showering, bathing, and dishwashing. Foods and beverages can be additional sources of DBP exposure.

The amount of chemical ingestion is usually linked to daily fluid intake. Global data on fluid intake are limited. In studies from Canada, the US, the UK, and the Netherlands, the average daily *per capita* fluid intake was less than 2 liters (WHO 1996). In a US and Canada study, average total fluid intake was 2.2 L/d for 19 – 30 years old women (Kleiner 1999; IMNA 2004). In other studies, an average total fluid intake ranged from 1.7 to 2.2 L/d (Ershow and Cantor 1989; Shimokura *et al.* 1998; Jacobs *et al.* 2000; USDA 2000; Valtin 2002). Average total tap water intake in the US and Canada ranged from 0.8 to 1.4 L/d (CMNHW 1981; Ershow and Cantor 1989; Shimokura *et al.* 1998; Jacobs *et al.* 2000).

Among pregnant women, 67% of all tap water beverages were consumed at home and 33% were consumed at work and outside the home (Shimokura *et al.* 1998). The average tap water consumption was 0.78 L/d in the US population (Shimokura *et al.* 1998). Pregnant women consumed 2.1 L/d cold tap water and 1.1 L/d of cold water-based beverages, while non-pregnant women consumed 1.1 L/d cold water and 0.7 L/d of cold water-based beverages (Zender *et al.* 2002). In the UK, pregnant women consumed an average of 2.7 L/d of total fluid and 0.6 L/d of cold tap water (Kaur *et al.* 2004). In Canada, about 1.0 L/d of tap water was used for drinking, adding to beverages and cooking (Levallois *et al.* 1998).

There was good agreement (R=0.78) between the questionnaire data and diary records for estimating drinking water intake (Shimokura *et al.* 1998). The correlation of cold tap water intake using the questionnaire vs. the seven days diary was good (R=0.79) for home consumption, but not for the workplace consumption (Kaur *et al.* 2004). The variability of water consumption patterns mainly resulted from individual differences (volume of water consumption and activities) (Shimokura *et al.* 1998).

Other water-based DBP exposure arises from showering and bathing. The average daily showering time in the US was 10 minutes for women (Shimokura *et al.* 1998) and 14 min for pregnant women (Zendar *et al.* 2002). Bathing was not a daily activity. Pregnant women usually took a bath averaging 28 min three times per week (Zender *et al.* 2002). In the UK, pregnant women spent about 8 min/d for showering or bathing (Kaur *et al.* 2004).

Heating water was observed to alter the concentrations of DBPs (Weisel and Chen 1994). An increase in the THM concentrations and a decrease in the haloacetonitriles and halopropanones concentrations in the air were observed after heating water for showering or bathing. The effect of boiling water on DBPs was investigated by Krasner and Wright (2005) and Savitz *et al.* (2005). THM concentrations in tap water were not detected after boiling (Savitz *et al.* 2005). TCAA concentrations decreased 9% to 37% over time upon boiling of tap water,

while DCAA or DBAA concentrations increased (25%–68%) after water was boiled (Krasner and Wright 2005; Savitz *et al.* 2005).

The concentrations of DBPs changed after filtering tap water. THM concentrations in tap water were not detected after using POU filtration devices (Savitz *et al.* 2005). TCAA and DCAA concentrations in tap water decreased 65% and 45% respectively after using POU devices.

2.2.3 Exposure Measurement Errors

Measurement errors occur frequently in exposure assessment. The exposure measurement error or misclassification of exposure is any discrepancy between the true exposure and the measured exposure (Armstrong *et al.* 1992; Thomas *et al.* 1993). Error in measurement of the exposure can be random or systematic (Armstrong 1998). Systematic error occurs when the measured exposure is not distributed randomly around the true exposure. Bias is a systematic error that is different in direction or size in one of the groups under study.

Measurement errors can be classified into differential or nondifferential. Differential exposure error is the error in which the outcome depends on the source of the error in the exposure. Nondifferential error is the error in which the outcome does not depend on the source of the error in the exposure. One result of measurement error is bias in the measure of association between the exposure and the outcome. Any bias resulting from nondifferential error of an exposure may be toward the null value on the measure of association such as odds ratio or correlation if misclassification is independent of other error (Armstrong *et al.* 1992; Rothman and Greenland 1998).

In observational epidemiological studies, measurement error results from faults in the design of instruments, the methods or protocols of measurement used, selection bias, data collection, unmeasured confounders, and data analysis (Elwood 1988; Armstrong *et al.* 1992). In the design of a questionnaire, error can arise from lack of inclusion of all exposure sources, non-relevant exposure agents, inaccurate exposure period, or inaccurate questions being asked of participants. Error in measurement protocols can occur when detailed steps of measurement procedures and standardization of instruments are not sufficiently addressed. Improper handling and analysis of biological samples during data collection or influence of participants' characteristics during interviews can cause measurement error. Selection bias such as poor recall of exposure is one source of differential measurement error. If additional sources of exposure to a confounder such as chloral hydrate (CH) for TCAA exposure assessment are not measured, measurement error can occur. Errors can result from data entry or data analysis.

Epidemiological studies pertaining to DBP exposure in drinking water have suggested weak associations between exposure to some DBPs and the incidence

of human cancer such as bladder cancer as well as adverse reproductive and developmental outcomes (Nieuwenhuijsen *et al.* 2000a, 2000b). In these studies, the exposure assessment relied on some surrogates such as individual residence, water sources, routine THM monitoring in treatment plants or distribution systems, and volume of water intake. These methods were inadequate to classify an individual exposure during a specific time window (the last trimester of gestation or the long latent period of cancer development) (Arbuckle *et al.* 2002). Exposure misclassification is a major obstacle to obtaining accurate rates of association between adverse health outcomes and exposure, and is usually expected to cause attenuation in health risk estimates, provided that exposure misclassification is random and non-differential.

In order to improve the accuracy of exposure assessment in reproductive and developmental studies, the collection of personal exposure data requires knowledge of the type of disinfection process in the residential area, the volume of tap water consumption, the types of other water intake, the specific DBP compounds being studied, the concentrations of specific DBP compounds during the critical exposure period, the variation of DBPs in water supplies, the DBP exposure activities, and the exposure routes other than ingestion (Nieuwenhuijsen *et al.* 2000a, 2000b).

Quantitative indices of measurement error are developed for evaluating measurement error distribution (Thomas *et al.* 1993). The methods include validity analysis, which is used to assess the accuracy of information, and reliability analysis to assess its reproducibility. Validity reflects the agreement between the measured value and its true value. Validity can be quantified by comparing the measurements with the true values. Poor validity reduces the precision of a single measurement and the ability to characterize relationships between variables in descriptive studies.

2.3 Biomarker of Exposure

2.3.1 Overview

A biomarker of exposure is a xenobiotic and/or its metabolites, or an event in the relation to exposure of interest that can be qualitatively or quantitatively identified in a biological system or in samples such as biological fluids, tissues, and expired air. A biomarker indicates the occurrence and extent of exposure in humans (Dor *et al.* 1999). The types of exposure biomarkers can be classified into two groups:

1) Xenobiotics and their metabolites related to the internal dose in a biological system; and
2) Macromolecular adducts such as DNA or protein adducts related to the internal dose.

The internal dose reflects the amount of the parent xenobiotic that is actually absorbed into the systemic circulation of the human body shortly following external exposure, during the preceding day, or as a result of past exposure (Sampsom *et al.* 1994; WHO 1996; Hrudey *et al.* 1996a). The biological processes have direct or indirect relationships to exposure. The level of the biomarker of exposure can be determined by biological monitoring. Biomonitoring is designed to directly estimate the internal doses of parent xenobiotics or their metabolites over their entire time in the human body, to quantify macromolecular adducts, or to indirectly estimate the amount of external doses of a xenobiotic to which humans are exposed (Bernard and Lauwerys 1987; WHO 1996). Biomonitoring takes into consideration all exposure sources and routes and the physiochemical and biological factors that influence the absorption, distribution, metabolism, and excretion.

Some biomarkers of exposure to environmental contaminants have been identified in the general population (Pirkle *et al.* 2005). Measurement of blood levels of a biomarker can quantitatively evaluate the exposure for most xenobiotics (Hrudey *et al.*1996b). For example, the THMs resulting from showering, bathing, and swimming were measured in blood and breath samples in the general population (Wallace *et al.* 1984; Aggazotti *et al.* 1990, 1995, 1998; Jo *et al.* 1990a; Aiking *et al.* 1994; Levesque *et al.* 1994; Weisel and Shepard 1994; Cammann and Hubner 1995; Weisel and Jo 1996; Gordon *et al.* 1998; Weisel *et al.* 1999; Brugnone *et al.* 1994; Backer *et al.* 2000; Lynberg *et al.* 2001, Miles *et al.* 2002, Whitaker *et al.* 2003). Because in most cases the blood concentration and the urine concentration of a biomarker are proportional, measurement of the cumulative concentration of a xenobiotic in urine is employed to roughly estimate the exposure (Hrudey *et al.* 1996b). For example, as an indicator of the internal dose, TCAA was measured in urine from groups of the population who had drunk tap water containing DBPs (Kim *et al.* 1999; Weisel *et al.* 1999; Bader *et al.* 2001, 2004; Froese *et al.* 2002; Calafat *et al.* 2003).

2.3.2 Strengths and Limitations

Biomarkers of exposure have to be validated according to their ability to assess the presence or absence of an exposure and to quantify the exposure. The application of biomarkers of exposure in epidemiological studies has many advantages (Hogue and Brewster 1988; Hulka and Wilcosky 1988; Hulka *et al.* 1992; Schulte 1989; Harris 1991, Shields and Harris 1991; Menselos 1991; Schulte 1991a, 1991b, 1992, 1993a, 1993b, 1995a; Schulte and Talaska 1995; Pirkle *et al.* 1995a, 1995b, 2005; Toniolo *et al.* 1997; Mayeux 2004). These include:

- Indication of the relationship between a biomarker and the biological phenomenon of interest;

- Improvement of sensitivity and specification of the measurement of exposure or risk factors;
- Provision of information on integrated multiple sources and routes of exposure;
- Identification of correlations between exposure and internal dose or exposure and biological response;
- Identification of reference ranges of a biomarker in given populations;
- Identification of mechanisms by which exposures and outcomes are related;
- Reduction of misclassification of exposure, risk factors, or outcomes when compared to use of historical characteristics and external exposure measurement;
- Improvement of validity for different conditions such as reducing bias in the measurement of exposure or risk factors;
- Evaluation of variability and effect modification;
- Evaluation of priority toxicants; and
- Enhancement of individual and group risk assessment.

The potential limitations include:

- Introduction of intraindividual and interindividual variability;
- Difficulty in balancing the best design of epidemiological study with the easiest logistics;
- Difficulty in identifying the sources of different exposures and confounding factors;
- Difficulty in establishing a normal range of a biomarker of exposure in a large population, or in sub-populations;
- Relatively high cost; and
- Ethical issues involving sampling from human subjects.

Variability is a major limitation in the application of a biomarker of exposure (Mayeux 2004). Interindividual variability is related to source variation, external exposure variation, and metabolism variation among individuals. Such variability can cause exposure misclassification (Wilcosky *et al.* 1990). For example, if two individuals are exposed to the same amount of TCAA in drinking water, urinary TCAA concentrations may differ considerably in the two individuals. The variation could arise from other exposure sources such as trichloroethylene (TCE) which can metabolize into TCAA, differences in metabolic rate and kidney function, and different routes of excretion. Thus, the misclassification occurs due to interindividual variability.

Intraindividual variability is a characteristic of most biomarkers of exposure. It is related to laboratory errors or other conditions, or exposure unique to the individual. Laboratory errors, personnel, methods, sample transport, and storage

procedures may affect the reliability of a biomarker. Exposure misclassification occurs due to intraindividual variability if a measure from a single sample in an individual is used to estimate long-term exposure status and chronic health outcomes (Wilcosky *et al.* 1990). In case-control studies, if intraindividual variability is random and non-differential, this exposure misclassification is likely to lead to attenuation in health risk estimates (Hunter 1997). This attenuation of relative risks can be proportional to the extent of intraindividual variability or considerably larger.

The collection of human samples is required in epidemiological studies using a biomarker of exposure approach. Sometimes, the best design of an epidemiological study and the easiest logistics of sample collection are in conflict (Potter 1997). The timing of sample collection is critical. A perspective cohort study is a favored design for cancer epidemiological studies. A biomarker of exposure with a long elimination half-life is required for such study design. If a biomarker of exposure with a short elimination half-life (such as TCAA with a half-life less than 6 days) is selected, a greater frequency of sampling is needed. In this case, the logistics of sampling are difficult.

The measured levels of a biomarker of exposure in biological samples represent all sources of exposure and confounding factors. The levels of urinary TCAA could arise from exposure to TCA and chloral hydrate (CH) in drinking water. CH can be metabolized into TCAA. The measurement of urinary TCAA does not distinguish CH as a confounding factor.

The levels of exposure to an environmental contaminant in the general population vary depending on geographical locations. The establishment of a normal range of a biomarker of exposure in the general population is not easy. The baseline of a biomarker of exposure needs to be evaluated in the study area.

The selection of a biomarker of exposure needs to be guided by the study question and the financial resources. Cost is often a concern. The estimated cost for a study population of 2000 couples using TCAA biomarker would be more than $1.5 million over a 2-year period (Arbuckle *et al.* 2002).

The use of a biomarker of exposure for epidemiological studies presents potential ethical issues because a biomarker is obtained from human tissues or body fluids (Toniolo *et al.* 1997). People may perceive that biomarkers are associated with some degree of health risk. Confidentiality and privacy are also concerns.

2.3.3 Validation of a Biomarker of Exposure

A candidate exposure biomarker is the most representative of a particular component in the continuum of the exposure event. This biomarker provides both qualitative and quantitative information about external exposure. Validation of a

biomarker of exposure results from an evaluation of the factors that influence the biomarker to predict exposure and allow it to be used in appropriate epidemiological studies.

Validation can be divided into three types of studies: development, characterization, and application (Toniolo *et al.*1997). Developmental studies include determining the biological relevance, toxicokinetics, temporal and spatial variability, persistence, laboratory methodology, and optimal conditions for sample collection, processing, and storage. Characterization studies involve examining the factors that influence the ability of analysis to predict exposure in a given population (Schulte 1987; Hulka and Margolin 1992; Rothman *et al.* 1995; Schulte and Perera 1997). Application studies assess the relationship between a biomarker of exposure and related events such as external exposure, biological effects, and susceptibility.

Validation processes include understanding of the purpose of using a biomarker, knowledge about the natural history of a biomarker and the relationship between a biomarker and exposure, toxicokinetics, temporal and spatial variability, persistence, laboratory methodology and types of specimens, determination of background level, evaluation of reliability (intraindividual and interindividual variability), validity (sensitivity, specificity, and predictive value) and relevant statistical analysis, selection of an appropriate biomarker (representative, surrogate, correlation, and prediction), and distinguishing confounder factors and their effect on the marker (age, sex, ethnicity, and lifestyle).

Biological Relevance and Selection of an Appropriate Biomarker

Biomarkers of exposure assume that a causal relationship exists between a xenobiotic in the environment and the toxicity response in the putative target sites in the human body (Schulte and Talaska 1995; Handy *et al.* 2003; Savitz *et al.* 2005). A biomarker of exposure indicates that a xenobiotic enters the human body from the environment. A xenobiotic may be distributed to some sites at the highest concentration but not elicit major toxicity while its metabolites may reach critical organs and cause some degree of toxicity. In this case, an exposure-dose relationship must be available for this specific marker (Hrudey *et al.* 1996a). If the quantitative relationship between external exposure and internal dose is identified, this xenobiotic relative to exposure can be selected as a potential biomarker of exposure. A biomarker of exposure can be used as an index of exposure to estimate the exposure intensity as well as to establish the biological relevance. If a relationship between a biomarker and a biological effect is not identified, the biomarker can still be correlated to external exposure as a surrogate for the agent causing a biological effect and thereby be indirectly related to the biological effect.

In most epidemiological studies, weak to modest association between exposure to chlorinated drinking water or THMs and carcinogenicity and adverse reproductive and developmental of effects were reported (Boorman *et al.* 1999; Nieuwenhuijsen *et al.* 2000b; IPCS 2000; Graves *et al.* 2001; Bove *et al.* 2002; IARC 2004). In animal models, DCAA and TCAA elicited renal carcinogenic and/or liver tumor promoting activity only at concentrations massively higher than any plausible drinking water exposure (Herren-Freund *et al.* 1987; Pereira *et al.* 2001; Ge *et al.* 2001; Bull *et al.* 2004; Tao *et al.* 1998, 2005). DCAA and TCAA increased chloroform toxicity (liver and kidney) (Davis 1992). Teratology studies related to DCAA and TCAA were conducted in rodents. Cardiac malformation was reported in female rats that were treated at doses of 330 mg/kg/day and 2730 mg/kg/day of TCAA (Smith *et al.* 1989; Johnson *et al.* 1998), and at a dose of 2400 mg/kg/day of DCAA (Epstein *et al.* 1992). In another study, cardiac malformation was not observed in rats after DCAA- and TCAA-treatment at doses of 3000 μM (Hunter *et al.* 1996). DCAA has been found to induce testicular toxicity at doses of 1500 and 3000 mg/kg (Linder *et al.* 1997). In a case-control study, haloacetic acid exposure was not directly associated with stillbirth risk after controlling for THM exposure (King *et al.* 2005). THMs, TCAA, and DCAA have some biological relevance for selection as potential biomarkers of exposure in epidemiological studies of cancer and reproductive and developmental outcomes.

Most epidemiological studies on reproductive and developmental outcomes have emphasized the relationship between THM exposure and reproductive and developmental outcomes. The biological relevance of HAA exposure in drinking water pertaining to adverse reproductive and developmental outcomes has not been well-documented. More research is needed to establish any relationship between HAA exposure and reproductive and developmental outcomes in the human population.

Biological relevance is not the only requirement for selection of a biomarker. Furthermore, biological relevance need not be based only on causation of an adverse effect, but rather on whether the prospective biomarker behaves in a biologically relevant manner, as discussed below under toxicokinetics, for it to serve as an effective biomarker of exposure. Selection of an appropriate biomarker of exposure also requires a biomarker being representative and a good surrogate, with good correlation and prediction between biomarker and exposure.

Toxicokinetics

Biological monitoring of exposure depends on knowledge of toxicokinetics including absorption, distribution, metabolism, and excretion (Hrudey *et al.* 1996a). Knowledge of toxicokinetics is critical for selecting the appropriate biomarkers and biological media for laboratory analysis, setting up sampling

strategies such as frequency and timing, and assistance in the interpretation of the data.

TCAA is readily absorbed in humans and animals (Muller *et al.* 1974; Curry *et al.* 1991; Larson and Bull 1992). It is metabolized into CO_2, DCAA, and GOG (Green and Prout 1985; Larson and Bull 1992). In rats and mice, about 48% to 78% of the administered doses were excreted in urine (Muller *et al.* 1974; Larson and Bull 1992). After oral administration of TCAA in humans, 23% to 50% of the administrated dose was recovered in urine (Muller *et al.* 1974; Humbert *et al.* 1994). TCAA can also be excreted in feces and bile (Green and Prout 1985; Larson and Bull 1992; Schultz *et al.* 1999).

Fluctuations in blood TCAA were observed in animals (Prout *et al.* 1985). Such fluctuations may be related to biliary excretion and enterohepatic recirculation (Green and Prout 1985). A change of pH in urine can alter the excretion rate. The amount of TCAA excreted during night (0–8 am) was lower than during the day (Monster *et al.* 1979). In practice, parent TCAA is measured in urine. The measurement of parent TCAA has less variability compared to the measurement of its metabolites, which results in greater interindividual variability for a given ambient exposure.

The magnitude of a biomarker of exposure can be influenced by genetic factors. Cytochrome P450 (CYP) enzymes are responsible for the metabolism of numerous xenobiotics in the environment. The form of CYP 2E1 was involved in TCAA and DCAA metabolism (Larson and Bull 1992). CYP 2E1 has three polymorphisms (Uematsu *et al.* 1991; Hu *et al.* 1990). An individual's capability to metabolize TCAA and DCAA can be altered by carrying the variant alleles of CYP 2E1. Therefore, the degree of excretion of TCAA and DCAA in urine can be altered from an individual to another because of genetic differences.

Temporal and Spatial Variability

Many factors such as abiotic or biotic factors (e.g., genotype) and physiological processes can contribute to the variability in biomarkers of exposure measurement (Hinwood *et al.* 2002; Handy *et al.* 2003). These factors vary with time and space. Understanding of temporal and spatial variability is important for developing sampling strategies and interpreting the results (Schulte and Talaska 1995).

Spatial variability and seasonal variation of the concentrations of DBPs in drinking water were observed. In a 1994 Canadian drinking water survey, the concentrations of TCAA in the water treatment system were 7.9 μg/L in Ottawa, 13 μg/L in Hull, and 5.9 μg/L in Buckingham (Health Canada 1996). The concentrations of TCAA in these three locations varied from one month to another. Thus, timing of sampling and interpretation of data based on different geographic locations should be considered in designing an epidemiological study related to DBP exposure.

Variability of xenobiotic concentrations was observed in samples when testing included spot-checks of urine samples taken at different times. Xenobiotics have different elimination half-lives in biological media. The time of appearance, persistence, and disappearance of the biomarkers is related to the time of external exposure or the fluctuation in time of the internal dose. The temporal relationship between external and internal exposure influences the ability to detect a response and substantially contributes to exposure misclassification.

The use of a biomarker of exposure with a long-term or a short-term exposure depends on the study question. For xenobiotics with a short elimination half-life, the concentrations in blood or urine reflect recent exposure. In this case, the time of sampling is critical. A standardization of sample collection time and repeated sampling are required to obtain meaningful results. For example, DCAA has a half-life between 20 min and 60 min in humans due to extensive metabolism (WHO 2000). In practice, measuring DCAA in biological media for an epidemiological study will not be effective as an exposure biomarker because the use of a biomarker with a relatively short half-life to identify long-term exposure may result in misclassification of exposure.

Persistence

Persistence of a biomarker of exposure in the human body can be employed to estimate past, current and future exposure. The persistence of a biomarker relies on kinetic models, kinetic parameters, and the availability of biological media. The elimination half-life of a xenobiotic is the time required to eliminate half of the amount of the xenobiotic from the body. The elimination half-life reflects the affinity of the xenobiotics for biological matrices, the efficiency of excretion, and metabolic processes of elimination. Therefore the elimination half-lives in different parts of the body can vary considerably.

For xenobiotics with a long elimination half-life, higher concentrations will be attained in body fluids if exposure is continuous or repeated. If a continuous exposure is three, five, and seven half-lives, the blood concentrations of a xenobiotic will reach approximately 90%, 97%, and 99% of the steady-state concentration respectively. In practice, a continuous exposure for three and five half-lives should be sufficient for stable measurement.

TCAA concentrations were approximately two-fold lower in the whole blood than in plasma in humans, likely because of a lack of oenetration of TCAA into red cell (Paykoc and Powell 1945). A high level of binding of TCAA to plasma proteins has been reported(Marshall and Owens 1954; Sellers and Koch-Weser 1971; Muller *et al.* 1972; Monster *et al.* 1976). About 94% to 66% of TCAA was bound to plasma protein at 10 to 300 μg/ml of plasma TCAA levels (Sellers and

Koch-Weser 1971; Muller *et al.* 1972). The bound fraction was relatively constant, with a mean of 82% over a 3.7-order of increase in TCAA concentrations (Lumpkin *et al.* 2003). Saturation of binding was observed in human plasma. The bound TCAA cannot be filtered through the kidney. TCAA can also bind with conjugates such as glucuronides (Fisher *et al.* 1991). Free TCAA in blood was rapidly eliminated by glucuronidation (Nomiyama and Nomiyama 1979). The half-lives of TCAA elimination after direct TCAA ingestion ranged from 30 hours to 6 days in humans (Paykoc and Powell 1945; Muller *et al.* 1974; Monster *et al.* 1976; 1979; Humbert *et al.* 1994; Fisher *et al.* 1998; Bader *et al.* 2004). TCAA levels in biological media reflect 65% of the steady-state condition after the 6^{th} day of exposure and almost 90% steady-state condition after the 12^{th} day of exposure, assuming a median urinary excretion half-life of 4 days. TCAA has adequate persistence (a long enough elimination half-life) in blood to allow the measuring of current exposure to TCAA in drinking water.

Laboratory Methodology

From a laboratory perspective, validity is the ability of an assay to detect the presence or absence of a designated biomarker in the specified biological medium (e.g., breath, blood, urine). The validation processes include well-characterized accuracy and precision, detection limits (sensitivity), exposure specificity, and reliability (Sampson *et al.* 1994; Schulte and Perera 1997). Laboratory variability is a function of instrumentation, reagents and human errors in sampling, labeling, preparation, and analysis performance (Stites 1991). The major errors can result from errors in the laboratory method, measurement protocols and sample collection (sampling constraints, number of samples for precision, availability and stability of storage, contamination, and xenobiotic deterioration, evaporation, precipitation, and adsorption).

TCAA in drinking water is a source of DBP exposure in the general population. Laboratory methods such as the US EPA Method 552.2 and Standard Method 6251B are used in most water utility laboratories. Techniques used for analysis include high-performance liquid chromatography (HPLC), HPLC with mass spectrometry (MS), capillary electrophoresis (CE), HPLC-MS with negative ion electrospray ionization-tandem MS and solid-phase extraction (SPE) (O'Donnell *et al.* 1995; Martinez *et al.*1998a, 1998b, 1999, 2000; Aher and Buchberger 1999; Kuklenyik *et al.* 2002). A new technique, electrospray ionization-high field asymmetric waveform ion mobility spectrometry-mass spectrometry (ESI-FAIMS-MS), was developed (Ells *et al.* 1999, 2000a, 2000b, 2000c; Gabryelski *et al.* 2003). FAIMS provides fast and sensitive analysis for drinking water samples. For urine samples, the salt content limits the achievement of sensitivity of analysis by FAIMS. Solid-phase microextraction (SPME) integrates sampling, extraction, concentration and sample introduction in a single step (Sarrion *et al.* 1999, 2000a, 2000b, 2002, 2003; Wu *et al.* 2002).

Wu *et al.* (2002) developed a liquid-liquid microextraction (LLME) and SPME combined with GC-ECD method to analyze TCAA, DCAA, and other DBP compounds in samples of tap water, urine, and blood using only 50–100 μl of sample volume. The LLME-SPME-GC-ECD allowed analysis of DBP compounds in water and biological samples with acceptable speed and precision. This method yielded relative standard deviations of 1.1 to 14% for DCAA concentrations ranging from 10 to 4600 μg/L and 0.5 to 13% for TCAA concentrations ranging from 6 to 600 μg/L for water samples. Recoveries of DCAA and TCAA were 86 to 110% in rodent urine and 82 to 110% in rodent blood.

Types of Specimens

Determination of the types of sample collection depends on the study purpose, exposure conditions, elimination half-life, and sensitivity of analytical methods. The preferred samples are adequately concentrated to ensure detection of a biomarker of interest (Brunzel 1994). Three types of samples are frequently used in environmental epidemiological studies: breath, blood, and urine.

The collection procedure for breath samples is non-invasive. For volatile organic xenobiotics such as THMs, the concentrations in alveolar air can be used to estimate the current exposure (Weisel *et al.* 1999; Xu and Weisel 2005; Nuckols *et al.* 2005). The major exposure routes from showering, bathing, or swimming are inhalation and dermal contact. The THMs can be easily inhaled or absorbed through dermal contact, metabolized to carbon dioxide and/or carbon monoxide in the liver, and rapidly exhaled (Fry *et al.* 1972; NAS 1987). Human exposure may come from airborne THMs such as chloroform released from tap water (Jo *et al.* 1990a, 1990b). The pulmonary excretion of chloroform occurs between 15 min and 2 hours (WHO 2000). The most practical type of sample for measuring THMs is exhaled breath. The concentrations of THMs in alveolar air can fluctuate very rapidly. The time of sampling is critical (WHO 1996). Breath or blood sampling was not commonly used for people exposed to THMs via drinking water ingestion because THMs are metabolized rapidly in the liver.

The collection procedure for blood samples is invasive. The blood concentration of a xenobiotic is related to current exposure or to past exposure for compounds such as PCBs. HAAs are semi-volatile compounds and can be measured in the blood. The permeability of HAAs via the skin is very low (Xu *et al.* 2002; Xu and Weisel 2003). The daily exposure dose resulting from showering, bathing, or swimming is insignificant for HAAs (Kim and Weisel 1998; Xu *et al.* 2002; Xu and Weisel 2003). For example, the proportion of absorbed doses of TCAA from daily bathing via dermal contact is about 0.005% to 0.5% of the daily ingestion doses of TCAA (Cleek and Bunge 1993; Xu *et al.* 2002). TCAA enters the human body mainly via tap water intake. After absorption, TCAA is highly bound to

plasma proteins in blood (Paykoc and Powell 1945; Marshall and Owens 1954; Sellers and Koch-Weser 1971; Muller *et al.* 1972; Monster *et al.* 1976). TCAA measured in whole blood reflects the total body burden of TCAA and is related to the duration of exposure or to concentrations stored in body tissues. For continuing exposure to TCAA in drinking water over two weeks, blood TCAA reflects the equilibrium between daily intake and excretion. TCAA in serum or urine reflects the free TCAA that can be eliminated from the body. The analysis of TCAA in blood can be used because of less extensive metabolism of TCAA and sufficiently long elimination half-life. Therefore the blood level of TCAA is an important exposure index with a high specificity.

Urine is an ultrafiltrate of the plasma. Urine samples are usually used to measure water soluble metabolites or parent xenobiotics. The concentrations of a xenobiotic or metabolite in urine reflect its proportional blood concentrations during the period of urine accumulation in the bladder (WHO 1996). Kidney function will affect the urinary concentration of a xenobiotic.

The procedure of urine sample collection is non-invasive. There are four types of urine samples: first morning, random, fractional, and timed samples (Brunzel 1994). The first morning urine (FMU) sample is ideal for detecting xenobiotics which can be concentrated in the urine and its results display less variation from weighted-average concentrations (Que Hee 1993; Kissel *et al.* 2005). The FMU involves urine collection spanning the period from bedtime until the first urine void the following morning. Random urine samples (collected at any time) are used for routine screening and cytological studies. Fractional urine sampling (collecting the second urine sample after discarding the FMU sample) is used to compare the concentration of a xenobiotic in urine to that in blood. Timed urine samples are collected over a 12 to 24 hour period. This type of sample is used to determine the concentrations of xenobiotics with long half-lives and to compare excretion patterns. The timed urine sample is not frequently used in routine biomonitoring because of the burden that it places on the sampling subjects.

The relationship between TCAA ingestion and excretion was studied by Kim *et al.* (1999). FMU and random samples were collected and compared to measure urinary TCAA concentrations. Correlations between TCAA ingestion and excretion were observed for the FMU samples, but not for random samples. FMU samples were also used in other studies (Weisel *et al.* 1999; Froese *et al.* 2002; Bader *et al.* 2004).

Urinary TCAA concentrations are affected by the rate of urine production. The traditional practice for reducing this variation is to correct to creatinine excretion. The determination of urinary creatinine is recommended to normalize over-diluted or over-concentrated urine samples. Creatinine is the metabolic product of muscle tissue and is a normal constituent of urine. Creatinine is excreted by glomerular filtration at a relatively constant rate of 1.0–1.6 g/day (Rosenberg *et al.* 1989) but 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 (Elkins 1974; Boeniger *et al.* 1993; Mage *et al.* 2004; Barr *et al.* 2005; Kissel *et al.* 2005). The creatinine concentration is inversely related to fluid intake. Analyses performed on very dilute (less than 0.3 g/L) or concentrated (greater than 3 g/L) urine samples must be interpreted with caution (Rosenberg *et al.* 1989). When the extremes of the creatinine variability are observed, the creatinine correction is not valid. Some studies report that the correction of the excretion of some compounds to urinary creatinine improved biological monitoring to a limited extent (Edwards *et al.* 1969; Bailey and Wardener 1970; Curtis and Fogel 1970; Greenblatt *et al.* 1976; Wilson and Crews 1995).

Background Levels

Understanding the range values of a biomarker of exposure in the general population is essential to evaluate intraindividual and interindividual variability (Schulte and Talaska 1995). The baselines of THMs in blood ranged from 2.3 to 3.3 μg/L (Backer *et al.* 2000). The mean blood chloroform concentration ranged from 11.4 to 21.7 ng/L in three communities in North Carolina (Savitz *et al.* 2005). Seasonal variation of blood THMs was observed. No clear correlation of blood THM levels with THM concentrations in tap water was observed (Miles *et al.* 2002; Savitz *et al.* 2005). Elevated breath concentrations of chloroform have been observed after showering (Xu and Weisel 2005; Nuckols *et al.* 2005). The median background exhaled breath concentration of THMs was 3.5 $\mu g/m^3$ (Wallace *et al.* 1984). An exposure-biomarker relationship has been identified between THM breath concentrations and both water THM concentrations and THM exposure from a shower (Weisel *et al.* 1999).

TCAA was identified in urine samples in the general population and demonstrated an exposure-biomarker relationship between urinary TCAA and ingestion of TCAA-containing water (Kim *et al.* 1999; Weisel *et al.* 1999; Bader *et al.* 2001, 2004; Froese *et al.* 2002; Calafat *et al.* 2003). 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).

Measurement of Variability – Reliability

The major sources of biomarker variability that influence epidemiological studies are intraindividual and inteindividual variability, and variability resulting from measurement errors (Vineis 1997). Evaluating intraindividual and interindividual variability for a biomarker of exposure in the general population is a priority in the validation process.

Interindividual variability is related to source variation, external exposure variation, and metabolism variation among individuals. It can arise from complex interactions affected by genetic factors such as ethnicity and gender or other characteristics such as lifestyle (Vineis 1997). Appropriate biological markers can take into consideration the sources of interindividual variability. The effect of interindividual variability can be reduced by increasing the sample size and study power.

In TCAA biomarker studies, sources of interindividual variability can arise from exposure to other compounds that may compete for the same biotransformation sites or metabolize to TCAA in the human body. CH is one of the DBP compounds measured in drinking water. CH is rapidly metabolized in the liver and other tissues to trichloroethanol, trichloroethanol-glucuronide, and TCAA (Breimer *et al.* 1974; Marshall and Owens 1954; Reimche *et al.* 1989; Gorecki *et al.* 1990; Humbert *et al.* 1994). A small fraction of CH is oxidized to TCAA and a large amount of CH is reduced to alcohol (Butler 1948). In humans, 8% of the administered dose of CH is directly oxidized to TCAA and additional TCAA is formed during enterohepatic circulation of trichloroethanol (Lash *et al.* 2000). Thirty five percent (a range of 5%–47%) of an initial dose of CH is converted to TCAA (Marshall and Owens 1954; Sellers *et al.* 1972; Allen and Fisher 1993; 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).

External exposure variation of blood THM concentrations was observed in a study conducted by Savitz *et al.* (2005). THM concentrations in both blood and tap water showed substantial seasonal variation, with higher levels in summer and lower levels in winter. Such external exposure variation contributes to interindividual variability.

The differing rates of metabolism of a xenobiotic in humans is attributable to interindividual variability. The contribution of cytochromes P450 (CYP) 2E1 and 2B1/2 to chloroform metabolism has been identified in animals (Nakajima *et al.* 1995). Metabolism of TCAA generates free radicals and induces lipid peroxidation (Larson and Bull 1992; Ni *et al.* 1996). CYP2E1 is the major isozyme for the metabolic activation of TCAA. Wide interindividual variability was found in the CYP2E1 activities (Peter *et al.* 1990; Chang *et al.* 1993; Raucy *et al.* 1995; Lieber 1997; Clewell III *et al.* 2000; Pastino *et al.* 2000; Bebia *et al.* 2004).

Intraindividual variability can result from various sources such as sample collection, transportation, storage, and random errors inherent in laboratory analysis (Vineis 1997). The effects of the storage conditions of urine samples on measured levels of some environmental contaminants were studied (Hoppin *et al.* 2005). The duration of storage had a larger impact on the measured levels of xenobiotics in urine than did the storage temperature.

Biological factors unrelated to measurement errors can influence variability as well. Individual daily diet, physical activity, and lifestyle are determinants of variability for a biomarker. Intraindividual variation can influence considerably the misclassification of exposure. The effect of intraindividual variability can be reduced by taking multiple samples.

Both intraindividual and inteindividual variability were observed in the Froese *et al.* study (2002). The variability of urinary TCAA excretion over 12 days of tap water ingestion was examined in 10 participants. The relative standard deviation (RSD) ranged from 14% to 67% for TCAA-containing tap water ingestion. Large variability of TCAA concentration between different homes, distribution systems, and sample dates (RSD=100%) and volume of fluid intake (RSD 30%–300%) was observed. The urinary TCAA excretion variability varied from 15% to 71% of the RSD. This study revealed that interindividual variability was substantial in TCAA ingestion and excretion.

The intraindividual and interindividual variability related to random errors can be evaluated through multiple sampling/measurements in a large-scale background survey (Hopkins 2004). The multiple measurements involve (1) multiple analytical measurements of the same sample, (2) multiple measurements of urinary TCAA for one individual over time to estimate the intraindividual temporal variability, and (3) multiple measurements across different individuals to estimate the interindividual variability.

A commonly used measure of the extent of variability for continuous data is the coefficient of variation (Vineis 1997). Reliability analysis is a quantitative analysis of random and non-random variation (bias). Quantitative indices of the extent of random variation of a biomarker can provide insight into whether the reliability of a measure is sufficient for the purpose of study.

Types of reliability include (1) inter-observer reliability, (2) test-retest reliability, (3) internal consistency reliability, and (4) parallel-forms reliability (Nunnally 1978; Koepsell and Weiss 2003; Trochim 2005). Inter-observer reliability refers to the measurement of the extent to which different observers provide consistent estimates of the same phenomenon. Statistics used for this type are Kappa coefficients for nominal/categorical data, Kendall's *tau* for ordinal data, and Pearson's correlation coefficient for continuous data (Cohen 1960; Fleiss 1980; 1986; Thomas *et al.* 1993; Koepsell and Weiss 2003). Parallel-form reliability refers to the assessment of the consistency of the values of two tests or two forms of instruments constructed in the same way (Trochim 2005).

Test-retest reliability refers to the assessment of the consistency (stability) of a measurement (Kelsey *et al.* 1996). Intraindividual reliability refers to the agreement of measurements within the same individual when tests are repeated. Interindividual reliability refers to the agreement of measurements between individuals. Interindividual reliability is affected by all the sources of error

contributing to intraindividual reliability as well as any differences between individuals. Often, high interindividual reliability is sufficient. If the interindividual reliability is low, it may be necessary to continue to an intraindividual reliability study to locate the source of unreliability.

There are three components of statistics for test-retest reliability: change in mean, change in standard error of measurement (SEM), and retest correlation (reliability coefficient) (Hopkins 2004). The change in mean provides the overall variance. The SEM provides an index of the degree of error in an individual's value. Reliability coefficients such as the Pearson correlation coefficient, or the Spearman *rho*, or the intraclass correlation coefficient (ICC) indicate the overall precision of a set of test estimates.

The reliability coefficient refers to the proportion of the total variance in the measurements resulting from the "true" differences between individuals. This "true" difference consists of the error variance and the variance between individuals (Trochim 2005). The magnitude of the coefficient is directly related to the variability between individuals. The product-moment (Pearson) correlation coefficient measures the correlation between the first and second sets of imperfect measurement (Kelsey *et al.* 1986). The ICC reflects differences in mean estimates and the degree of correlation between the two sets of measures, which is the analysis of variance (Shrout and Fleiss 1979; Snedecor and Cohran 1980, Fleiss 1986, Kelsey *et al.* 1986). The values range from 0 to 1.0. The larger the ICC, the more reliable the measurement. The ICC also indicates the extent of between-individual variability relative to total variability. Total variability includes reproducibility, repeatability, and sampling variation. The ICC approach was used for reliability analysis in some environmental epidemiological studies (Ryan *et al.* 2000; Pang *et al.* 2001; Hoppin *et al.* 2002; Hryhorczuk *et al.* 2002; Hauser *et al.* 2004; Meeker *et al.* 2004).

Internal consistency is a measure of the extent to which items in a test are homogeneous. It consists of the average inter-item correlation, the average item total correlation, and split-half reliability (Hopkins 2004; Trochim 2005). The average inter-item correlation is a calculation of the correlation of each item with all other items. The average item total correlation is a calculation of the correlation of each item with total items. Split-half reliability is determined by randomly dividing the test into two parts and calculating the correlation for each part. Cronbach's α is used for continuous data and Kuder-Richardson for dichotomous data. Cronbach's α estimates the reliability and is obtained by combining a given number of separate measures into a single composite. It is the proportion of the observed variance resulting from true differences among individuals in the sample (Cronbach 1951). The values range from 0 to 1.0. The larger the Cronbach's α, the more consistent the measurement.

Validity

Biomarker sensitivity refers to external exposure levels that can be detected by means of a biomarker. Highly sensitive biomarkers can detect a low level of external exposure. In the four TCAA biomarkers studied, urinary TCAA excretion was sensitive to TCAA ingestion in tap water (Kim *et al.* 1999; Weisel *et al.* 1999; Froese *et al.* 2002; Bader *et al.* 2004).

Biomarker specificity refers to the probability that the biomarker is an indicator of actual exposure to the specific xenobiotic in the environment. For mixed exposure, highly specific biomarkers can be used to identify the xenobiotic nature of exposure. A single biomarker sampling can be used to perform exposure screening in the general population. Based on current knowledge, the parent TCAA can be specifically measured in urine because it has a relatively longer half-life as compared to THMs and DCAA (Kim *et al.* 1999; Weisel *et al.* 1999; Froese *et al.* 2002; Calafat *et al.* 2003; Bader *et al.* 2004). If a good correlation between TCAA and THMs in water treatment plants or distribution systems can be identified, urinary TCAA can also be used as a surrogate for THM exposure in drinking water. Urinary TCAA can also be used as an exposure indicator for screening DBP exposure in the general population.

A predictable relationship between external exposure and a biomarker has to be identified in a validity study of that biomarker (Decaprio 1997). When kinetics are linear and exposure distribution is stationary, the mean value of a biomarker measured repeatedly in an individual over time should be proportional to the mean exposure over the same period (Rappaport *et al.* 1993, 1995). The linear relationship is measured by determination of a coefficient. The linear relationship reflects the kinetic process, the variability in the rate of absorption, distribution, metabolism and elimination, the specificity of a biomarker, and intraindividual and inteindividual variability. Poor correlation indicates either a nonlinear kinetic process, or other factors associated with exposure routes, individual differences, specificity, sample size, or lack of precision of the laboratory assay.

Unmeasured Confounders and Effect of Markers

Confounding factors are often difficult to measure in exposure assessment. Such unmeasured confounders can cause measurement error. For example, some solvents such as TCE, tetrachloroethylene (PERC), trichloroethanol, trichloroethane (TRI), and tetrachloroethane can be metabolized to TCAA in the human body (Nomiyama 1971; Monster 1979; Caperos *et al.* 1982; Bruckner *et al.* 1989; Inoue *et al.* 1989; Fisher *et al.* 1998, 2000; Volkel *et al.* 1998; Bloemen *et al.* 2001, Furuki *et al.* 2003). These solvents are used as vapour for metal degreasing or in cold cleaning agents, dry cleaning, printing, printing ink, and some consumer products such as typewriter correction fluid, paint remover, adhesive, stain remover, and rug-cleaning fluid (Aggazzotti *et al.* 1994a, 1994b; WHO 1985; IARC 1995). TCE was detected in treated water in Canadian water supply facilities at levels ranging from 0.1 to 1.0 μg/l (Otson *et al.* 1982; Health

Canada 1992; WHO 2000). One to three percent of the absorbed PERC, 0.5% to 6% of TRI, and 20% to 40% of TCE were metabolized to TCAA in humans (Monster 1979; Nomiyama 1971; ATSDR 1995, 1996). In a survey of background levels, statistically significant correlations between urinary TCAA and blood TRI/TCE were observed (Calafat *et al.* 2002). In biomarkers of exposure survey, the use of the above solvents may not be identified as contributing to urinary TCAA.

Other factors (age, sex, ethnicity, and lifestyle) can affect the measurement of a biomarker of exposure. The measured TCAA concentration in blood and urine depends mainly on TCAA concentrations in water and on the volume of water consumed by individuals. Water consumption patterns vary greatly among different ages, genders, and ethnic groups, different geographic areas, and time-activities within individuals. Children or youth aged 11 to 19 consumed higher volumes of community water than adults 20 years and older (EPA 2000; Raman *et al.* 2003). Men ingested more community water than women (EPA 2000; Raman *et al.* 2003). Significant ethnic variation in water intake was observed in terms of availability of water sources (Williams *et al.* 2001). Pregnant women ingested more tap water at home than they did at work or otherwise outside the home (Shimokura *et al.* 1998). The quantity of a biomarker of exposure can change within an individual over time as a result of changes in diet, health status, and time-activities (Vineis 1997).

2.4 Summary

Disinfection by-products are a set of compounds that are formed during chlorination for disinfection of drinking water. Chlorinated and brominated compounds have attracted the greatest attention. More than 250 DBPs can be identified or measured in treated water at water treatment plants, within the distribution system, and in tap water. The two most abundant classes of DBPs are volatile compounds of THMs and non-volatile compounds of HAAs. DCAA and TCAA are the principal species of HAAs. The TTHMs correlate strongly with chloroform because it is usually the largest component of TTHMs. Depending on the nature of water treatment and storage of each system, TTHMs may not be a good surrogate of exposure for other specific THMs or specific DBPs, especially brominated species. The correlation between THMs and HAAs depends on water treatment processes, bromide content, and potential for volatilization.

In reproductive and developmental epidemiology studies on DBPs, the exposure assessment relied on some surrogates such as residences, water sources, routine THM monitoring in treatment plants or distribution systems, and volume of water intake. These methods were inadequate to classify an individual exposure during a specific time window and are expected to cause attenuation in health risk estimates if exposure misclassification is random and non-differential.

TCAA can be selected as a potential biomarker for DPB exposure in drinking water. TCAA is readily absorbed by the human body via ingestion of drinking water. It can be metabolized into CO_2, DCAA, and other compounds. The elimination half-life ranges from 30 hours to 6 days in humans. Background urinary TCAA levels have been measured in an urban population and a relationship between TCAA ingestion and excretion has been observed. Laboratory methodology has been developed to allow detection of low levels of TCAA in water, blood, and urine samples.

Validation processes for a biomarker of exposure include understanding of the purpose of using a biomarker, knowledge about the natural history of a biomarker and the relationship between a biomarker and exposure, toxicokinetics, temporal and spatial variability, persistence, laboratory methodology and types of specimens, determination of background level, evaluation of reliability (intraindividual and interindividual variability), validity (sensitivity, specificity, and predictive value) and relevant statistical analysis, selection of an appropriate biomarker (representative, surrogate, correlation, and prediction), and identifying the effects of confounder factors (e.g., age, sex, ethnicity, and lifestyle) on the marker.

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CHAPTER 3 CHARACTERIZATION OF EXPOSURE

In past decades, many epidemiological studies addressing adverse human reproductive and developmental outcomes associated with exposure to drinking water DBPs have been published (Aschengrau *et al.* 1989, 1993; Aggazzotti *et al.* 2004; Bove *et al.* 1995, 1996; Cedergren *et al.* 2002; Deane *et al.* 1992; Dodds *et al.* 1999; Dodds *et al.* 2004; Dodds and King 2001; Fenster *et al.* 1992; Fenster *et al.* 2003; Gallagher *et al.* 1998; Graves *et al.* 2000; Hert-Piccitto *et al.* 1989; Jaakkola *et al.* 2001; Kallen and Robert 2000; Kanitz *et al.* 1996; King *et al.* 2000; King *et al.* 2005; Klotz and Pyrch 1999; Kram *et al.* 1992; Magnus *et al.* 1999; Savits *et al.* 1995; Savitz *et al.* 2005; Shaw *et al.* 1990, 1998, 2003; Toledano *et al.* 2005; Tuthill *et al.* 1982; Waller *et al.* 1998; Windham *et al.* 1992; Windham *et al.* 2003; Wrensch *et al.* 1992; Wright *et al.* 2003;Yang *et al.* 2000a, 2000b, 2004). A major limitation of all of these studies was the lack of accurate exposure classification (Arbuckle *et al.* 2002). In order to improve current exposure assessment of epidemiological studies pertaining to DBP exposure in drinking water, development of a useful biomarker of exposure is critical.

Research exploring urinary TCAA for DBP exposure was carried out in recent years (Kim *et al.* 1999; Weisel *et al.* 1999; Bader *et al.* 2004; Froese *et al.* 2002; Calafat *et al.* 2003). Some characteristics of urinary TCAA excretion associated with TCAA ingestion were examined in two pilot studies conducted by the Environmental Health Sciences group of the University of Alberta (Bader *et al.* 2004; Froese *et al.* 2002). In these two pilot studies, the elimination half-lives of TCAA, which ranged from 2.1 to 6.3 days, were examined in eight volunteers. In the Froese *et al.* study (2002), large variation of urinary TCAA excretion was observed within and between individuals. These results indicated the need for better controlled exposure studies of a large cohort to validate urinary TCAA as a potential biomarker of exposure to DBPs in drinking water.

As the follow-up of the suggestions from the pilot study, the validation TCAA biomarker study of a large cohort was designed for this project. The objectives of this study were to provide an understanding of the relationships between TCAA ingestion and urinary TCAA excretion and/or blood loading, to assess intraindividual and interindividual variability, to identify potential confounding factors, and to evaluate the feasibility of the use of TCAA as a biomarker in field studies. The re-evaluation of the elimination half-lives of TCAA in this large cohort was not included in order to limit participants' commitment to a maximum of 15-days tap water consumption, a reasonable level for volunteer participation.

In this chapter, the details of the study design, recruitment process, sample collection, laboratory methodology, QA/QC for the laboratory analysis, statistical methods, and the results of the exposure gradient to TCAA, and background and measured levels of TCAA in water and biological samples are described and the results are discussed.

3.1 Materials and Methods

3.1.1 Recruitment

The proposal for this research project was approved by the Health Research Ethics Board at the University of Alberta, Edmonton, Alberta, Canada. All volunteers were recruited based on the following criteria:

- Female
- Aged between 18 to 45 years old
- Healthy (no known health conditions and no medications in use)
- Non-pregnant
- Living in City B (in City B tap water contains very low TCAA concentrations) between January 2003 and April 2004
- Drinking tap water
- Literacy in English

The volunteers were students and staff at the University of Alberta. Recruitment information was posted in the newsletter of the Graduate Students' Association. A total of 209 individuals responded via e-mail to the request for volunteers. Detailed study information was sent to all respondents.

Seventy-four (35%) individuals were interested in being interviewed by telephone after receiving the detailed information. During the telephone interview, the following information was collected: demography, 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.

Fifty-six individuals were selected for the second in-person interview. During the second interview, the volunteers signed consent forms, answered a few questions about their detailed volume 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. Twenty-two people from the original respondents were not recruited after these two interviews because of use of medication or inappropriate schedules for sample collection. A total of 52 volunteers were finally selected for participation in the study.

3.1.2 Exposure Groups

The fifty-two participants were randomly stratified into five sub-groups. Each exposure level was assigned to a different exposure status. Tap water shipped from City A was used as the major TCAA exposure source in this study. City A is

one of larger cities in Canada. In City A, TCAA concentrations in tap water were relatively higher than those in City B.

These five exposure levels were defined as follows:

Group 1: Control group – TCAA-free bottled water;
Group 2: 12.5% TCAA-containing tap water from original tap water shipped from City A (87.5% TCAA-free bottled water);
Group 3: 25% TCAA-containing tap water from original tap water shipped from City A (75% TCAA-free bottled water);
Group 4: 50% TCAA-containing tap water from original tap water shipped from City A (50% TCAA-free bottled water); and
Group 5: 100% TCAA-containing original tap water shipped from City A.

The number of participants in each exposure group was determined according to the requirements for sample size and distribution of TCAA ingestion and excretion values that would allow the researchers to achieve a linear relationship in a statistically significant manner. An initial data analysis was performed in the mid-study. The largest variation in the relationship between TCAA ingestion and excretion was observed among the individuals assigned to the higher exposure groups. More individuals were recruited to the higher exposure groups to accommodate the final data analysis.

Participants were asked to commence the study on the first Wednesday after the completion of their menstrual cycle to preclude the likelihood of pregnancy. Each participant ingested supplied water every day for a 15-day period. A total of 3 L of water in three 1-L Nalgene bottles were 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. Three supplied bottles were collected from each participant the following morning. The remaining volume of water in the bottle(s) was recorded. Each day in their diaries, participants recorded the physical activities of the day, and their beverage consumption in addition to water consumption.

3.1.3 Water Samples

Fresh tap water in 20-L polycarbonate carboys was shipped from City A to City B every Tuesday via overnight courier. Spring bottled water was delivered based on a commercial company's delivery schedule (about 1.5 per week). The water was stored in a cool, clean room in the Provincial Public Health Laboratory for Microbiology. In order to protect water from external contamination, a trained research assistant regularly cleaned the spigot of the cooler using an alcohol-swab.

Water samples were sent to the Provincial Public Health Laboratory for Microbiology for water quality testing from Monday to Friday. These water quality tests included the heterotrophic plate count (HPC), total coliforms presence/absence test, and *Escherichia coli* presence/absence test.

Tap water samples from each carboy were sent to the Environmental Health Sciences Laboratory (EHS Lab) every Monday and Thursday for laboratory analysis of TCAA, DCAA, chloral hydrate (CH), chloroform (TCM), bromodichloromethane (BDCM), dichloroacetonitrile (DCAN), and 1,1,1-trichloropropanone (TCP). Spring water samples were sent to the EHS Lab every Wednesday for the same chemical analysis. Tap water samples were also sent to the Centre for Toxicology Laboratory for trace metal analysis. A total of 22 trace metals were analyzed: aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, molybdenum, nickel, selenium, silver, thallium, titanium, vanadium, and zinc.

All 1-L Nalgene bottles and sample bottles were cleaned using dishwasher detergent, then rinsed with tap water from City B followed by Milli-Q water. Plastic bottles were dried in the air and glass bottles were dried in the oven. All cleaned bottles were stored in the cool, clean room.

All Nalgene bottles were labeled with each participant's temporary identifier, date of ingestion, and number order of the bottles. Each bottle was filled with tap water with designated concentrations based on each participant's exposure level. Three bottles were delivered to each participant per day from Monday to Thursday. Nine bottles were delivered to each participant on Friday for water consumption from Saturday to Monday. All bottles were stored in the refrigerators in the participants' homes.

3.1.4 Biological Samples

Urine

A urine collection kit with instructions for urine collection was prepared for each participant. The kit included one funnel, one 1-L clean urine collection container labeled with each participant's ID and date of collection, and one Ziploc plastic bag. All items were packed into a small size cooler with one ice-pack.

The urine samples were collected on Day 1 before supplied tap water consumption and on Day 2, 8, 13, 14, 15, and 16 after supplied tap water consumption. The urine collection kit was delivered to each participant one day prior to urine collection. Participants were advised to avoid water consumption close to bedtime. They collected the entire volume of urine within 30 minutes after waking up in the morning and before drinking any liquid so that it 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 hours of urine collection and immediately delivered to the EHS Lab. The volume of urine was measured and recorded. The bottle was refrigerated at 4 °C prior to chemical analysis.

Blood

Provision of a blood sample was optional for each participant. Blood samples were collected on Day 1 before supplied water consumption and on Day 8, 14, and 15 after supplied water consumption. A list of designated clinical lab sites for blood collection was provided to each participant. Whole blood samples were collected in a private laboratory and delivered to the EHS Lab within 24 hours for TCAA analysis. Serum samples were collected for serum creatinine analysis. The 200 µl whole blood samples were stored in the Provincial Public Health Lab for Microbiology.

3.1.5 Laboratory Analysis

Water (0.1 mL), or urine (0.1 mL) or blood (25–50 µL) and 0.1 M acetate buffer (0.2 mL, pH 5.2) were combined and vortex-mixed in a 1.5 mL polypropylene microcentrifuge vial in a fume hood. 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 and DCAA were extracted with 0.6 ml methyl *tert*-butyl ether (MTBE). After extraction, the organic layer (MTBE) was placed in a 2-ml autosampler 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 and DCAA were derivatized at 80 °C for 20 min. After derivatization, the sample was cooled down 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), desorbed for 2 min in the injection port of the GC, and detected with an ECD detector. 2,3 DCPA was used as an internal standard.

Analyses using liquid-liquid microextraction (LLME) headspace SPME were performed on a Varian CP 3800 GC-ECD coupled with an 8200 autosampler (Wu *et al.* 2002). A DB-1 MS fused silica capillary column (20 m x 0.18 mm I.D) with 0.4 µm film thickness was used, with helium as the gas carrier 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 injector temperature was maintained at 200 °C and the detector temperature

was 260 °C. Two μl liquid was injected in pressure pulse splitless mode at 30 psi for 0.1 min in the LLME method. The SPME fiber was desorbed for 2 min in splitless mode in the LLME SPME method.

The estimated detection limits (EDL) were 0.5 μg/L for DCAA and 0.2 μg/L for TCAA in this study. The EDL is the method detection limit or a level of compound in a sample yielding a peak in the final extract with a signal to noise ratio of approximately five (USEPA 1995). The method detection limit (MDL) is a statistically defined method attribute. Measured results falling at or above this point indicate the presence of an analyte in the sample with a specified probability (>99% confidence) with assumption of controlling sources of error in identification or biases in measurement (IUPAC 1997). In this study, the MDL was calculated as SD x 3.143, where SD was the standard deviation of the concentration obtained from a replicate standard (N=7) fortified with TCAA and DCAA at concentrations that produce peak intensities approximately five times the intensity of the background noise (Wu *et al.* 2002). The value of 3.143 refers to the Student's t value for 99% confidence with 6 degrees of freedom.

A blank was analyzed at the beginning of each analysis set. Milli-Q water and HPLC-grade water were used as reagent blanks for the analysis of water, urine, and blood samples. Laboratory fortified sample matrix analysis was performed by using human urine and blood samples. The samples were donated from a volunteer who consumed drinking water from City B. The purpose of this performance is to determine whether the urine and blood sample matrix contributes bias to the analytical results (USEPA 1995). Duplicate analysis was performed for all types of samples. Triplicate analysis was performed in one of eight water samples and in one of five urine or blood samples. Some urine samples were analyzed four times.

Laboratory multiplicate analysis was performed for samples of water, urine, and blood. 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 determined by extraction of a sample fortified with known concentrations of the TCAA standard. The calculation of the percent recovery (R) is

$$R = 100\ (A - B)/C$$

where A is the total measured concentration in the fortified sample for background concentration, B is the measured concentration in the unfortified sample, and C is the fortifying concentration.

3.1.6 Statistical Analysis

The data from 52 participants were included for variability analysis related to fluid and tap water intake. Total fluid intake refers to direct ingestion of water, coffee, tea, soft drinks, milk, beer, wine, and other beverages. Tap water intake refers to direct ingestion of supplied tap water. Three types of measurements were used in this study: (1) Self-Reported Total Fluid Intake was obtained from the questionnaire and was based on participants' recall of previous consumption patterns before the study period, (2) Self-Measured Total Fluid Intake was obtained from records in participants' diary booklets during the 15 days plus measured tap water in supplied bottles, and (3) Measured Tap Water Intake was directly measured each day from supplied bottles during the 15-day period. These three measurements were slightly skewed, but can be considered as approximately normal distributions. Pearson's correlation coefficients (R) were calculated for the three measures: self-reported total fluid intake, self-measured total fluid intake, and measured tap water intake.

The Exposure Day refers to the day that a participant ingested supplied tap water. The Urine Collection Day refers to the day that a urine sample was collected after 24 hours of ingesting supplied tap water. Analysis of urinary TCAA/DCAA excretion was based on the data collected from Urine Collection Days 13 to 16 because the steady-state of urinary TCAA/DCAA was expected to be reached after three or more elimination half-lives of TCAA/DCAA.

A statistical summary (arithmetic mean, median, standard deviation, range, and 95% confidence interval) of TCAA/DCAA levels in various samples is calculated as follows:

- TCAA/DCAA concentration in tap water: Exposure Days 1 to 15 for each individual;
- Amount of TCAA/DCAA ingestion: Exposure Days 1 to 15 for each individual;
- Urinary TCAA/DCAA excretion: Urine collection on Days 13 to 16 for each individual;
- Creatinine-adj. Urinary TCAA/DCAA: Urine collection on Days 13 to 16 for each individual;
- Amount of TCAA/DCAA excretion: Urine collection on Days 13 to 16 for each individual; and
- Blood TCAA/DCAA level: Blood collection on Days 14 to 15 for each individual.

Descriptive statistics and boxplots were performed for exploring the data. A t-test for independent samples was used to compare the means of TCAA excretion between different groups. A Pearson correlation coefficient was calculated to explore the relationship between two variables. The coefficient of variation (CV%) was calculated as:

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

where SD is the standard deviation and mean is the average of TCAA or DCAA measures in various samples.

3.2 Results and Discussion

3.2.1 General Information

The general information on the individuals is summarized in Table 3-1. A total of 52 women of reproductive age participated in the study. The criteria for recruitment were determined in order to control some important factors associated with the TCAA exposure in this human experimental study. Because of the concern about reproductive and developmental outcomes associated with TCAA and DCAA exposure in animal studies (Smith *et al.* 1989; Epstein *et al.* 1992; Linder *et al.* 1997; Johnson *et al.* 1998), women of reproductive age are highly relevant for recruiting to this study. Pregnant women were not selected in this study to avoid exposure to any unknown risk factors for them. In an epidemiological study related to reproductive and developmental outcomes, teleological alteration during pregnancy should be considered. Pregnant women experience physiological, biochemical, and anatomical changes (Koos and Moore 2003). During pregnancy, women may increase food and total fluid intake. The increased fluid intake can result in increased TCAA ingestion. Functional alteration of metabolism in the liver occurs during pregnancy, such as a decrease of production of albumin which is a protein in plasma that binds TCAA. The amount of protein-bound TCAA in plasma may decline. The glomerular filtration rate increases during pregnancy by about 50%.The renal plasma flow rate increases by as much as 25-50%. The renal alteration may increase the TCAA excretion rate in pregnant women.

Table 3-1 General Information

Item		Parameter
Period		May 2003 – April 2004
Sample Size		N = 52
Gender		Female
Age (yr)		Mean = 27, Range: 19 – 41
Education	Undergraduate	N = 9
	Graduate	N = 43
Compliance with Water Intake	50 out of 52	96%
Compliance with Urine Collection	47 out of 52	90%
Donation of Blood Samples	36 out of 52	69%

Healthy women were selected so that some confounders derived from other exposure sources and alteration of health conditions could be controlled. Chloral hydrate (CH) can be used as medicine. A proportion of CH can be converted to TCAA in the human body (Marshall and Owens 1954; Sellers *et al.* 1972; Allen and Fisher 1993; Humbert *et al.* 1994). Some health conditions such as kidney malfunction can influence the excretion rates of TCAA in urine. Thus, women who used any medications or had any health conditions were not included for the study.

Women who live in City B were selected. Drinking water in City B is drawn from a river within the city limits and treated once with chlorine in the water treatment facilities. The chlorine is converted to chloramine before entering the distribution system. Chloramine is a weaker oxidant and has a stable residual time. The use of chloramines reduces the formation of TCAA (Singer 1993). Thus, lower concentrations of TCAA and other DBP compounds were observed in the tap water of City B. Thus, women living in City B have a relatively low level of TCAA exposure via drinking water ingestion. A low level of TCAA concentration in tap water could also minimize additional TCAA exposure from sources such as washing, bathing, and showering. Women who drink tap water every day were selected to ensure the test protocol was as consistent as possible with their normal behavior. Because low levels of TCAA in tap water from City B contributed to TCAA levels in the human body, the examination of background levels of TCAA in the biological samples of all volunteers was critical. (See Section 3.2.4.)

Compliance with water intake and urine sample collection was 96% and 90%, respectively (Table 3-1). Sixty-nine percent of volunteers donated blood samples. The high rates of compliance resulted from this specific population which was restricted to undergraduade and graduate students on campus. The advantages of using this sub-population included (1) minimizing the effects of confounders resulting from a large general population, and (2) improving compliance with water consumption and sample collection by utilizing the logistics of sample collection and the understanding of research processes in this group of individuals.

The volunteers consumed supplied tap water from City A. DBP levels in tap water were considerably different between City A and City B. 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 into the distribution system. The lake source disinfected by chlorination at several points along the water supply system results in a high level of DBPs in the tap water of City A.

Water quality tests were performed for all tap and bottled water supplied to the volunteers to ensure the safety of drinking water. The HPC only indicates contamination from all kinds of bacteria. During the entire study period of time, presence/absence tests for total coliforms and *E. coli* were negative. Sometimes,

HPC levels in the commercial water exceeded the standard. In this case, the water was immediately discarded after receiving the lab reports. The concentrations of trace metals measured in tap water were within the ranges of the *Guidelines for Canadian Drinking Water Quality* (Health Canada 1996a).

Quality assurance and quality control (QA/QC) of the laboratory analysis was performed during entire study. A total of 1460 water, urine, and blood samples, and 108 blank samples were analyzed. The recovery was performed based on the fortified sample matrix. A summary of the recovery of TCAA in the water, urine, and blood samples is presented in Table 3-2. Recovery of TCAA in the water samples ranged from 70% to 126% except for one sample 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 with a low value (51%) and five samples with high values (137%–149%).

Table 3-2 Recovery of TCAA in Various Samples

Concentration (μg/L)	Median	Mean	SD	Min.	Max.	CV
Water (N=9)						
10–100	91	89	22	61	126	23
Urine (N=9)						
4–100	86	90	11	77	108	12
Blood (N=76)						
4–200	101	104	18	51	149	17

3.2.2 Exposure Groups

The distributions of TCAA intake are illustrated in Figure 3-1 and Figure 3-2. The distribution of TCAA concentrations in supplied tap water is right-skewed. The distribution of amount of TCAA ingestion is approximately normal except for group 4. Statistics of TCAA concentrations, volume of fluid intakes and amount of TCAA ingestion for one control group (1) and four exposure groups (2–5) are summarized in Table 3-3. The median TCAA concentrations in four exposure groups ranged from 8 to 85 μg/L and the median amount of TCAA ingestion ranged from 23 to 171 μg/d. The mean volume of tap water intake was 2.3 L/d. The self-report method showed a mean volume difference of total fluid intake of 0.8 L/d compared to the self-measured method and the difference was 0.1 L/d between the self-reported method and the measured tap water method. There were no observed correlations between the self-report total fluid intake and the self-measured total fluid intake and between the self-reported total fluid intake and the measured tap water intake (R: 0.21–0.34, $p>0.01$). The correlation between the

self-measured total fluid intake and the measured tap water intake was good (R=0.87, p<0.001).

Table 3-3 TCAA Exposure in Various Groups

Group	Median	Mean	SD	Min.	Max.	95% Confidence Interval	
						Lower	Upper
TCAA Concentration in Tap Water (μg/L)							
1 (n = 6)	0	0	–	–	–	–	–
2 (n = 6)	8.3	9.5	2.9	7.4	14.6	6.5	12.5
3 (n = 9)	21.4	20.3	5.6	14.7	29.2	16.1	24.6
4 (n = 14)	38.7	39.1	8.3	29.5	53.2	34.3	43.9
5 (n = 17)	85.1	79.6	18.2	54.8	104.4	70.2	88.9
Volume of Fluid Intake (L/d)							
1-5 (Self-measured)[a]	3.0	3.0	0.8	1.7	5.0	2.8	3.2
1-5 (Tap water)[b]	2.5	2.3	0.6	0.9	3.0	2.3	2.7
1-5 (Self-reported)[c]	2.0	2.2	.9	.9	5.0	1.9	2.5
Amount of TCAA Ingestion (μg/d)							
1 (n = 6)	0	0	–	–	–	–	–
2 (n = 6)	23.2	25.0	10.0	13.6	43.1	14.5	35.4
3 (n = 9)	39.3	45.8	23.2	21.4	87.6	28.0	63.6
4 (n = 14)	84.7	95.4	31.9	52.5	159.4	77.0	113.8
5 (n = 17)	170.8	173.8	62.9	89.1	268.9	141.5	206.2

a: Self-Measured Total Fluid Intake (L/d) obtained from recorded values in diary booklet plus measured tap water intake; b: Measured Tap Water Intake (L/d); c: Self-Reported = Self-Reported Total Fluid Intake (L/d).

The amount of chemical ingestion is usually linked to daily fluid intake. Global data on fluid intake are limited. Studies from Canada, the US, the UK, and the Netherlands showed that the average daily *per capita* consumption was less than 2 liters (WHO 1996). In some studies from the U.S. and Canada, an average of total fluid intake ranged from 1.7 to 2.2 L/d (Ershow and Cantor 1989; Levallois *et al.* 1998; Shimokura *et al.* 1998; Jacobs *et al.* 2000; USDA 2000; Valtin 2002) and 2.2 L/d for 19- to 30- year-old women (IMNA 2004). An average of total tap water intake in the U.S. and Canada populations ranged from 0.8 to 1.4 L/d (CMNHW 1981; Ershow and Cantor 1989; Shimokura *et al.* 1998; Jacobs *et al.* 2000).

The average of self-measured total fluid intake was 3.0 L/d, ranging from 1.7 to 5.0 L/d in our study. The higher values of total fluid intake may result from the higher tap water intake. The average of tap water intake was 2.3 L/d. All participants were advised to drink supplied tap water as much as possible. The majority of participants had considerable physical activity. These factors may influence the higher values of tap water intake observed.

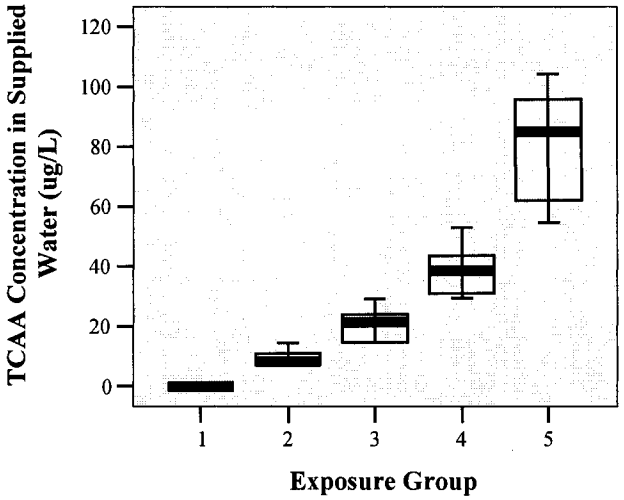

Figure 3-1 TCAA Concentrations in Supplied Water in Various Groups

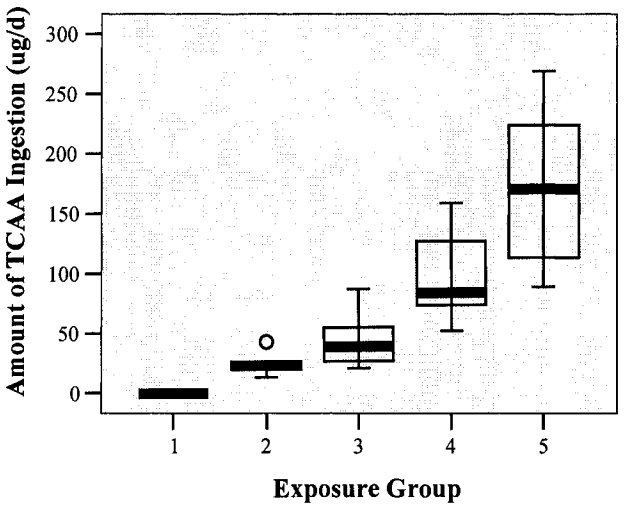

Figure 3-2 Amount of TCAA Ingestion in Various Groups

A good agreement (R=0.78) between questionnaire and diary responses for estimating drinking water intake has been reported (Shimokura *et al.* 1998). The correlation of cold tap water intake by using the questionnaire vs. the seven-day diary was good (R=0.79) at home, but not at workplaces (Kaur *et al.* 2004). The variability of water consumption patterns mainly resulted from individual differences such as volume of water intake and water consumption habits (Shimokura *et al.* 1998). In our study, the correlation of total fluid intake when comparing questionnaire and self /direct measures was not strong. The result may be due to the high volume of tap water intake by individuals during the15-days study periods of time. The results indicated that the rates of fluid/tap water intake from this study might not represent the rates from a general population.

The average of daily showering time was 10 min for women in the US (Shimokura *et al.* 1998) and 14 min for pregnant women (Zendar *et al.* 2002). Bathing was not a daily activity. Pregnant women usually took a bath averaging 28 min each time, three times per week (Zender *et al.* 2002). In the UK, pregnant women spent about 8 min/d for showering or bathing (Kaur *et al.* 2004). In our study, 50% of individuals took a bath during the study period of time. The average was 5 baths per 15 days, 23 min per time. Ninety-six percent of the individuals took showers with an average of 11 times per 15 days, 12 min per time. Seventy-nine of the individuals did dishwashing with an average of 7 times per 15 days, 12 min per time. The times of bathing and showering in our study were similar to those in US or UK populations.

3.2.3 TCAA and THMs in Tap Water

Seven DBP compounds were analyzed in tap water samples from City A and City B. The concentrations of measured DBPs, weekly variation of the concentrations, and correlations among measured DBPs are presented in Appendix II. The concentrations of TCAA and two major THM species, TCM and BDCM, in tap water are summarized in Table 3-4. Inter-city differences in the concentrations of three compounds were observed. The levels of the three compounds in tap water were higher in City A than in City B. The r values of the concentrations of TCAA with TCM and BDCM were 0.57 and 0.58 ($p<0.001$) in tap water from City A, and 0.02 and -0.33 ($p>0.05$) in tap water from City B.

Table 3-4 Concentrations of TCAA, TCM and BDCM in Tap Water

Concentration (μg/L)	Mean	Mean	SD	Min.	Max.	95% CI for Mean	
						Lower	Upper
City A (N=59)							
TCAA	79	80	20	45	130	75	86
TCM	134	132	23	77	168	126	138
BDCM	8.0	8.5	1.8	5.0	12	8.0	8.9
City B (N=28)							
TCAA	6.9	6.7	2.4	2.1	12	5.7	7.6
TCM	18	19	5.0	15	39	17	21
BDCM	1.4	1.2	0.7	0.1	2.1	1.0	1.5

THMs and HAAs are two predominant groups of DBPs in drinking water (Krasner *et al.* 1989; Singer *et al.* 1995). Because THMs were routinely monitored in the water treatment plants, the concentrations of THMs were used in most reproductive and developmental studies over the past decades. It is important to examine the relationship between THMs and HAAs in drinking water in order to understand the ability and limitations of THMs and HAAs to

represent other DBPs. In addition to variations in formation and stability of these two groups of DBPs, their respective physical/chemical properties also affect the relevance of different human exposure routes. Because THMs are much more volatile that HAAs, inhalation exposure in showering and bathing is a major human exposure route for THMs, but not for HAAs. Likewise, because THMs are much more lipophilic than HAAs, dermal exposure is much more important for THMs than it is for HAAs.

The median concentrations of total HAAs were approximately equal to the median total THM concentrations in US treated drinking water systems according to data from the USEPA's Information Collection Rule (Roberts *et al.* 2002). There was a strong correlation ($R=0.96$, $n=140$) between total THMs and the sum of 19 individual halogenated DBPs in the US drinking water (Krasner *et al.* 1989).

In another US study, TTHMs were moderately correlated with HAAs ($R=0.667$, $p<0.001$, $n=40$) (Hinckley *et al.* 2005). In a UK study, good correlations between TTHMs and chloroform ($R=0.98$, $p<0.01$, $n=1494$) or BDCM ($R=0.62$, $p<0.01$, $n=1494$) were observed (Keegan *et al.* 2001). In another UK study, correlations between TTHMs and THAAs in the water supply from three water companies varied from no correlation ($R=0.10$, $p>0.01$, $n=27$) to high correlation ($R=0.87$, $p<0.01$, $n=37$) (Malliarou *et al.* 2005).

When treatment conditions were relatively uniform and the water had a low concentration of bromide, a good correlation ($R=0.907$, $n=93$) between THMs and HAAs was observed in North Carolina drinking water (Singer *et al.* 1995). In the study from Spain, a good correlation between TTHM and THAA ($R=0.815$, $p<0.0005$, $n=18$) was reported (Villanueva *et al.* 2003). Some specific HAAs were correlated with specific THMs. For example, TCAA was fairly correlated to chloroform ($R=0.66$, $p=0.003$, $n=18$) in Spanish drinking water (Villanueva *et al.* 2003). In Nova Scotia, TTHMs for 140 household water samples were fairly or highly correlated with chloroform ($R=0.97$), BDCM ($R=0.63$), THAAs ($R=0.74$), DCAA ($R=0.70$), and TCAA ($R=0.65$) in tap water (King *et al.* 2004). In eastern Ontario, TTHMs for 214 household water samples were fairly or highly correlated with chloroform ($R=0.96$), THAAs ($R=0.52$), and TCAA ($R=0.56$) in tap water (King *et al.* 2004). TTHMs were weakly correlated with BDCM ($R=0.26$) and DCAA ($R=0.39$).

In a study from the Canadian prairies, strong correlations between DCAA plus TCAA and TCM plus BDCM (R: 0.92–0.97, $p<0.01$, $n=32–36$) were observed in drinking water from water treatment plants and distribution sites in a city in Alberta (Rizak *et al.* 2000). Conventional treatment is used in these water treatment plants. In the same study, water monitoring was performed for City A. The correlation between THAAs and TTHMs in one reservoir site was fair ($R=0.72$, $p<0.01$, $n=26$). Poor correlations were observed in the distribution system (R: 0.26–0.52, $p>0.01$, $n=18–36$). The poorer correlation likely resulted from loss of volatile THMs in the open storage reservoir in this city and possible

biodegradation of HAAs in the distribution system (Chen and Weisel 1998; McRae *et al.* 2004).

In our study, the correlation between TCAA and TCM/BDCM in tap water from City A was relatively low (R: 0.57–0.58, p <0.001). The results are consistent with Rizak's study. The poorer correlation may result from loss of volatile THMs in the open storage reservoir in City A and biodegradation of HAAs in the distribution system (Rizak *et al.* 2000; Chen and Weisel 1998). HAAs would not provide a good surrogate for THM exposure in drinking water from City A because of the loss of THMs that occurs in this system relative to the more stable, semi-volatile HAAs.

No correlation between TCAA and TCM/BDCM was observed in tap water from City B. This may arise from the use of chloramines in water treatment and the reduction in the levels of free chlorine and the formation of TCAA. HAAs cannot be used as a good surrogate for THM exposure in drinking water from City B.

The findings from our study and other studies indicated that whether or not TCAA can be used as a surrogate for DBP exposure in drinking water depends on the nature of both the water treatment and distribution systems, making the use of TCAA for this purpose very site-specific.

3.2.4 TCAA in Biological Samples

Background Levels

The background levels of TCAA in the urine and blood of participants are summarized in Table 3-5. Normal distribution of background TCAA levels was observed in urine samples. The variation of background levels of TCAA was larger in the urine measurements (CV: 150%–187%) than in the blood measurements (CV=92%).

Table 3-5 Background Levels of TCAA in Biological Samples

Group	Median	Mean	SD	CV%	Min.	Max.	95% CI for Mean	
							Lower	Upper
Urinary Excretion (n=52)								
TCAA Conc.	3.5	6.6	10	150	nd	52	3.8	9.4
Cr-adj. TCAA	2.2	5.3	10	187	nd	56	2.5	8.0
Amount of TCAA	2.6	6.2	10	167	nd	57	3.4	9.0
Blood (n=35)								
TCAA Conc.	8.7	13	12	92	2.3	54	9.2	17

Note: TCAA Conc (μg/L), Cr-adj. TCAA (μg/g cr), and Amount of TCAA (μg/d).

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). In our study, the median concentration of urinary TCAA was 3.5 μg/L (2.2 μg/g Cr) in a group of women. The background levels of TCAA in biological samples may result mainly from a low level of TCAA concentration in tap water from City B and possibly other minor TCAA sources.

Steady-State Levels

A previous pilot study in the Environmental Health Sciences Laboratory (Bader *et al.* 2004) was used to test and develop the experimental protocol for this study as well as to determine the urinary excretion half-life with a small group of volunteers (5) from the research group. The volunteers consumed TCAA-containing tap water obtained from City A. This study established excellent evidence for the urinary excretion half-life of TCAA (Figure 3-3) and found that it varied from 2.1 to 6.3 days with the observed coefficient of variation for the single compartment exponential decay (R^2: 0.76–0.94). Although they were obtained from a small cohort, these data were consistent with the experimental range of 30 hours to 6 days from the literature (Paykoc and Powell 1945; Muller *et al.* 1974; Monster *et al.* 1976, 1979; Humbert *et al.* 1994; Fisher *et al.* 1998; Bader *et al.* 2004).

The volunteer cohort for the current study was required to observe a rigorous set of requirements for the 15-day duration of their participation. The requirements of consistent consumption for 2 weeks plus collection of first morning urine samples for 3 to 4 weeks during the washout period as designed in the Bader *et al.* study was not realistic for volunteers from the community.

In this study, the urine samples were collected on the 1st day of study (before tap water consumption) and on the 2nd, 8th, 13th, 14th, 15th, and 16th days (after previous 24 hour water consumption). The urinary TCAA levels were relatively stable after the 8th day of urine collection (Figure 3-4). The urinary TCAA levels were statistically significantly higher after the 8th day of urine collection compared with the 1st and the 2nd days ($p<0.001$). There were no statistically significant differences among the TCAA levels on the 8th, 13th, 14th, 15th, and 16th days of urine collection ($p>0.05$).

The whole blood samples were collected on the 1st day of study (before tap water consumption) and on the 8th, 14th, and 15th days (after 24 hour water consumption). Blood TCAA concentrations were relatively stable after the 8th day (Figure 3-5). The blood TCAA levels were statistically significantly higher after the 8th day of blood collection than on the 1st day ($p<0.001$). There were no statistically significant differences of the TCAA levels among the 8th, 14th, and 15th days of blood collection ($p>0.05$).

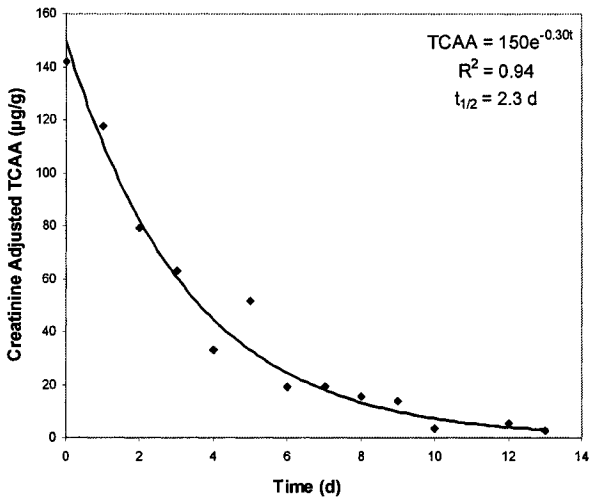

Figure 3-3 Example of Half-Life Determination of TCAA

[Source: previous pilot study done in the Environmental Health Sciences Lab at the University of Alberta by Bader *et al.* (2004), with permission]

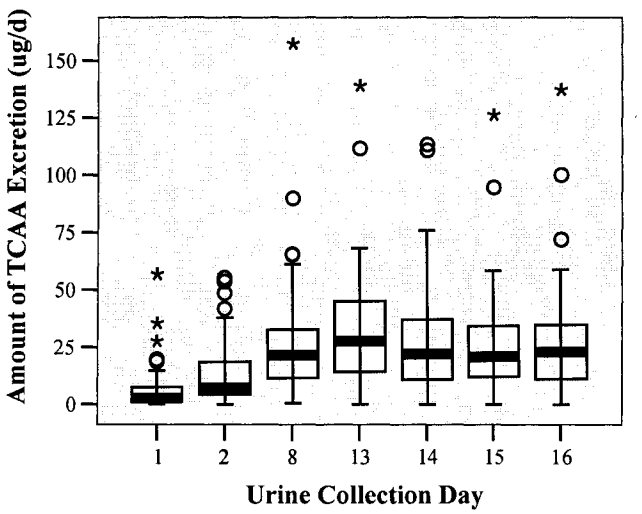

Figure 3-4 TCAA Levels in Urine with Increased Exposure Days

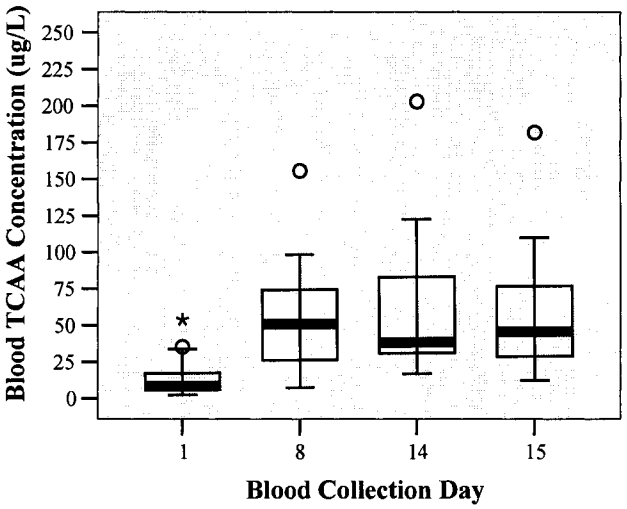

Figure 3-5 Blood TCAA Concentrations with Increased Exposure Days

The data obtained using the 15-day protocol described is not applicable to the direct determination of the excretion half-life that was already obtained in the previous pilot study. But the results indicated that the urine and blood TCAA levels reached quasi-steady-state levels after the 7-day tap water consumption.

TCAA Levels in Urine and Blood

The summary of TCAA excretion levels and blood concentration in different exposure groups after the 12th day of supplied tap water consumption are presented in Table 3-6. The distributions of urinary and blood TCAA levels were skewed (Figure 3-6). The rationale for calculating TCAA levels was that the steady state of TCAA levels was reached in the body after the 12th day of exposure in most individuals.

The median values in the control group were 1.5 μg/L for urinary TCAA concentrations, 1.4 μg/g Cr for Cr-adj. urinary TCAA, 1.2 μg/d for amount of TCAA excretion, and 6.1 μg/L for blood TCAA concentration. After the 12th day of supplied tap water consumption, the median values of TCAA in the four exposure groups ranged from 9 to 33 μg/L for urinary concentrations, 7 to 25 μg/g Cr for Cr-adj. concentration, 8 to 33 μg/d for amount of urinary excretion, and 26 to 73 μg/L for blood concentration.

TCAA levels in all types of samples increased significantly with increased exposure levels between the control group and the highest exposure group 5 ($p<0.05$). The results indicated that the increased urinary TCAA resulted from the ingestion of TCAA in tap water.

The variation of urinary TCAA measurements was larger in the control group (CV: 93%–130%) than in the four exposure groups (CV: 44%–75%). The variation of TCAA measurements was larger in the urine samples of the exposure groups (CV: 44%–75%) than in the blood samples of the exposure groups (CV: 31%–65%). The results indicated that the use of blood samples provided much more stable measurement compared to the use of urine samples.

Table 3-6 TCAA levels in Various Exposure Groups

Group	Median	Mean	SD	CV%	Min.	Max.	95% CI for Mean	
							Lower	Upper
Urinary TCAA Concentration (μg/L)								
1 (n = 6)	1.5[a]	1.5[a]	1.4	93	nd	3.0	0.01	3.0
2 (n = 6)	9.0[b]	7.5[b]	4.5	60	nd	11	2.8	12.2
3 (n = 9)	13	13	6.9	53	2.0	24	7.3	10
4 (n = 14)	26	25	11	44	5.8	43	19	40
5 (n = 17)	33	39	23	59	13	95	28	51
Cr-adj. Urinary TCAA Conc. (μg/g Cr)								
1 (n = 6)	1.4[c]	2.0[c]	2.6	130	nd	7.1	-0.8	4.7
2 (n = 6)	6.7	7.8	5.7	73	nd	14	1.8	14
3 (n = 9)	9.7	12	7.4	62	2.5	26	5.9	17
4 (n = 14)	25	28	18	64	5.4	75	17	38
5 (n = 17)	25	34	23	68	13	97	22	46
Amount of TCAA Excretion (μg/d)								
1 (n = 6)	1.2[a]	1.4[a]	1.5	107	nd	4.0	-0.2	2.9
2 (n = 6)	8.0	11	8.2	75	nd	23	1.9	19
3 (n = 9)	14	16	11	69	4.2	40	7.7	24
4 (n = 14)	28	33	22	67	7.9	93	20	46
5 (n = 17)	33	39	26	67	13	111	26	52
Blood TCAA Concentration (μg/L)								
1 (n = 4)	6.1[c]	6.3[c]	3.4	52	2.4	11	0.9	12
2 (n = 4)	26	26	8.0	31	17	35	13	38
3 (n = 7)	27	31	20	65	17	75	12	50
4 (n = 8)	49	54	19	35	32	86	38	71
5 (n = 13)	73	80	44	55	36	193	53	106

a: $p<0.05$ non-exposure vs. all exposure groups;
b: $p<0.05$ exposure group 2 vs. exposure group 4 &5; and
c: $p<0.05$ non-exposure group vs. exposure groups 2 & 5.

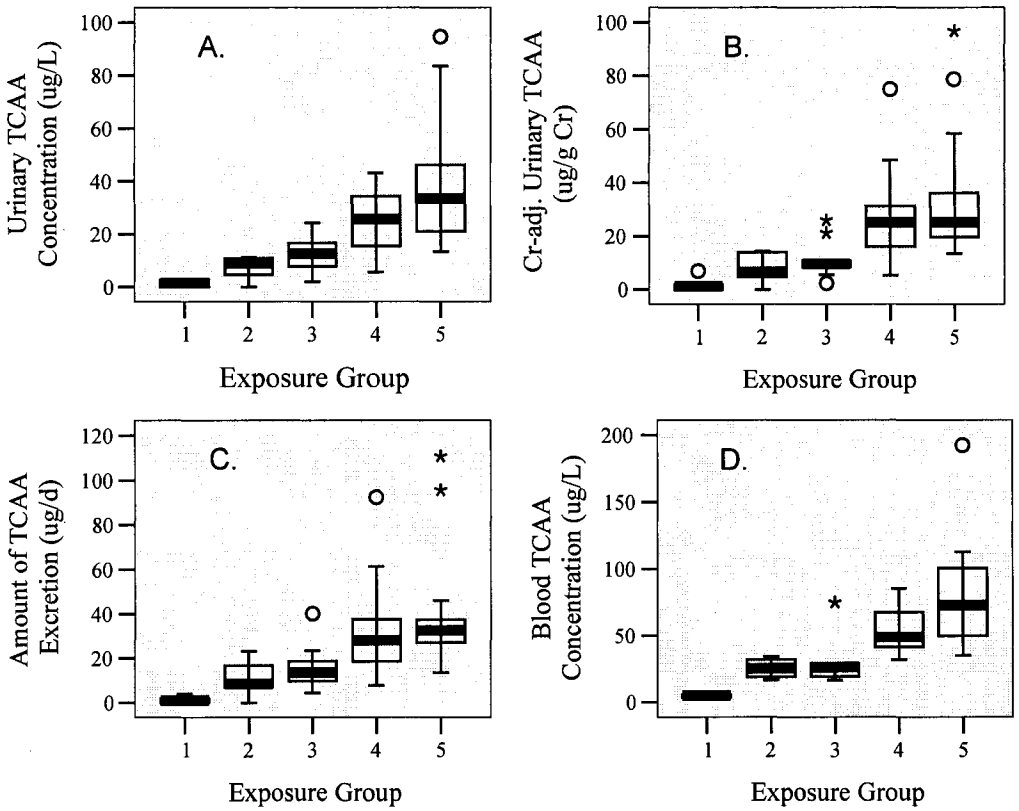

Figure 3-6 TCAA Levels in Urine and Blood in Various Exposure Groups

3.2.5 DCAA in Biological Samples

The DCAA levels in tap water and biological samples after the 12th day of tap water consumption are summarized in Table 3-7. The mean concentrations of DCAA (71 μg/L) and TCAA (80 μg/l) in tap water from City A were similar. The amounts of ingestion of DCAA and TCAA from drinking water were similar, but the urinary DCAA excretion was considerably lower than that of TCAA (Figure 3-7). This is due to extensive metabolism and shorter half-life of elimination (2–60 min) of DCAA (Larson and Bull 1992; Schultz *et al.* 1999).

Table 3-7 DCAA Levels in Urine and Blood Samples

	Median	Mean	SD	Min.	Max.	95% CI for Mean	
						Lower	Upper
Conc. in Tap Water (μg/L)	68	71	25	16	110	75	86
Urinary Conc. (μg/L)	2.4	3.1	3.8	nd	22.1	2.0	4.2
Cr-adj. Conc. (μg/g Cr)	1.6	3.6	5.7	nd	30.1	2.0	5.2
Amount of Excretion (μg/d)	1.9	3.7	4.7	nd	20.3	2.4	5.0
Blood Conc. (μg/L)	0.56	0.7	0.4	0.2	2.6	0.5	0.8

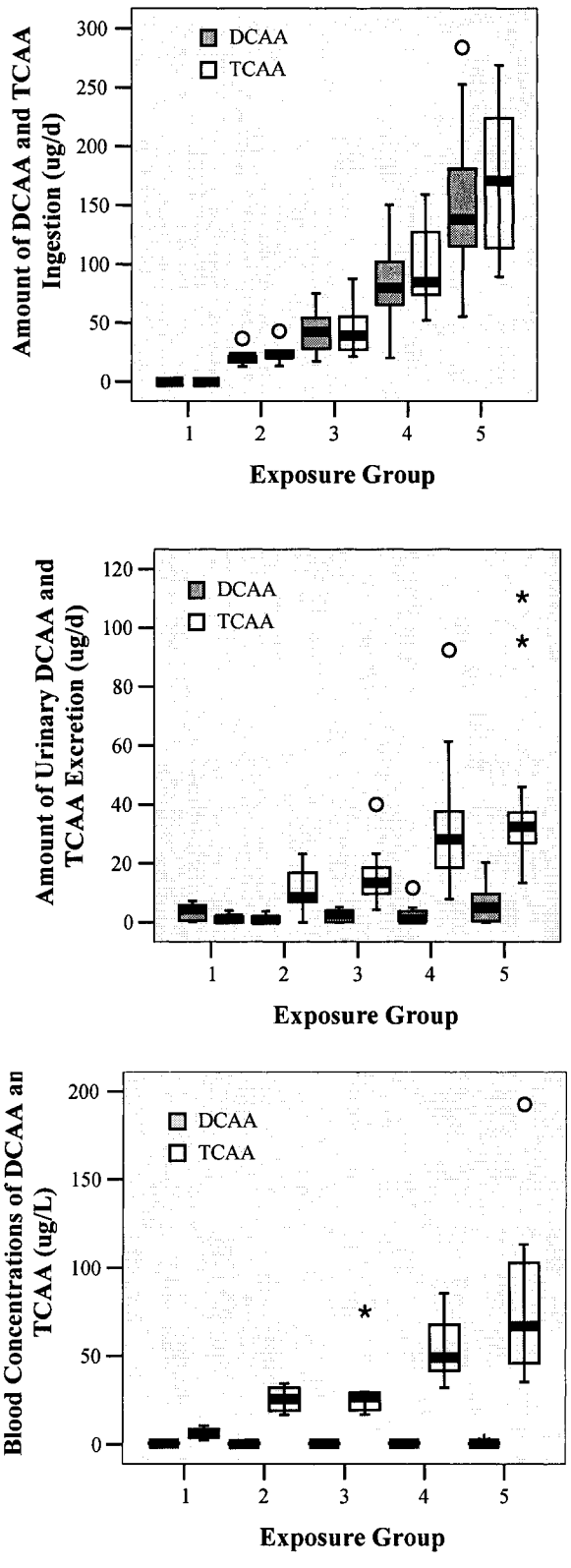

Figure 3-7 Amount of DCAA and TCAA Ingestion and Excretion

There was no correlation between the amount of DCAA ingested and the amount of DCAA excreted after the 12th day of supplied water consumption (r=0.05, p>0.05) (Figure 3-8). There was no correlation between urinary DCAA excretion and urinary TCAA excretion (r=0.002, p>0.05) (Figure 3-9).

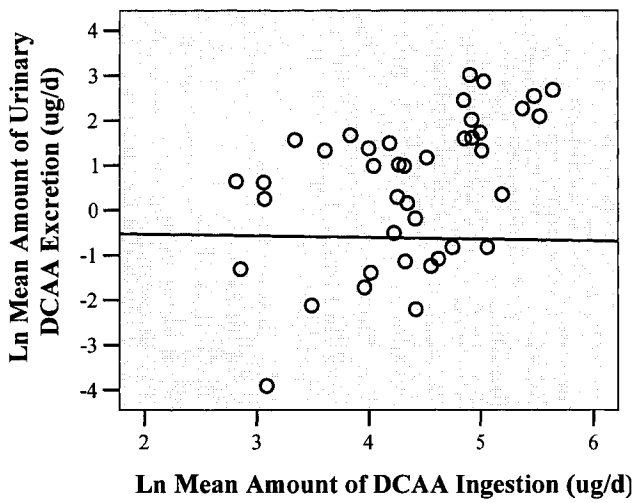

Figure 3-8 Correlation between DCAA Ingestion and Excretion

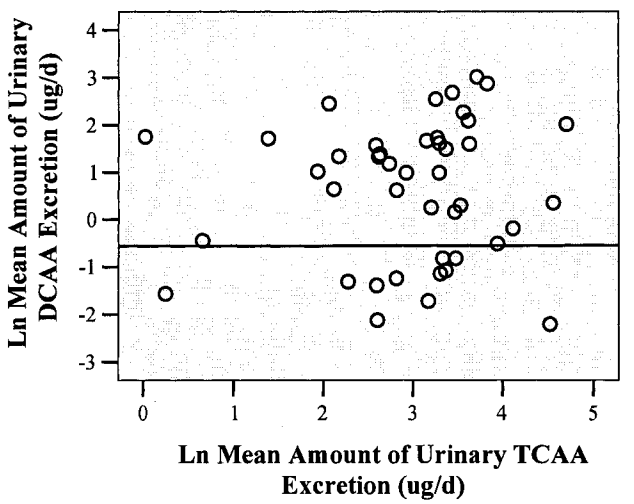

Figure 3-9 Correlation between Urinary DCAA and TCAA Excretion

TCAA and DCAA are two major species of DBPs. Exposure to TCAA and DCAA were associated with adverse reproductive and developmental outcomes in animal studies (Smith *et al.* 1989; Epstein *et al.* 1992; Linder *et al.* 1997; Johnson

et al. 1998). The amount of DCAA recovered in the urine was about 2% of the initial dose in rats and mice (Larson and Bull 1992). DCAA was readily metabolized to CO_2 (28%), GOG (14%), and thiodiacetic acid (6%). Characteristics of DCAA ingestion and excretion were examined in human studies (Kim *et al.* 1999; Weisel *et al.* 1999). The DCAA amount excreted in the urine represented 2–5% of the ingested amount of DCAA in drinking water. The DCAA ingestion was not statistically significantly related to the urinary DCAA excretion. These results are consistent with the findings from our study. Our findings indicated that urinary DCAA was not a useful biomarker of exposure to DBPs and that urinary TCAA excretion could not predict urinary DCAA excretion.

3.3 Conclusions

TCAA concentrations measured in tap water depended on the nature of the water treatment and distribution system. The correlation between TCAA and THMs relied on the nature of water treatment as well. Whether TCAA can be used as a surrogate of THMs depended on the nature of water treatment and storage of each system. This makes the possibility of TCAA being an effective surrogate for THMs very site-specific.

Background TCAA levels were detected in urine and blood. The urinary and blood TCAA levels in the higher exposure groups increased with the increased exposure to TCAA in tap water. The findings indicated that increased urinary TCAA levels resulted mainly from the ingestion of TCAA in tap water. In contrast, the concentrations of DCAA in urine and blood were very low due to the extensive metabolism and shorter half-life of elimination of DCAA. There were no correlations between DCAA ingestion and excretion, and between urinary DCAA excretion and urinary TCAA excretion. TCAA was a useful biomarker of exposure for DBP exposure in drinking water, but DCAA was not a useful biomarker of exposure.

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CHAPTER 4 RELIABILITY ANALYSIS

A major limitation to the use of biomarkers of exposure is variability. The sources of variability consist of interindividual variation, intraindividual variation, sampling variation, laboratory variation, and others such as diet, lifestyle, or personal characteristics (Janetos 1988; Vineis 1997). The interindividual and intraindividual variability contribute to background noise in biological monitoring and epidemiological studies. Evaluation of the variability reveals the potential for misclassification of exposure (Mayeux 2004). The interindividual and intraindividual variability should be characterized to understand the utility of a biomarker as an aid to reduce exposure misclassification.

Interindividual variability is related to source variation, external exposure variation, and metabolism variation among individuals (Vineis 1997; DeCaprio 2000; Pastiono *et al.* 2002). Intraindividual variability includes sampling, laboratory errors, exposure unique to individual samples, or biological factors (Schulte and Perera 1997; Vineis 1997). This variability can influence the misclassification of exposure because a given sample may not accurately represent the typical characteristics of an individual.

Reliability analysis is a major component in validation processes for a biomarker of exposure. In 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 study evaluated the intraindividual and interindividual variability in a substantially larger cohort 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. To perform this analysis, multiple measurements of TCAA levels in various samples were obtained at consistent times for each individual. The strategies for estimating reliability for continuous data include estimation of coefficients of variation within individuals, intraclass correlation coefficients (ICC) between individuals, and Cronbach's coefficient α. The ICC is the analysis of variation (ANOVA) tradition and estimates the equivalence of repeated measurements, that is, reproducibility. Cronbach's coefficient α estimates the reliability of a summation of items, that is, internal consistency.

4.1 Materials and Methods

Recruitment procedures, sample collection, and laboratory methodology are described in Section 3.1 of Chapter 3. The statistical methods used in reliability analysis are discussed below.

The data from 46 participants who provided urine samples in exposure groups were included in the reliability analysis related to TCAA ingestion and excretion. The data for estimating the reliability of TCAA ingestion include those from Tap Water Consumption Day 1 to 15 (fifteen days). Considering a half-life of 30 hours to 6 days for TCAA in the human body, the data collected from the 12^{th}, 13^{th}, 14^{th}, and 15^{th} days of supplied water consumption, that is, from Urine Collection Days 13 to 16 (four days), were used for reliability analysis of TCAA excretion to allow measurements at a steady state (i.e., the same quantity).

The data from 25 participants who donated blood samples in exposure groups were included in the reliability analysis. The data collected from blood samples at the14^{th} and 15^{th} days after supplied water consumption, that is, Blood Collection Days 14 and 15, were used for analysis.

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

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

where SD is the standard deviation and mean is an average of TCAA measures in multiple samples on the same day for an individual.

Reproducibility of measurements of TCAA ingestion and excretion between individuals in different daily samples was evaluated by the ICC. The ICC is an estimate of expected correlation between two randomly chosen measures, which is the analysis of variance (Shrout and Fleiss 1979; Snedecor and Cohran 1980; Fleiss 1986; Kelsey *et al.* 1986). The ICC is expressed as:

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

where CV_b is the coefficient of variation between-individuals and CV_w is the coefficient of variation within-individuals.

The values of ICC range from 0 to 1.0. 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 and Newell 1996). 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, a minimum reliability of 0.7 was recommended when the measurement was used for research (Nunnally 1994). In our study, we selected 0.7 the cut-off point consistent with an acceptable level of reliability.

A one-way random analysis for a single measure was performed by using the software SPSS 13.0 package.

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

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

where ICC is the average correlation between any two measures and n is the number of measures pooled. Cronbach's coefficient α is an index to estimate the reliability 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). The logical justification for using this index is that the sum of many measures is more reliable than any single measure because increasing the number of measures decreases the weight of the error variance compared to the true measure variance (Bravo and Potvin 1991).

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 and Newell 1996). In our study, 0.8 is the cut-off point for an acceptable level of reliability. The α values were calculated manually based on the above equation.

4.2 Results and Discussion

4.2.1 Sample Collection and Laboratory Analysis

Coefficients of variation for analysis of multiple urine samples are listed in Table III-1 in Appendix III. A total of 347 samples were analyzed for urinary TCAA, with 257 samples done with duplicate analysis, 73 with triplicate analysis, and 17 with quadruplicate analysis. The ranges of analytical CVs and proportion of samples are listed in Table 4-1. The CVs were under 20% in 91% samples.

Table 4-1 Analytical Coefficient of Variation in the Urine Samples

Range of Analytical CV (%)	Proportion of Samples	
0 – 10	171	49%
11 – 20	146	42%
21 – 30	26	8%
31 – 40	4	1%
Total	347	100%

Technical variability is a function of instrumentation and reagents as well as the possibility of human error in sampling, labeling, preparation, and analysis (Stites 1991). It is important to develop good protocols for sampling, transportation, and sample storage. Laboratory Quality Assurance and Control (QA/QC) should be carried out throughout laboratory analysis.

The procedures for sample collection and laboratory analysis were carefully selected. These included 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. Duplicate analysis was performed for all types of samples. Triplicate analysis was performed in one out of five urine or blood samples. Some samples were analyzed four times.

For laboratory analysis, the US EPA Method 552.2 and Standard Method 6251B are used in most water utility laboratories. Techniques used for analysis included high-performance liquid chromatography (HPLC), HPLC with mass spectrometry (MS), capillary electrophoresis (CE), HPLC-MS with negative ion electrospray ionization-tandem MS, and solid-phase microextration (SPE) (O'Donnell *et al.* 1995; Martinez *et al.*1998a, b, 1999, 2000; Aher and Buchberger 1999; Kuklenyik *et al.* 2002). A new technique, electrospray ionization-high field asymmetric waveform ion mobility spectrometry-mass spectrometry (ESI-FAIMS-MS), was developed (Ells *et al.* 1999, 2000a, b, c; Gabryelski *et al.* 2003). FAIMS provides fast and sensitive analysis for drinking water samples. For biological samples, a small volume of samples limits the achievement of sensitivity of analysis by FAIMS. Solid-phase microextraction (SPME) integrates sampling, extraction, concentration, and sample introduction in a single step (Sarrion *et al.* 1999, 2000a, b, 2002, 2003; Wu *et al.* 2002).

In our study, liquid-liquid microextraction (LLME) and SPME combined with GC-ECD was used to analyze TCAA, DCAA, and other DBP compounds in samples of tap water, urine, and blood with only 50–100 μl of sample volume (Wu *et al.* 2002). The LLME-SPME-GC-ECD allowed analysis of DBP compounds in water and biological samples with speed and acceptable precision. The results of the study indicated that variation in laboratory analysis contributed to intraindividual variability to a limited extent.

4.2.2 Ingestion, Loading in Blood, and Urinary Excretion

Variation in Tap Water

Within a single water supply, TCAA and DCAA levels can vary greatly. The differences 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 and Singer 2003). The weekly variation of the concentrations of TCAA and DCAA in City A is shown in Figure 4-1. In City A, the levels of DCAA and TCAA in tap water were higher during the cold water months. In two Canadian National Surveys (Health Canada 1995 and 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. Mean DCAA levels changed very little within the distribution system in winter and summer.

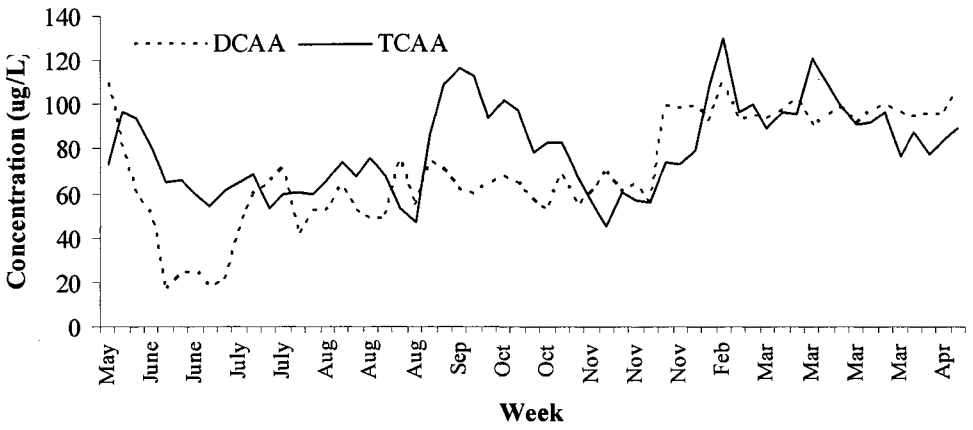

Figure 4-1 Weekly Variation of Concentrations of TCAA and DCAA

Ingestion

The ICCs of measurements of TCAA ingestion in all exposure groups are summarized in Table 4-2. The ICCs of three TCAA ingestion measures (concentration in tap water, volume of tap water intake, and amount of TCAA ingestion) within any two days of the 15-days water consumption among all individuals ranged from 0.69 to 0.97. The interindividual reliability was very high for TCAA concentrations measured in tap water and fair for the volume of tap water intake.

Table 4-2 Intraclass Correlation Coefficients for TCAA Ingestion Measures

Concentration in Tap Water (μg/L)	Volume of Tap Water Intake (L/d)	Amount of Ingestion (μg/d)
0.97	0.69	0.89

Although weekly variation of measured TCAA was observed in this study, 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 (0.97) of measurement of TCAA concentration in tap water reflects this controlled factor in an experimentally designed manner. In a field study, interindividual reliability of measurement of tap water TCAA concentrations will not be as high as shown in our study because TCAA concentrations in water treatment systems vary greatly depending on the nature of water at different sites and in different seasons (Health Canada 1996).

A large variation in the water intake rate was observed among individuals in other studies (Raman *et al.* 2004). In our study, 31% variation for the volume of tap water consumption could be attributed to intraindividual variability (ICC=0.69). Intraindividual 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 (WHO 1996; Williams *et al.* 2001; Raman *et al.* 2004). In our study, the reliability of the volume of water consumption is acceptable.

Variation in measurements of the amount of TCAA ingestion was high (ICC=0.89) even with little variability in measurement of tap water TCAA concentrations. In addition to the above two sources of variation, variation of TCAA ingestion can be derived from other exposures depending on 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, visiting dry-cleaning shops, or using chlorinated bleach and other solvents.

In our study, the source of water consumed was limited to TCAA-containing tap water plus TCAA free bottled water. Participants consumed beverages (e.g., coffee, tea, juice, milk, or liquor) every day. TCAA has been detected in food and beverages such as coffee, tea, fruit juice, and canned soups (Raymer *et al.* 2000). The influence of coffee and tea on the levels of urinary TCAA has not been documented. One participant used supplied tap water to make coffee (500–700 mL/d) and tea (250–500 mL/d). The urinary TCAA concentrations were not detectable, but the blood TCAA level was detected. The relationship between the use of coffee/tea and the urinary TCAA levels would be interesting to investigate in the future.

In our study, a questionnaire was administered to each individual to evaluate non-ingestion TCAA sources. Some DBP compounds such as THMs have been measured in blood and breath samples in the general population as a result of showering, bathing, dishwashing, and swimming (Wallace *et al.* 1984; Aggazotti *et al.* 1990, 1995, 1998; Jo *et al.* 1990a, 1990b; Aiking *et al.* 1994; Levesque *et al.* 1994; Weisel and Shepard 1994; Cammann and Hubner 1995; Weisel and Jo 1996; Lindstrom *et al.* 1997; Gordon *et al.* 1998; Weisel *et al.* 1999; Brugnone *et al.*

1994; Backer *et al.* 2000; Barbone *et al.* 2002; Chu and Nieuwenhuijsen 2002; Miles *et al.* 2002; Whitaker *et al.* 2003). The major exposure routes from these activities are inhalation and dermal contact. THMs can be easily inhaled through breath or absorbed through dermal contact. HAAs are non-volatile compounds. The permeability of HAAs via the skin was very low (Xu *et al.* 2002; Xu and Weisel 2003) so the daily exposure dose resulting from the above activities was insignificant for HAAs (Kim and Weisel 1998; Xu *et al.* 2002; Xu and Weisel 2003). For example, the proportion of absorbed doses of TCAA from daily bathing via dermal contact is about 0.005% to 0.5% of the daily ingestion doses of TCAA (Cleek and Bunge 1993; Xu *et al.* 2002). In our study, most individuals took baths or showers or washed dishes about 10 to 15 min per day using water from City B. No significant patterns between bathing/showering/dishwashing and TCAA excretion were observed. This may result from a low level of TCAA in tap water from City B.

Many DBP compounds and TCAA are formed in swimming pools (Aggazzotti *et al.* 1987, 1990, 1993, 1995, 1998; Aiking *et al.* 1994; Lindstrom *et al.* 1997; Kim and Weisel 1998; Fantuzzi *et al.* 2001; Kim *et al.* 2002). In our study, four participants swam during the study period. In one case the urinary TCAA levels declined. In another case, the urinary TCAA levels increased slightly after one week of the study. In two cases, the urinary TCAA levels remained stable. Thus, no clear relationship between swimming and urinary TCAA levels emerged.

Some solvents such as trichloroethylene (TCE), tetrachloroethylene (PERC), trichloroethanol, trichloroethane (TRI), and tetrachloroethane can be metabolized to TCAA in the human body (Nomiyama 1971; Monster 1979; Caperos *et al.* 1982; Bruckner *et al.* 1989; Inoue *et al.* 1989; Fisher *et al.* 1998; Volkel *et al.* 1998; Fisher 2000; Bloemen *et al.* 2001; Furuki *et al.* 2003). These solvents are used as vapour 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 (Aggazzotti *et al.* 1994; WHO 1985; IARC 1995). TCE was detected in treated water at Canadian water supply facilities at levels ranging from 0.1 to 1.0 μg/L (Otson *et al.* 1982; Health Canada 1992; WHO 2004). One to three percent of the absorbed PERC, 0.5% to 6% of TRI, and 20% to 40% of TCE were metabolized to TCAA in humans (Monster 1979; Nomiyama 1971; ATSDR 1995, 1996). In a background survey, statistically significant correlations between the urinary TCAA and blood TRI/TCE were observed (Calafat *et al.* 2002). TCAA has been used as a biomarker of exposure for assessing long-term exposure to TCE in occupational settings (Ulander *et al.* 1992). The use of household cleaning solutions and products containing solvents was recorded by three participants in our study. No clear patterns between urinary TCAA and solvent use were observed.

Exposure to several compounds may compete for the same biotransformation sites or metabolize to TCAA in the human body. Chloral hydrate is rapidly metabolized in the liver and other tissues to trichloroethanol, trichloroethanol-

glucuronide, and TCAA (Breimer *et al.* 1974; Marshall and Owens 1954; Reimche *et al.* 1989; Gorecki *et al.* 1990; Humbert *et al.* 1994). A small fraction of CH was oxidized to TCAA and a large amount of CH was reduced to the alcohol (Butler 1948). In humans, 8% of the administered dose of CH is directly oxidized to TCAA and additional TCAA is formed during enterohepatic circulation of trichloroethanol (Lash *et al.* 2000). An average of 35% (a range of 5%–47%) of the initial dose of CH is converted to TCAA (Marshall and Owens 1954; Sellers *et al.* 1972; Allen and Fisher 1993; 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 in tap water were measured in tap water. The ratio of CH to TCAA was 1:20. The blood CH levels 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.

Loading in Blood

The ICC of blood TCAA concentration measurement was 0.90 in this study. The result indicates that using the blood measurement, the intraindividual variability was small and interindividual reliability was high. The blood TCAA concentration was related to current TCAA exposure. TCAA is a semi-volatile compound and is readily absorbed in humans and animals (Muller *et al.* 1974; Curry *et al.* 1991; Larson and Bull 1992). After absorption, TCAA is highly bound to plasma proteins in blood (Paykoc and Powell 1945; Marshall and Owens 1954; Sellers and Koch-Weser 1971; Muller *et al.* 1972; Monster *et al.* 1976). 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). Saturation of binding has been observed in human plasma. TCAA concentrations in whole blood are two-fold lower than those in plasma. TCAA measured in whole blood reflects the total body burden of TCAA related to duration of exposure. For continued exposure to TCAA in drinking water over two 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 bind with conjugates such as glucuronides (Fisher *et al.* 1991). Free TCAA in blood is rapidly eliminated by glucuronidation (Nomiyama and Nomiyama 1979). Theoretically, the analysis of TCAA in blood is more reliable because of the less extensive metabolism of TCAA, its sufficiently long elimination half-life time, and less variability within individuals as compared to the measurement of urinary TCAA. The blood level of TCAA is an important exposure index with a high specificity. Use of blood samples in a larger cohort within an epidemiological study is limited because of the invasive sampling procedure.

Urinary Excretion

The ICCs of measurements of TCAA excretion in all exposure groups are summarized in Table 4-3. The ICCs of three TCAA excretion measures within any two days of the urine collection of the last 4-days among all individuals ranged from 0.73 to 0.77. The interindividual reliability for all measurements except for the measurement of the amount of TCAA excretion is relatively high (ICCs≥0.7).

The ICCs were performed for each sub-group and the estimates ranged from 0.26 to 0.79. The ICCs within each sub-group were based on small sample sizes, but illustrate that restricting the range of exposure lowers the reliability of individual measurements.

Table 4-3 Intraclass Correlation Coefficients in TCAA Excretion Measures

Urinary Concentration (μg/L)	Cr-adj. Concentration (μg/g Cr)	Amount of Excretion (μg/d)
0.75	0.73	0.77

The ICCs for TCAA ingestion and blood TCAA measurements were relatively higher than those for 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 elimination of TCAA from the body. The rates of absorption, metabolism, and excretion of TCAA vary from individual to individual and are influenced by age, sex, and physical conditions. The extent of metabolic enzymes is different in different individuals. The differences account for the different levels of TCAA in body fluids between individuals.

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 first morning urine (FMU) is a traditional practice for this purpose. The individual voids 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 more concentrated compounds.

Urinary TCAA concentrations are affected by the rate of urine production. The traditional practice for reducing this variation is to correct for creatinine excretion. The determination of urinary creatinine is recommended to normalize over-diluted or over-concentrated urine samples. Creatinine is the metabolic product of muscle tissue and is a normal constituent of urine. Creatinine is excreted by glomerular filtration at a relative constant rate of 1.0–1.6 g/day 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 (Elkins 1974; Boeniger *et al.* 1993; Mage *et al.* 2004; Barr *et al.* 2005; Kissel *et al.* 2005). The creatinine concentration is inversely related to fluid intake. Analysis performed on very dilute (less than 0.3 g/L) or concentrated (greater than 3 g/L) urine samples must be interpreted with caution (Rosenberg *et al.* 1989). When the extremes of creatinine variability are observed, creatinine correction is not valid. Some studies 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 and Wardener 1970; Curtis and Fogel 1970; Greenblatt *et al.* 1976; Wilson and Crews 1995). In our study, the ICC of Cr-adj. TCAA concentration (0.73) was similar to that of urinary TCAA concentration (0.75). The correction of urinary TCAA excretion for urinary creatinine seems not to improve the results of intraindividual variability.

TCAA is metabolized into CO_2, DCAA, and GOG (nonchlorinated acids glyoxylate, oxalate, and glycolate) (Green and Prout 1985; Larson and Bull 1992). In rats and mice, excretion rates in urine vary from about 48% to 78% of the administered doses (Muller *et al.* 1974; Larson and Bull 1992). After oral administration of TCAA in humans, 23% to 50% of the administrated dose can be recovered in urine (Muller *et al.* 1974; Humbert *et al.* 1994).

TCAA can be excreted in urine, feces, and bile (Green and Prout 1985; Larson and Bull 1992; Schultz *et al.* 1999). Fluctuation of blood TCAA has been observed in animals (Prout *et al.* 1985). This fluctuation may be related to biliary excretion and enterohepatic recirculation (Green and Prout 1985). The change of pH in urine can alter the excretion rate. The amount of TCAA excreted at night (0–8 am) is lower than in the daytime (Monster *et al.* 1979). The half-life of TCAA elimination after direct TCAA ingestion ranges from 30 hours to 6 days in humans (Paykoc and Powell 1945; Muller *et al.* 1974; Monster *et al.* 1976, 1979; Humbert *et al.* 1994; Fisher *et al.* 1998; Bader *et al.* 2004).

Metabolism of TCAA generates free radicals and induces lipid peroxidation (Larson and Bull 1992; Ni *et al.* 1996). CYP2E1 is the major isozyme for the metabolic activation of TCAA. Wide interindividual variability was found in the

CYP2E1 (Peter *et al.* 1990; Chang *et al.* 1993; Raucy *et al.* 1995; Lieber 1997; Clewell *et al.* 2000; Pastino *et al.* 2000; Bebia et al. 2004). The activities of CYP2E1 were not assessed in individuals in this study.

4.2.3 Multiple Samples

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. Cronbach's α estimates for four different ingestion and excretion measurements in different pooled sampling days are illustrated in Figure 4-2. 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 to 4 days of sampling. The results indicated an increased reliability with repeated measures. However, it is 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 steady-state TCAA level in the body is reached and the exposure variability is relatively high.

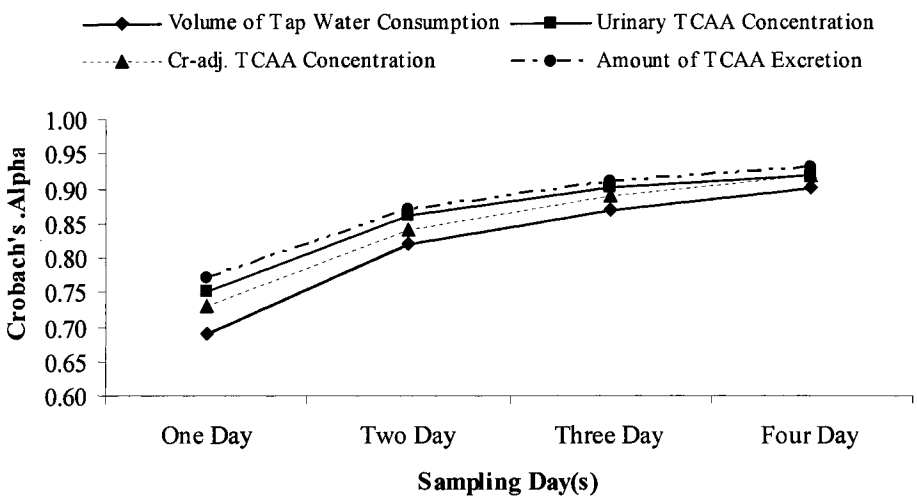

Figure 4-2 Cronbach's α Estimates in Expected Sampling Day(s)

4.3 Conclusions

By using blood TCAA as a biomarker of exposure, the intraindividual variability was small and the interindividual reliability was very high. By using urinary TCAA as a biomarker of exposure in a substantial cohort, the intraindividual

variability contributed to background noise to a certain extent, but the interindividual reliability was relatively high in measurements of TCAA ingestion and urinary TCAA excretion. Variation of laboratory analysis contributed slightly to intraindividual variability. No clear patterns of non-ingestion TCAA sources (bathing, showering, swimming, and solvent contact) and physical activities related to urinary TCAA excretion were observed. The correction of urinary excretion for urinary creatinine improved the result of intraindividual variability to a limited extent.

For sampling strategies, one-day blood sampling and two-day urine sampling are sufficient to achieve reliability in a study if a steady-state TCAA level in the body is reached and the exposure variability is relatively high. Because a relatively high interindividual reliability was observed, the measurements of TCAA ingestion, TCAA loading in blood, and urinary TCAA excretion are relatively reliable for use in epidemiological studies.

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CHAPTER 5 VALIDITY ANALYSIS

Some epidemiological studies have reported weak associations between exposure to several DBPs and occurrence of carcinogenicity and adverse reproductive and developmental effects (Boorman *et al.* 1999; Nieuwenhuijsen *et al.* 2000; IPCS 2000; Graves *et al.* 2001; Bove *et al.* 2002; IARC 2004). THMs were generally selected as surrogates of DBP exposure in these epidemiological studies. THMs 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, the most promising sampling method for measuring THMs as a biomarker is exhaled breath. The median background exhaled breath concentration of THMs was 3.5 $\mu g/m^3$ (Wallace *et al.* 1984). 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 using as a biomarker in urine or blood to measure DBP exposure because of the transient presence of THMs in the body (Weisel *et al.* 1999).

TCAA is one of the principal species of HAAs. TCAA is readily absorbed into the blood following ingestion of water (Muller *et al.* 1974; Curry *et al.* 1991; Larson and Bull 1992) and is bound to plasma proteins (Paykoc and Powell 1945; Marshall and Owens 1954; Sellers and Koch-Weser 1971; Muller *et al.* 1972; Monster *et al.* 1976). The protein-bound TCAA cannot be filtered through the kidney. TCAA can also bind with conjugates such as glucuronides (Fisher *et al.* 1991). TCAA is metabolized into CO_2, DCAA, and GOG (nonchlorinated acids glyoxylate, oxalate, and glycolate) (Green and Prout 1985; Larson and Bull 1992). The TCAA levels in whole blood reflect the total burden of TCAA and those in serum or urine reflect the free TCAA that can be eliminated from the body via the kidney. Free TCAA in blood is also rapidly eliminated by glucuronidation (Nomiyama and Nomiyama 1979). TCAA can be excreted in urine, feces, and bile (Green and Prout 1985; Larson and Bull 1992; Schultz *et al.* 1999). The half-life of urinary elimination after direct TCAA ingestion ranges from 30 hours to 6 days in humans (Paykoc and Powell 1945; Muller *et al.* 1974; Monster *et al.* 1976, 1979; Humbert *et al.* 1994; Fisher *et al.* 1998; Froese *et al.* 2002; Bader *et al.* 2004).

TCAA has been measured in urine samples in the general population and demonstrates an exposure-response relationship between urinary TCAA and ingestion of TCAA-containing water (Kim *et al.* 1999; Weisel *et al.* 1999; Froese *et al.* 2002; Calafat *et al.* 2003; Bader *et al.* 2004). These findings, combined with a 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 in drinking water. This study addresses the validity of using urinary TCAA as a biomarker of exposure to DBPs by ingestion of drinking water by a direct experiment in a relevant human cohort.

5.1 Materials and Methods

Recruitment procedures, sample collection and laboratory methodology are described in Section 3.1 of Chapter 3. The statistical methods used in reliability analysis are discussed below.

The data from 52 participants who provided urine samples in the control and exposure groups were included in the validity analysis. Considering a half-life of 30 hours to 6 days for TCAA in the human body, the data collected from urine samples on the 12^{th}, 13^{th}, 14^{th}, and 15^{th} days of supplied water consumption, that is, from Urine Collection Days 13 to 16 (four days), were used for analysis of TCAA excretion to allow measurements at a steady state (i.e., stable over time).

A total of 31 participants in the control and exposure groups donated blood samples on the 14^{th} and 15^{th} day of supplied tap water consumption, that is, Blood Collection Days 14 and 15. These data are included in the validity analysis.

A logarithmic transformation of the values was performed to correct the skewing of the distribution of urinary and blood TCAA measures and TCAA ingestion measures. The data under detection limits were reported as zero, which became missing values during log transformation. In order to include missing values in the analysis to avoid the effect of restriction of the range exposure, a value of 0.0001 was added to all data before log transformation.

Pearson's correlation analysis and linear regression model were performed to the log-transformed data. The variables included three TCAA ingestion measurements, three urinary TCAA excretion measurements, and one blood TCAA measurement. The ratio of urine to blood TCAA concentration was calculated as an average ratio for the 0, 1^{st}, 7^{th}, 13^{th} and 14^{th} day of tap water consumption.

For analysis of the correlation between the volume of tap water intake and urinary TCAA excretion, original (non-transformed) data were used. Because of the large range of concentrations of TCAA in tap water (0–121 μg/L) intentionally provided combined with the restriction of range of the volume of tap water intake (up to 3L per day per individual), the observed relationship between volume of tap water intake and urinary TCAA excretion could be small. In this case, the four-days data in each item (e.g. D12, 13, 14 &15 of TCAA conc. in tap water) were combined into a single variable to perform partial correlation analysis. The effect from the large range of the concentration of TCAA in tap water was controlled for this data analysis. Partial correlation analysis between volume of tap water intake and the urinary TCAA excretion parameters (Urinary TCAA conc., Cr-adj. TCAA Conc., Amount of TCAA Excretion) was performed using SPSS 13.0.

5.2 Results and Discussion

5.2.1 Ingestion and Urinary Excretion

The urinary TCAA concentrations increased with increased levels of TCAA ingestion of supplied tap water in 96% (44 out of 46) of participants who were assigned to exposure groups. Elevated TCAA concentrations were not detected in urine but were detected in blood after consumption of supplied tap water in one participant. An unexpected downward trend of the urinary TCAA concentrations was observed in one other participant.

The correlation coefficients (r) between TCAA ingestion and urinary TCAA excretion are listed in Table 5-1. Regression lines between TCAA ingestion and urinary TCAA excretion are shown in Figure 5-1 and 5-2.

Table 5-1 The Correlation among TCAA Ingestion and/or Urinary Excretion

Urine Collection [a]	Day 13	Day 14	Day 15	Day 16	2 Days	3 Days	4 Days
TCAA Concentration in Supplied Tap Water (μg/L) vs.							
uc[b]	.77	.62	.63	.57	.78	.78	.81
ucr[b]	.77	.63	.61	.56	.78	.78	.83
ua[b]	.77	.62	.61	.62	.77	.77	.77
Amount of TCAA Ingestion (μg/d) vs.							
uc	.77	.63	.62	.57	.77	.77	.80
ucr	.77	.63	.61	.55	.77	.77	.82
ua	.77	.62	.61	.62	.77	.77	.77
Urinary TCAA Concentration (μg/L) vs.							
ucr Day 13	.99						
ucr Day 14	.84						
ucr Day 15	.83	.97					
ucr Day 16	.83	.95	.97				
ua Day 13	.99	.85	.84	.82			
ua Day 14	.85	.98	.98	.96			
ua Day 15	.83	.97	.99	.98			
ua Day 16	.86	.96	.98	.98			

Note: $p<0.001$ for all above r values.

a: 2 days = Day 13 + Day 14; 3 days = Day 13 + Day 14 + Day 15; and 4 days = Day 13 + Day 14 + Day 15 + Day 16.

b: *uc* = urinary TCAA concentration (μg/L), *ucr* = creatinine-adjusted urinary TCAA concentration (μg/g Cr), and *ua* = amount of urinary TCAA excretion (μg/d).

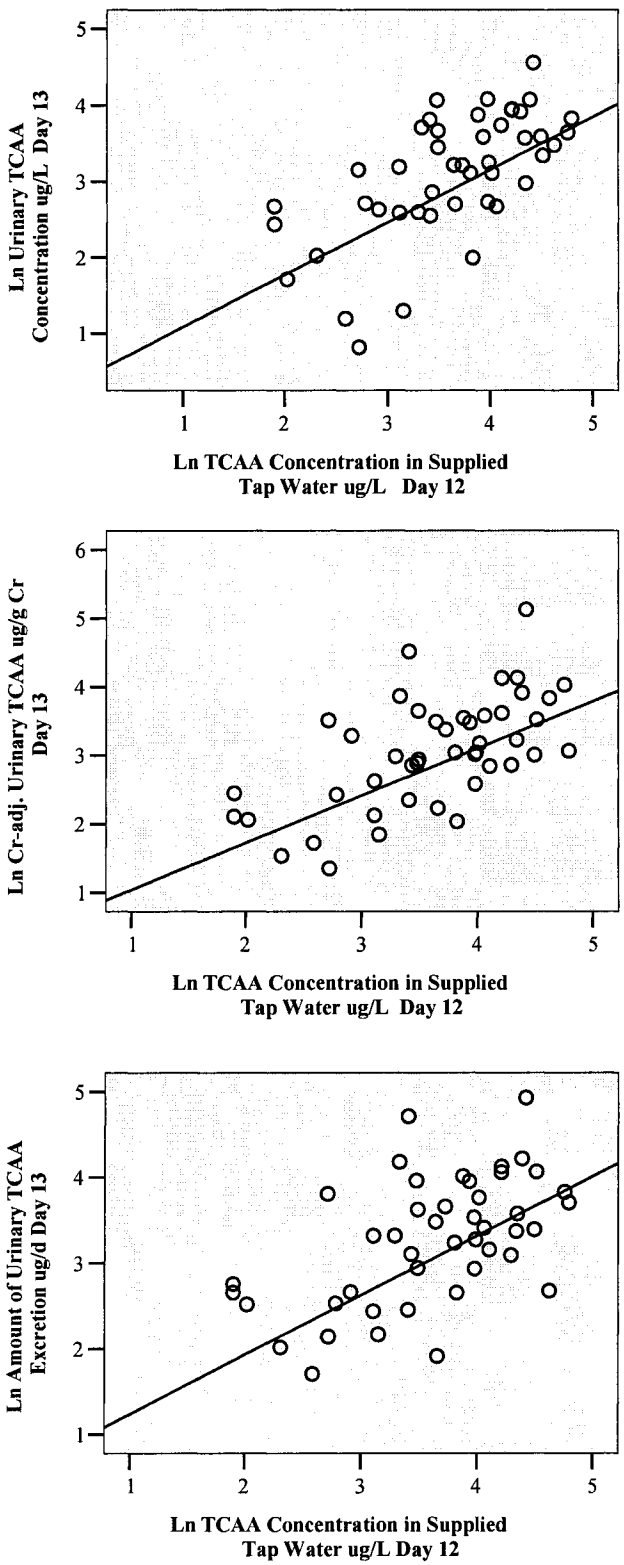
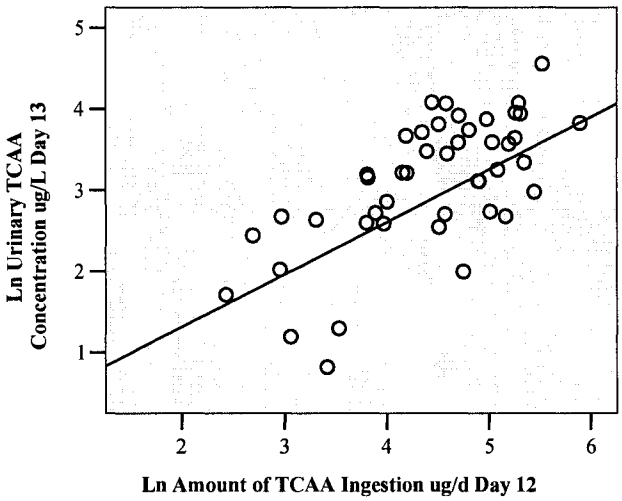
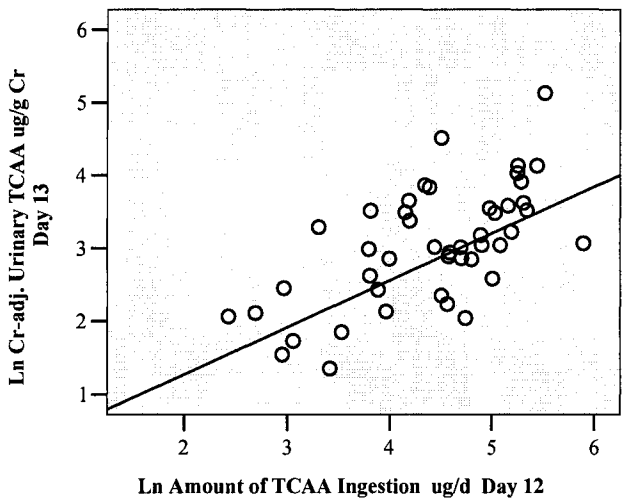


Figure 5-1 Linear Regression Lines Between TCAA Concentrations in Supplied Water and Urinary TCAA Excretion





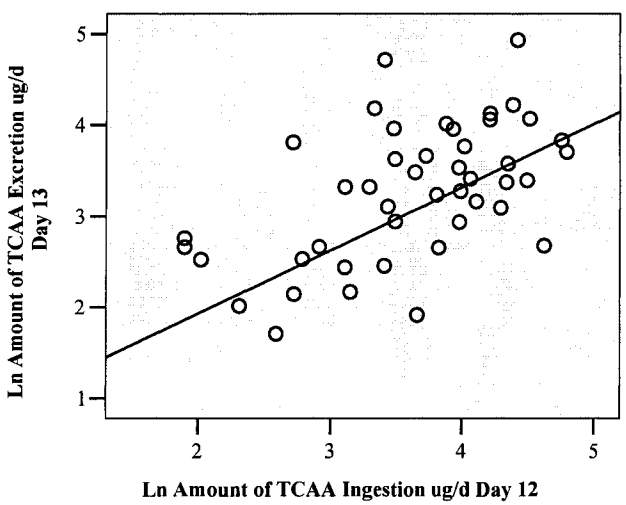


Figure 5-2 Linear Regression Lines Between Amount of TCAA Ingestion and Urinary TCAA Excretion

Correlations between urinary TCAA concentration on each Urine Collection Day (13, 14, 15, or 16) and TCAA concentration in tap water on any day of supplied tap water consumption were observed. The r values ranged from 0.57 to 0.77 ($p< 0.001$) and the r^2 values ranged from 0.33 to 0.60 ($p<.001$). Correlations between the amount of urinary TCAA excretion on each Urine Collection Day and the amount of TCAA ingestion on any day of supplied tap water consumption were observed. The r values ranged from 0.55 to 0.77 ($p<0.001$) and the r^2 values ranged from 0.31 to 0.60 ($p<0.001$).

Within the same urine collection day, the r values between any one of two TCAA ingestion measurements and any one of three urinary excretion measurements were similar (Table IV-1 in Appendix IV). Compared to a single day measure between ingestion and excretion, the r values were higher on the 13th Urine Collection Day (0.77) and lower on the 16th Day (0.55–0.62). The r values for combined urine collection days (2 days, 3 days, or 4 days) ranged from 0.77 to 0.83 ($p<0.001$). The prediction of TCAA exposure was improved by using 4-days combined urinary TCAA measures.

The correlations among three urinary TCAA measurements ranged from 0.82 to 0.99 from one urine collection day to another (Table 5-1). The results suggested that all three measurements could be considered to be similar for measuring TCAA in urine. The Cr-Adj. measurement did not improve the prediction of TCAA ingestion and excretion suggesting that it is not necessary to use the Cr-adj. measurement in a field study. This finding is a little surprising because the excretion amount should be related with ingestion amount better than excretion concentration would be related to excretion concentration. Some of the reduction in expected prediction may relate to uncertainty added by extrapolating FMU excretion to a 24 h excretion amount.

The relationship of the volume of tap water intake to urinary TCAA excretion was examined by using partial correlation analysis. The partial correlation coefficients obtained are listed in Table 5-2. After controlling for variability of the concentrations of TCAA in tap water, volume of tap water intake was weakly correlated with urinary TCAA excretion, particularly with Cr-adj. urinary TCAA concentrations and amount of TCAA excretion ($r=0.27$, $p<0.001$). The results indicated that an increase of volume of tap water intake increased urinary TCAA excretion in individuals, but the effect was very small compared to the effect of the concentration of TCAA in tap water ($r=0.56$ to 0.83).

Table 5-2 The Correlation Between the Volume of Tap Water Intake and Urinary TCAA Excretion

Controlled Variable	**Variables**	*Urinary TCAA Conc. (μg/L)*	*Cr-adj. TCAA Conc. (μg/g Cr)*	*Amount of TCAA Excretion (μg/d)*
TCAA Conc. in tap water *(μg/L)*	*Volume of Tap Water Intake (L/d)*	0.13	0.27	0.27
	p value	0.059	<0.001	<0.001

A predictable relationship between external exposure and a biomarker has to be identified in a validity study of that biomarker (Decaprio 1997). When the kinetics is linear and the exposure distribution is stationary, the mean value of a biomarker measured repeatedly in an individual over time should be proportional to the mean exposure over the same period (Rappaport *et al.* 1993, 1995). The extent of linear relationship is measured using a coefficient of determination (r^2). The linear relationship reflects the kinetic process, variability in the rate of absorption, distribution, metabolism, and elimination, the specificity of a biomarker, and intraindividual and interindividual variability.

The relationships between TCAA concentration in tap water and urinary TCAA excretion have been investigated in New Jersey by Kim *et al.* (1999) and Weisel *et al.* (1999). The characteristics of these two studies and our study are compared (Table 5-3). The advantages of our 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-hr recall questionnaire; (3) repeated water, urine, and blood samples from an individual were collected during the 15 day period of the study rather than a single sample, which allowed the reliability assessment of measures; and (4) the skewed distribution of original data was corrected using a the log normal distribution.

The discrepancy of the results between their study and ours was the correlation between TCAA concentration in tap water and urinary TCAA excretion. In our study, good correlations were observed between TCAA ingested from supplied tap water containing known concentrations of TCAA in 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 supplied TCAA-containing tap water. These controlled factors and a wide range of exposure among individuals may contribute to the good correlation between TCAA concentrations in tap water and urinary TCAA excretion. The lack of observed relationship between TCAA concentrations in tap water and urinary excretion from Kim and Weisel *et al.*'s studies may result from the effect of restriction of their narrow range of exposure because the TCAA concentrations in tap water measured in a single day were skewed to the low end and their sample size was relatively small.

From a laboratory perspective, validity is the ability of an assay to detect the presence or absence of a designated biomarker in the specified biological medium. The validation processes include well-characterized accuracy and precision, detection limits (sensitivity), exposure specificity, and reliability (Sampson *et al.* 1994; Schulte and Perera 1997). Analytical specificity refers to the probability that the biomarker is an indicator of actual exposure to the specific xenobiotic in the environment. Analytical sensitivity refers to the quantitative relationship in

Table 5-3 Characteristics of TCAA Biomarker Studies in Two Locations

Feature	Kim *et al.* (1999) & Weisel *et al.* (1999)	Current Study
Objective	The relationship between TCAA ingestion and urinary excretion	The same
Study Design	One-time survey (48 hours)	Experimental cohort (15 days)
Location	New Jersey, US	Alberta, Canada
Gender	Female	Female
Age (yr)	18–43	19–41
Sample Size	25–42 from the same study population	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 days
Volume Measured	Estimated using 48-hr recall questionnaire	Direct measurement per day
TCAA Conc. (μg/L) (a single day)	Range: 0.25–120 Mean: 18 Median: 5.7	0–121 41 33
Amount Ingested	WaterConc x (VolCold+0.61VolHot) (μg/48 hr)	WaterConc x Vol (μg/24 hr)
Urine Sample		
Type	First Morning Urine	First Morning Urine
Sampling per person	One sample during a home visit	7 samples during 15 days
Measurement	Urinary excretion rate	Urinary concentration Amount of urinary excretion
Blood Sample	No	4 samples during 15 days
Laboratory Analysis	LLE-GC-ECG	LLME-SPME-GC-ECG
Data analysis	Regression analysis	Log-transformed data Correlation and regression analysis
Findings	(1) No relationship between TCAA conc. in tap water and urinary TCAA excretion rate (2) Linear relationship between amount of TCAA ingested and urinary TCAA excretion rate (r^2 0.575 $p<0.0001$)	(1) Correlation between TCAA conc. in tap water and urinary TCAA excretion (2) Correlation between amount TCAA ingested and urinary TCAA excretion (3) Log-linear relationship between TCAA ingestion and urinary excretion (r^2 0.33-0.60 $p<0.001$)

which an external exposure level can be detected by the means of biomarker. 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 study, a liquid-liquid microextraction (LLME) and SPME combined with GC-ECD method was used to analyze TCAA and other DBP compounds 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. 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). However, 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). 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 (Elkins 1974; Boeniger *et al.* 1993; Mage *et al.* 2004; Barr *et al.* 2005; Kissel *et al.* 2005). 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 and Wardener 1970; Curtis and Fogel 1970; Greenblatt *et al.* 1976; Wilson and Crews 1995). In our study, the correlation coefficients for measurements of urinary TCAA concentrations and cr-adj. concentrations were similar. Adjustment of creatinine to correct the excretion of urinary TCAA did not improve the results of validity analysis.

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 longer elimination half-life (30 hours to 6 days) than dichloroacetic acid (20 to 60 min) (Paykoc and Powell 1945; Muller *et al.* 1974; Monster *et al.* 1976 and 1979; Humbert *et al.* 1994; Fisher *et al.* 1998; Bader *et al.* 2004; WHO 2000). This elimination half-life reflects the affinity of TCAA for plasma protein and the efficiency of excretion and metabolic processes of elimination (Paykoc and Powell 1945; Marshall and Owens 1954; Sellers and Koch-Weser 1971; Muller *et al.* 1972; Monster *et al.* 1976). TCAA has adequate persistence in blood and urine to allow the measuring of current TCAA exposure in drinking water.

Higher concentrations of TCAA were attained in blood or urine when exposure was continuous for 15 days. 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 our study, TCAA

levels in blood and urine reflected 65% of the steady-state condition after the 6th day of exposure and almost 90% steady-state condition after the 12th day of exposure, assuming a median urinary excretion half-life of 4 days. Thus, continuous exposure after the 12th day of exposure was sufficient for stable measurement. The reliability of urinary TCAA measurements at Urine Collection Days 13, 14, 15, and 16 was high, as discussed in Chapter 3. This is the basis for using the data from the last four days of urine collection for validity analysis.

5.2.2 Ingestion and Loading in Blood

The correlations between blood TCAA concentration and TCAA ingestion were high (r: 0.77–0.82, $p<0.001$) (Table 5-3). Correlations between blood TCAA concentration and urinary TCAA excretion were modest (r: 0.56–0.75, $p<0.001$). The results indicated that the source of TCAA in the blood arose from ingestion of TCAA-containing tap water, and that TCAA elimination in the urine was related to the blood TCAA concentration.

Table 5-4 The Correlation Between Blood, Ingestion and Excretion

Blood TCAA Concentration	Day 14	Day 15
Ingestion		
inc	.77	.82
ina	.78	.82
Urinary Excretion		
uc 14	.58	
uc 15		.74
ucr 14	.60	
ucr 15		.75
ua 14	.56	
ua 15		.73

Note 1: all values: $p < 0.001$

Note 2: inc = TCAA concentration in tap water (μg/L); ina = amount of TCAA ingestion (μg/d); uc = urinary TCAA concentration (μg/L); ua = amount of urinary TCAA excretion (μg/d); bc = blood TCAA concentration (μg/L).

Note 3: Number in column: day of urine collection

TCAA in whole blood refers to the total TCAA burden in the body. For continuous exposure to TCAA in drinking water over two weeks, the blood TCAA reflects the equilibrium between daily intake and excretion. In our study, the TCAA concentration ratio in urine to that in whole blood was 54%, and the ratio of the amount of TCAA excretion to ingestion was 35%. The small proportion of TCAA recovered in urine from the ingested dose and blood could result from plasma protein binding and metabolism of TCAA in the human body.

TCAA can be rapidly absorbed in the human body (Muller *et al.* 1974). A larger proportion of TCAA in the blood is bound to plasma protein at a relatively constant rate, and saturation of binding has been observed in human plasma. (Sellers and 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 a product of the larger number of binding sites and its higher levels of albumin. Free TCAA in blood is rapidly eliminated by glucuronidation and filtration through the kidney (Fisher *et al.* 1991; Nomiyama and Nomiyama 1979). The proposed pathway of TCAA metabolism is reductive dechlorination (Larson and Bull 1992). A one-electron reduction and hemolytic cleavage catalyzed by cytochrome P450 produces the dichloroacetyl radical. The free radical abstracts a hydrogen atom to yield DCAA. The microsomal enzyme-mediated dehalogenation process can yield CO_2 and GOG (nonchlorinated acids glyoxylate, oxalate, and glycolate). The proportion of metabolites is 6–8% for CO_2, 1–2.5% for DCAA, and 5–11% for GOG in rats and mice (Green and Prout 1985; Larson and Bull 1992). TCAA was eliminated via feces (1.4–3%). About 50% to 65% of unchanged TCAA was excreted in the urine in animals. 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 study, the recovered ratio of 35% in urine from the ingested dose is similar to the ratio reported in the literature.

The blood concentration of TCAA is related to current exposure with a high specificity, and it 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.

5.3 Conclusions

There were modest correlations between TCAA concentrations in supplied tap water and urinary TCAA excretion, and the amount of TCAA ingestion and urinary TCAA excretion. The major source of TCAA excretion came from supplied TCAA-containing tap water. TCAA concentration in tap water and amount of TCAA ingestion can be good surrogates for TCAA exposure from ingestion of drinking water. There were weak correlations between the volume of tap water intake and urinary TCAA excretion ($r=0.27$).

There were high correlations between blood TCAA concentration and TCAA ingestion or excretion. The source of TCAA in the blood resulted mainly from ingestion of TCAA-containing tap water. Urinary TCAA excretion correlated to the blood TCAA concentration. The blood concentration is the best biomarker of exposure to TCAA in drinking water but because it requires invasive sampling. TCAA in blood may not be the most practical biomarker for field use.

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CHAPTER 6 FEASIBILITY

Reliable and sensitive biomarkers of exposure are useful tools for understanding the nature and extent of exposure in the general population. A biomarker of exposure can be employed to identify and assess the exposure of individuals or a population to an environmental xenobiotic and can establish a relationship between external exposure and internal dose and between exposure and health outcomes in humans (Hrudey *et al.* 1996). In the public health field, a biomarker of exposure can be applied for screening and monitoring purposes. In epidemiological studies, a biomarker of exposure can be utilized to improve exposure assessment because it is expected that a biomarker of exposure can classify more clearly the exposure status in individuals (Groopman and Kensler 1999, 2005; DeBord *et al.* 2004).

The use of a biomarker of exposure in an epidemiological study is subject to the limitations introduced by intraindividual and interindividual variability. Such limitations can lead to substantial misclassification of exposure. It is important to understand the use of an appropriate biomarker of exposure for a study, and to disseminate and interpret data that are useful for individual or population-based exposure assessment.

The process of selection and validation of a biomarker of exposure requires selection of a relevant biomarker for study, logical consideration (conceptualization), development of laboratory methodology with acceptable accuracy and precision, establishment of quality assurance/control of laboratory methodology, practice for the field collection, elevation of reliability and validity, identification of predictive value, affordability, and applicability to the general population (WHO 2001).

The objective of this study was to validate trichloroacetic acid (TCAA) as a useful biomarker for exposure to disinfection by-products (DBPs) in drinking water. The conceptual framework of the selection process and the feasibility of the field study are illustrated in Figure 6-1. The feasibility of using TCAA as a useful biomarker of exposure to DBPs in drinking water is discussed in this chapter. Affordability is not discussed in this chapter because the cost-effectiveness of a biomarker approach versus other exposure assessment methods was not investigated in this study.

6.1 Understanding of Purpose of Study

There are two fundamental principles for the biomarker approach: (1) that a biomarker of exposure can be absorbed into the body via external exposure (bioavailability), and (2) that there is a relationship (identified or unidentified) between exposure and the biomarker response (Hrudey *et al.* 1996).

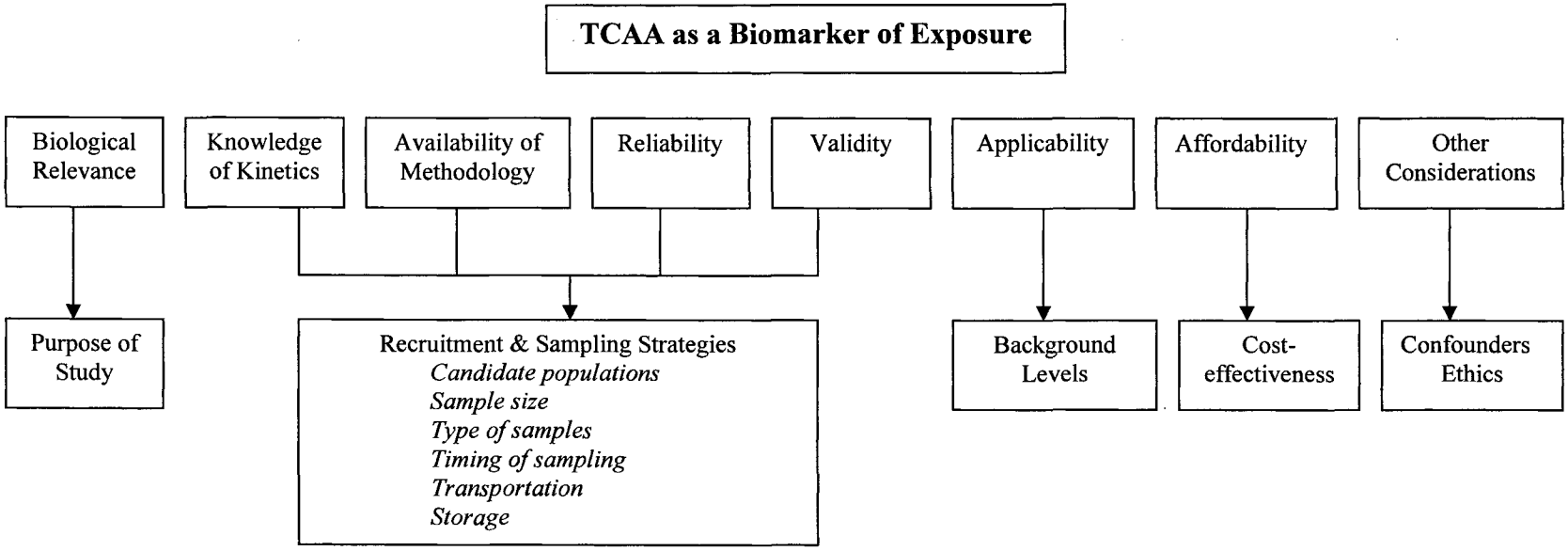

Figure 6-1 Conceptual Framework and Feasibility of Using TCAA as a Biomarker of Exposure

If these principles are satisfied, the biomarker will be a good indicator of exposure. The use of TCAA as a biomarker of exposure relies on these principles and on a full understanding of the study purposes or hypothesis.

From an exposure-biomarker response perspective, TCAA can be selected to explore the relationship between TCAA and reproductive and developmental outcomes or cancers in humans. From a public health perspective, TCAA can be employed for biomonitoring as a surrogate for screening exposure to a specific DBP compound or DBP mixture in drinking water in individuals, communities, and specific populations. The biomonitoring information in different groups of the population serves as the baseline of population-based exposure and provides the basis for policy-making. The measurement of biomarkers of exposure may not be the only approach for improving exposure assessment at a reasonable cost. An analysis of cost-effectiveness will be needed to assess alternative exposure measures for improving exposure assessment in the most practical manner.

6.1.1 Exploring the Relationship between Exposure and Biomarker Response

Research on the relationship between TCAA exposure and adverse health outcomes has not been extensively documented. In animal models, DCAA and TCAA elicited renal carcinogenic and/or liver tumor promoting activity only at concentrations massively higher than any plausible drinking water exposure (Herren-Freund *et al.* 1987; Pereira *et al.* 2001; Ge *et al.* 2001; Bull *et al.* 2004; Tao *et al.* 1998, 2005). DCAA and TCAA increase chloroform toxicity (liver and kidney) (Davis 1992). In two epidemiological studies, haloacetic acid exposure was not directly associated with reproductive outcomes such as stillbirth risk after controlling for THM exposure (King *et al.* 2005; Savitz *et al.* 2005).

Although the relationship between exposure to a biomarker and associated adverse outcomes is not clear, our findings show that urinary or blood TCAA provides a good indicator for current ingestion exposure to drinking water disinfection by-products. Reproductive and developmental outcomes are short-term endpoints. The TCAA biomarker is most useful in a prospective cohort study. Urinary or blood TCAA can be measured frequently at designated times of pregnancy to minimize non-differential measurement errors. The results of internal dose should be interpreted with other exposure source information (e.g., questionnaires and environmental monitoring).

TCAA persists in the body long enough to accumulate levels in blood or urine. If TCAA can be used as a surrogate of DBP exposure, it can be a valid indicator for cumulative exposure if pregnant women consume tap water during the entire pregnancy period. In this case, the TCAA biomarker can be used in a case-control study to provide information about the internal dose. Urinary or blood TCAA samples are obtained from cases and control at the time of the study.

Measurement of TCAA as an objective measure of exposure may possibly reduce differential measurement errors resulting from selection bias and recall bias. The exposure status may also need to be confirmed by questionnaire data.

The use of TCAA as a biomarker of exposure for long-term exposure to detect possible carcinogenic effects appears less promising. A prospective study is the only appropriate epidemiological approach to examine the biomarker-related relative risk of cancer because exposure is measured before outcome and the source of population is explicitly defined (Rothman *et al.* 1995; Hunter 1997). This study design is time-consuming and expensive, and involves a small number of cases, the difficulty of frequent sampling, degradation of samples during long-term storage, and a lack of detailed information on potential confounders (Potter 1997).

In a prospective cohort design for cancer study, the exposure of interest is a moving average exposure because the time-frame for induction of cancer is very long (years or decades). If individuals live in one location long enough (at least several years) and continue to consume DBP-containing tap water, urinary/blood TCAA can serve as a biomarker of chronic exposure in a prospective cohort study. The major limitations to its feasibility are the constraints of obtaining frequent samples in the larger cohort (Hunter 1997). In most studies, the sample collection takes place at a single point in time.

If individuals do not continue to ingest TCAA-containing tap water for a sufficiently long time, infrequent sampling will reflect the variation of TCAA exposure from time to time in individuals. Intraindividual variability is introduced. Measurement errors such as nondifferential misclassification are inevitable if intraindividual variability is random (Liu *et al.* 1979; Hunter 1997). However, increasing the sample size or repeated sampling from an individual to reduce intraindividual variability will increase the expense of sample collection considerably. An alternative approach is to estimate the extent of intraindividual variability in a study cohort. The extent of intraindividual variability is used to adjust for bias toward the null direction (Willett 1990; Hunter 1997).

A nested case-control design can be employed in a cancer study by using biomarkers of exposure (Hunter 1997). Biological samples may be collected from cohorts and stored early in the study. A biomarker of exposure will be measured at the time that each case is identified. The TCAA biomarker may not be suitable for this type of study because of degradation of TCAA during storage.

A biomarker of exposure has limited value for use in a case-control design of cancer study since cancer may alter any exposure marker or biological process (Potter 1997). The use of TCAA as a biomarker is not appropriate in a case-control design because it indicates a short-term exposure.

A biomarker of exposure may be considered in ecological study designs for cancer. Using long-term monitoring, a baseline of TCAA exposure in communities and populations, geographical differences, and time trends of TCAA exposure can be established. The TCAA biomarker, as an independent variable, can be used to assess cancer incidence and prevalence. The major disadvantage is that ecological study designs are inherently weak for providing insights on causation.

6.1.2 Public Health Surveillance

Public health surveillance is the ongoing systematic collection, analysis, and interpretation of health data (Schulte 2005). Measurement of biomarkers of exposure is one biomonitoring method for public health surveillance (Ogata *et al.* 1997; Morgan 1997; Morgan *et al.* 1999; WHO 2001). Biomarkers of exposure can be utilized to confirm exposure to xenobiotics in individuals and populations. Biomarkers of exposure are also used to routinely screen exposure in individuals or population groups to characterize exposure status and patterns, identify relationships between exposure and susceptibility factors (e.g., high exposure group), and provide a basis for prevention efforts.

Traditionally, biomonitoring is used for assessing exposure to a single xenobiotic. In many circumstances, people are exposed to xenobiotic mixtures. An indicator selected from the mixture can be useful for screening the mixture exposure if this indicator behaves in the organisms in a similar way to the toxic compounds of the mixture and the initial mixture is a stable composition (Aitio 2000).

In the past, trihalomethanes (THMs) have been used as surrogates of DBP mixture in epidemiological studies. In these studies, weak to modest association between exposure to chlorinated drinking water or THMs and carcinogenicity and adverse reproductive and developmental effects were reported (Boorman *et al.* 1999; Nieuwenhuijsen *et al.* 2000; IPCS 2000; Graves *et al.* 2001; Bove *et al.* 2002; IARC 2004). The THMs are the most prominent volatile compounds in DBP mixtures. Their major exposure routes are inhalation and dermal contact. The THMs can be easily inhaled or absorbed through dermal contact. They are metabolized to carbon dioxide and/or carbon monoxide in the liver, and rapidly exhaled (Fry *et al.* 1972; NAS 1987). It was rational to select THMs as surrogates for DBP exposure specifically to estimate current exposure via inhalation and dermal contact (e.g., showering, bathing, or swimming).

The instability of THMs limits their use as indicators in environmental and biological samples. The pulmonary excretion of chloroform occurs between 15 min and 2 hours (WHO 2000). Exhaled breath is the most practical sample method for measuring THMs. The concentrations of THMs in alveolar air can fluctuate very rapidly. The baselines of THMs in blood have been measured in U.S. populations (Backer *et al.* 2000; Savitz *et al.* 2005). Seasonal variation of

blood THMs was observed. Human exposure may come from airborne THMs such as chloroform released from tap water (Jo *et al.* 1990a, b) but studies found no clear correlation of blood THM levels with THM concentrations in tap water (Miles *et al.* 2002; Savitz *et al.* 2005). Breath and blood sampling were not commonly used for people exposed to THMs via drinking water ingestion because THMs are metabolized rapidly in the liver.

HAAs are the second most prominent compounds in DBP mixtures. They are semi-volatile. TCAA is one of the principal HAA compounds found in disinfected drinking water. TCAA in urine and blood was correlated with TCAA concentrations in tap water or the amount of TCAA ingestion, as discussed in Chapter 4. The findings indicated that TCAA is absorbed via ingestion of drinking water into the human body. TCAA is relatively common in disinfected drinking water compared to other DBP compounds except for THMs. TCAA is a relatively stable component of the mixture of DBPs that normally occur. Therefore, TCAA can in some instances serve as a surrogate to screen DBP exposure from drinking water.

Because it depends on the nature of the water treatment and distribution systems, the use of TCAA for this purpose is very site-specific. In some studies, TTHMs are moderately or strongly correlated with HAAs (Singer *et al.* 1995; Villanueva *et al.* 2003; Hinckley *et al.* 2005). In a UK study, the correlations between TTHMs and THAAs in the water supply from three water companies varied from no correlation to a high correlation (Malliarou *et al.* 2005). In Nova Scotia, Canada, TTHMs for 140 household water samples were fairly correlated with TCAA in tap water (King *et al.* 2004). In a study from western Canada, strong correlations between DCAA plus TCAA and TCM plus BDCM were observed in drinking water from water treatment plants and distribution sites in a city in Alberta (Rizak *et al.* 2000). Conventional treatment is used in these water treatment plants. In the same study, water monitoring was performed for City A (see definition of City A in Chapter 2). Fair or poor correlations between THAAs and TTHMs were observed in that distribution system. In our study, the correlation between TCAA and TCM/BDCM in tap water from City A was fair. The poorer correlation may have resulted from loss of volatile THMs in the open storage reservoir in City A and biodegradation of HAAs in distribution system (Rizak *et al.* 2000, Chen and Weisel 1998).

Dichloroacetic acid (DACC) is another principal HAA species present in drinking water. DCAA is metabolized quickly and has an elimination half-life between 20 min and 60 min in humans (WHO 2000). Therefore, measuring DCAA in biological media is not practical.

6.1.3 Exploring the Relationship between Exposure and Internal Dose

No exposure measure is ideal for all individuals. Each measure has a limited ability to correctly classify individuals into exposure categories (Rothman *et al.* 1995). A biomarker of exposure (internal dose) alone is not a perfect indicator for exposure. It needs to be considered along with other sources of data (questionnaire, environmental exposure measures, and medical records). The availability and quality of that data as well as the affordability must be considered.

Detection of TCAA in biological samples provides confirmation of exposure to TCAA or a DBP mixture in individuals. The internal dose of TCAA reflects a total dose integrated from multiple exposure routes, especially via ingestion of drinking water. The use of TCAA as a biomarker improves the classification of DBP exposure at an individual level to a certain extent. A major limitation of using TCAA as a biomarker is intraindividual and interindividual variability. In our study, interindividual reliability when measuring TCAA in urine or blood was relatively high, in part because our study design assured a substantial range of TCAA exposure. Urinary or blood TCAA is considered a reliable biomarker of exposure.

The sources of variability such as temporal or spatial variation are other influential factors. The extent of source variability affecting variation of urinary or blood TCAA in the human body was not assessed in our study. The concept of a homeostatic control system in the human body to protect the internal biological system from larger environmental fluctuations has been discussed (Handy *et al.* 2002). Ideally, the variability observed when measuring the TCAA biomarker should be less than the variability resulting from non-drinking water exposure sources.

In addition to variability, the use of TCAA as a biomarker of exposure does not control for confounding factors. In some cases, the use of a biomarker of exposure can introduce confounding factors into a study (IARC 1997). For example, chloral hydrate (CH) is often present in drinking water as a confounding variable. CH can be metabolized to TCAA. The measured urinary TCAA may then be partially attributable to ingestion of CH in drinking water.

Environmental measurements such as levels of DBP compounds in tap water, water treatment sites, and distribution systems are frequently used in epidemiological studies to classify the exposure status of individuals. Spatial variability and seasonal variation of the concentrations of DBPs in drinking water have been observed (Health Canada 1996). In our study, TCAA concentrations in tap water were strongly correlated to urinary or blood TCAA. The results indicated that measuring TCAA levels in environmental samples was an important variable for classifying exposure status. However, using this approach alone did not provide accurate information on exposure classification at an individual level.

The information on exposure patterns such as volume of water consumption, types of water (use of filtration devices and cold vs. hot tap water), locations of consuming tap water (home vs. workplace), diet, physical activities, and confounding factors is collected via questionnaires or interview approaches. Although this approach is subject to information bias, the data can be used to further refine exposure assessment.

In our study, the volume of tap water consumption was not directly correlated with TCAA excretion from the body. The use of the volume of tap water consumption alone does not enhance exposure assessment. The amount of TCAA ingestion (TCAA concentration x volume of tap water consumption) was directly associated with TCAA excretion. The volume of tap water consumption combined with TCAA concentrations in tap water provides a more accurate estimation of TCAA exposure.

The information on the types of tap water helps to adjust exposure in individuals because the concentrations of DBPs change after filtering tap water and after heating or boiling tap water. TCAA concentrations of 65% in tap water decreased after using Point-Of-Use (POU) devices (Savitz *et al.* 2005). Boiling may decrease TCAA concentrations of 9% to 37% (Krasner and Wright 2005; Savitz *et al.* 2005). POU devices were not a factor in our study because we provided the water to participants and they were instructed to use that water directly.

In epidemiological studies, when considering the strengths and limitations of each exposure measure, a combination of three exposure measures improves exposure assessment. However, the combined approach may not be practical in terms of cost-effectiveness. The use of TCAA as a biomarker may be more valid than the use of questionnaires to assess current exposure but the use of questionnaires and long-term monitoring of TCAA in water may be more valid than the use of the TCAA biomarker to evaluate past exposure and to identify confounding factors. Studies on correlations between the three types of exposure measurements and cost-effectiveness should be conducted to elevate the approach that is sufficient for achieving acceptable accuracy of exposure assessment with reasonable cost.

6.2 Recruitment of Candidate Populations

Selection of candidate populations depends on the purpose of study, the logistics of access to the candidate populations and sample collection, and ethical and social considerations. For public health surveillance, to explore the relationship between exposure and internal dose, the general population can be recruited. For reproductive and developmental outcome studies, pregnant women or women of reproductive age are eligible for selection. Exposure characteristics are different among different groups of populations. Pregnant women consumed more tap water than non-pregnant women (Zender *et al.* 2002; Kaur *et al.* 2004). Average

daily showering or bathing times are longer for pregnant women than non-pregnant women (Shimokura *et al.* 1998; Zendar et al. 2002; Kaur *et al.* 2004).

Accessibility to a candidate population and an information dissemination process are important for the recruitment process. In our study, two approaches were employed. The first approach was to post the study information in the newsletter of the Graduate Students' Association at the University of Alberta every two weeks via a website. A total of 209 individuals responded via e-mail to the request for volunteers. This group of responders consisted of students and staff on campus. Thirty-five percent (74 out of 209) of responders were interested in being interviewed after receiving the detailed study information. Twenty-five percent (52 out of 209) of eligible individuals were recruited to the study.

The second approach was to mail out letters to residents living in City B. The mailing list was obtained from a provincial health database. The letters were mailed to 400 residents. Six percent (24 out of 400) of the individuals responded to the request for volunteers. Of the 24 individuals, 9 (37.5%) expressed interest in receiving further information about the study. Fifty-four (13.5%) letters were returned because of incorrect mailing addresses. The individuals of 80.5% on the mailing list did not respond to the request to volunteer. This second group was not used because of the difficulty of access to the candidate population in this large city.

6.3 Sampling Strategies and Processes

6.3.1 Development of Protocols

A pilot study was conducted to examine logistics and feasibility by using all the protocols before beginning the main study. These protocols included questionnaires, recruitment, tap water shipment, water consumption, daily diary booklets, schedules for delivering water bottles, urine collection, blood collection, transportation and storage, chemical analysis, record keeping and database (appendix V). Eight individuals participated in this pilot study. In the pilot study, the time of urine collection was the 1^{st}, 2^{nd}, 8^{th}, 14^{th}, 15^{th}, and 16^{th} days after the supplied water consumption. The largest variation of urinary TCAA levels was observed on the 16^{th} day. In order to improve statistical analysis, additional urine samples were collected on the 13^{th} day from each participant in the main study. In the pilot study, the largest variation of urinary TCAA levels was also observed in participants assigned to the highest exposure group (consuming 100% supplied tap water shipped from City B). Based on this information, the sample size of the highest exposure group was increased in the main study to accommodate statistical analysis. Some details of the initial protocols such as labeling the study bottles, the schedule for delivering water and picking up urine samples, and methods for study information dissemination were revised in the main study.

6.3.2 Sample Size

The determination of sample size involves issues of feasibility, cost, and the possible effects of confounding factors (Schulte and Perera 1997). A good strategy for biomarkers of exposure is to select a control group and exposure groups with different exposure ranges matching potential confounding factors in both groups in order to enhance the precision of the measured association between exposure and outcome. This study design will decrease the study power (Hulka 1990).

Temporal and spatial variability influences the determination of sample size as well. Practical options to minimize the variability of a biomarker of exposure include (1) selection of "high exposure" and "low exposure" sites with similar water treatment processes for sampling; (2) repeated sampling during the study period; (3) systematic analysis of confounding factors; and (4) increase of sampling frequency to obtain an average of exposure rather than a fixed sampling time (IARC 1997). These strategies will increase the cost of a study. In practice, the determination of sample size relies on a balance between the statistical power and the cost of recruiting participants, sample collections and laboratory assays.

The implications for sample size were considered in the selection of TCAA as a biomarker. An expensive biomarker study may limit the study sample size. In order to reduce intraindividual variability, repeated samples from an individual may be required. It is important to consider the trade-off between increasing the number of participants and collecting multiple samples from an individual (IARC 1997). In our study, the relative extent of intraindividual variability was characterized (see Chapter 3). The reliability coefficients (ICC) were high (ICC>0.70) for all measurements of TCAA ingestion and excretion in four repeated urine samples and two blood samples from one individual. For a sampling strategy, one-day blood sampling and two-day urine sampling from an individual are sufficient to achieve reliability in a study if TCAA levels are steady-state in the body.

6.3.3 Type of Specimen

The preferred types of samples for measuring TCAA in biological media are blood and urine. In our study, a good correlation between TCAA concentrations in tap water and whole blood TCAA concentrations was observed (see Chapter 4). TCAA in whole blood reflects the total burden of TCAA while TCAA in serum reflects the free TCAA that can be eliminated via urine. Whole blood or serum TCAA is a good biomarker of exposure to DBP exposure in drinking water. The limitation of using blood TCAA in an epidemiological study is the invasive procedure required for sample collection.

Urinary TCAA is a useful biomarker of exposure to DBP exposure in drinking water and a good correlation was observed between TCAA ingestion in tap water and urinary excretion. The collection procedure for urine samples is non-invasive. Variations in concentrations of urinary TCAA depend on the rate of urine production. The traditional practice for reducing this variation is to correct for creatinine excretion. The determination of urinary creatinine is recommended to normalize over-diluted or over-concentrated urine samples. 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 (Elkins 1974; Boeniger *et al.* 1993; Mage *et al.* 2004; Barr *et al.* 2005; Kissel *et al.* 2005). The creatinine concentration is inversely related to fluid intake. Analysis performed on very dilute (less than 0.3 g/L) or concentrated (greater than 3 g/L) urine samples must be interpreted with caution (Rosenberg *et al.* 1989). When extremes of creatinine variability are observed, the creatinine correction is not valid. Some studies 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 and Wardener 1970; Curtis and Fogel 1970; Greenblatt *et al.* 1976; Wilson and Crews 1995). In our study, the findings reveal that the correction of the excretion of TCAA for urinary creatinine improves the results of intraindividual and interindividual variability and the validity to a limited extent.

6.3.4 Timing of Sampling

The elimination half-life of a xenobiotic is the time required to eliminate half of the amount of the current xenobiotic burden from the body. The elimination half-life reflects the affinity of the xenobiotics for the biological matrix, the efficiency of excretion, and metabolic processes of elimination. Therefore, the timing of sampling is critical in an exposure assessment. The persistence of a biomarker of exposure in the human body can be employed to estimate past, current, and future exposures. The persistence of a biomarker relies on kinetic models and parameters and the availability of biological media. The elimination half-life can vary considerably in different parts of the body. For xenobiotics with a long elimination half-life, higher concentrations of xenobiotics will be attained in body fluids if exposure is continuous or repeated. If a continuous exposure is three, five, and seven half-lives, blood concentrations of a xenobiotic will reach approximately 90%, 97%, and 99% of the steady-state concentration respectively. In practice, a continuous exposure for three and five half-lives should be sufficient for stable measurement.

The elimination half-life of TCAA after direct TCAA ingestion ranges from 30 hours to 6 days in humans (Paykoc and Powell 1945; Muller *et al.* 1974; Monster *et al.* 1976, 1979; Humbert *et al.* 1994; Fisher *et al.* 1998; Bader *et al.* 2004). TCAA levels in biological media will reflect 65% of the steady-state condition

after the 6th day of exposure and almost 90% steady-state condition after the 12th day of exposure, assuming a median urinary excretion half life of four days. TCAA has adequate persistence (a long enough elimination half-life) in blood or urine to allow a measure of the current TCAA to reflect recent exposure from drinking water.

In an epidemiological study, the timing of sampling should be determined after assessing exposure conditions in individuals. If an individual continues to drink tap water from local water supplies over two weeks, urine samples can be collected on any day to provide a relatively stable measure of urinary TCAA levels. If exposure to TCAA in tap water is not repeated over two weeks, urinary TCAA levels may not be stable and will result in larger intraindividual variability. Increasing repeated sampling in an individual will be necessary in this case.

6.3.5 Transportation and Storage

Conditions for transportation and storage of water and biological samples need to be assessed before field collection in order to reduce intraindividual variability in an epidemiological study. In our study, a stability test of TCAA concentration in tap water samples in different types of containers and storage temperatures was conducted before developing a sampling protocol. Changes in TCAA and DCAA concentrations in tap water stored in a cool room (4 °C) are illustrated in Figure 6-2 and 6-3.

TCAA and DCAA concentrations in tap water were very slightly different when using 20-L polycarbonate containers and 20-L amber glass containers. These two types of containers were used for shipping tap water from City A to City B. During 10 days of storage, TCAA and DCAA concentrations varied only slightly between a refrigerator (4 °C) and room temperature (20 °C). TCAA and DCAA concentrations declined with length of storage. TCAA had decreased by 8% on the 3rd day, 13% on the 5th day, and 37% on the 9th day. DCAA had decreased by 5% at the 3rd day, 28% on the 5th day, and 50% on the 9th day.

Changes in TCAA and DCAA concentrations in urine stored in a refrigerator (4 °C) and a freezer (-20 °C) are illustrated in Figures 6-4 and 6-5. TCAA concentrations in urine were only slightly different whether storied in a refrigerator or in a freezer; both declined with time. Urinary TCAA decreased about 20% within 13 days and 42%–48% over 21 days in a refrigerator. Urinary DCAA decreased about 15% within 21 days and 32% by the 36th day in a refrigerator.

The decrease of TCAA and DCAA concentrations in water and urine may result from biodegradation. Although the water was disinfected, it is not sterile. The dominant loss of TCAA in water due to biodegradation was reported by some researchers (Borthling *et al.* 1979; Ellis *et al.* 2001; McRae *et al.* 2004).

In summary, all tap water and urine collected for TCAA or DCAA analysis must be transferred to the analytical laboratory within 24 hours. Polycarbonate containers or bottles can be used for storing specimens. Storage of water and urine samples at 4 °C is preferred. TCAA and DCAA in tap water should be analyzed within three days after sample collection. TCAA and DCAA in urine should be analyzed within two weeks after sample collection.

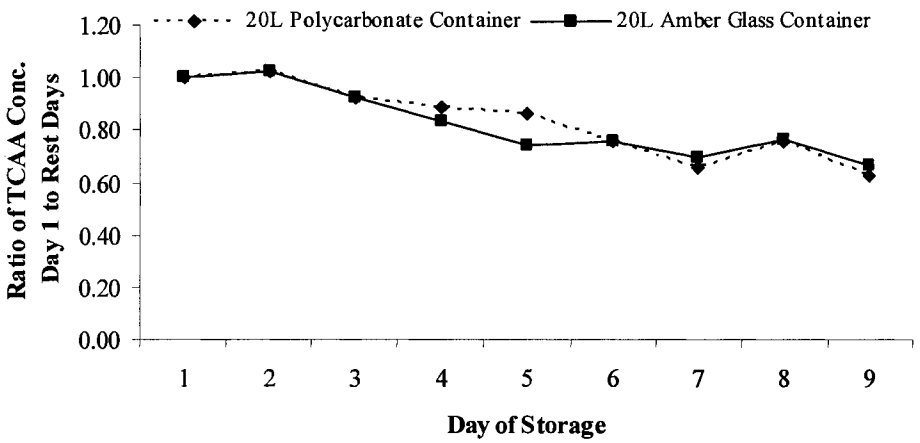

Figure 6-2 Tap Water TCAA Concentration Changes with Time during Storage

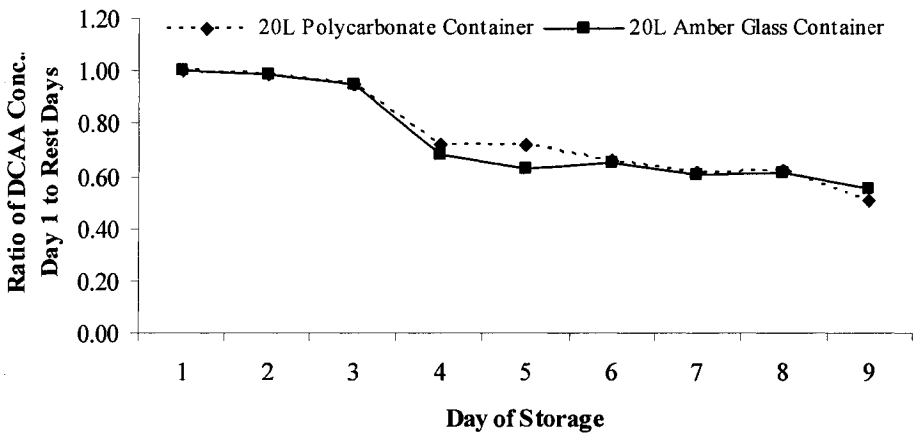

Figure 6-3 Tap Water DCAA Concentration Changes with Time during Storage

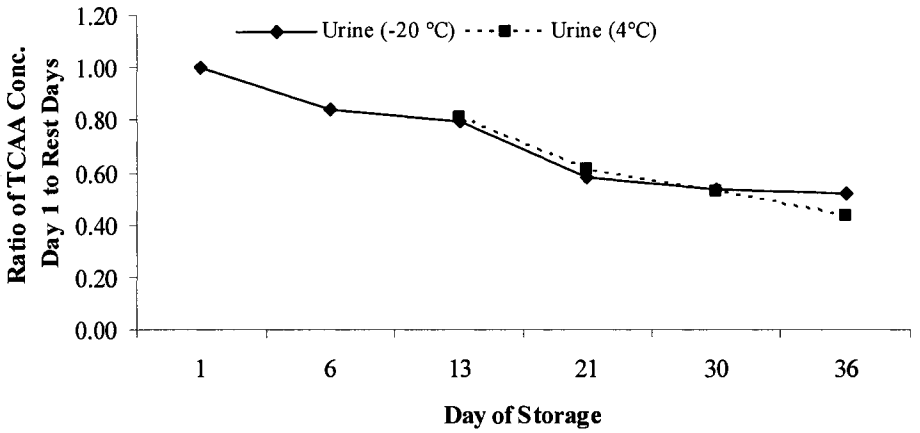

Figure 6-4 Urinary TCAA Concentration Changes with Time during Storage

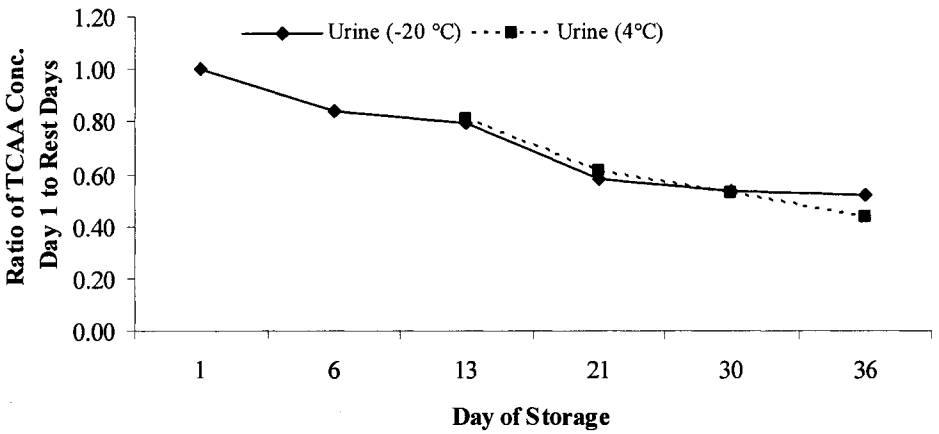

Figure 6-5 Urinary DCAA Concentration Changes with Time during Storage

6.4 Availability of Laboratory Methodology

A biomarker of exposure for individuals is only possible if that biomarker can be measured in biological media using valid analytical methods. Analytical considerations include the availability of instrumentation and appropriate methodology, sensitivity, specificity, appropriate precision and accuracy, and QA/QC protocols.

Laboratory methods for analyzing some DBP compounds in water such as the US EPA Method 552.2 and Standard Methods 6251B have been developed and certified by regulatory agencies. These methods are used for water analyses in most water utility laboratories. Techniques used for analysis include high-performance liquid chromatography (HPLC), HPLC with mass spectrometry (MS), capillary electrophoresis (CE), HPLC-MS with negative ion electrospray ionization-tandem MS and solid-phase microextraction (SPME) (O'Donnell *et al.* 1995; Martinez *et al.*1998a, 1998b, 1999, 2000; Aher and Buchberger 1999; Kuklenyik *et al.* 2002). Solid-phase microextraction (SPME) integrates sampling, extraction, concentration, and sample introduction in a single step (Sarrion *et al.* 1999, 2000a, 2000b, 2002, 2003; Wu *et al.* 2002). In our study, a liquid-liquid microextraction (LLME) and SPME combined with GC-ECD method developed by Wu *et al.* (2002) was employed to analyze TCAA, DCAA, and other DBP compounds in samples of tap water, urine, and blood with only 50–100 μl of sample volume.

The QA/QC program was well-documented in our study. The method detection limits (MDLs) using LLME-SPME-GC-ECD methods were calculated as SD x 3.143, where SD was the standard deviation of the concentration obtained from a replicate standard (N=7) fortified with TCAA and DCAA at concentrations that produce peak intensities approximately five times the intensity of the background noise. The value of 3.143 refers to the Student's t value for 99% confidence with 6 degree of freedom. The MDLs were 0.6 μ/L for TCAA and DCAA in our study.

The limits of detection (LOD) using the LLME-SPME-GC-ECG method are arbitrarily defined as SD x 3.143, where SD is the standard deviation with at least 6 degrees of freedom at concentrations near 0, obtained by extrapolation from the calibration curve. The estimated detection limit (DL) is extrapolated from the calibration curve to the hypothetical peak intensity three times greater than the average intensity of the background noise near the TCAA and DCAA peaks. The estimated DLs were 0.5 μg/L for DCAA and 0.2 μg/L for TCAA in our study.

A blank was analyzed at the beginning of each analysis set. Milli-Q water and HPLC-grade water were used as reagent blanks for the analysis of water, urine, and blood samples. For the urine samples with the lowest TCAA concentrations, human urine was used as the reagent blank. For the blood sample with the lowest TCAA concentrations, human blood collected from a volunteer who drank only tap water from City B was used as a baseline for quantifying TCAA concentrations.

Laboratory multiple analysis was performed for samples of water, urine, and blood. 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 determined by extraction of a sample fortified with known concentrations of a TCAA standard. Calculation of the percent recovery (R) is

$$R = 100\ (A - B)/C$$

where A is the total measured concentration in the fortified sample for background concentration, B is the measured concentration in the unfortified sample, and C is the fortifying concentration.

The mean recovery of TCAA in 12 water samples was 91%, ranging from 70% to 126% except for one sample from City A (61%). The mean recovery of TCAA in urine samples was 86%, ranging from 77% to 108%. The mean recovery in blood samples was 101%. Recovery of TCAA in 70 blood samples ranged from 70% to 130%. Recovery was 51% in 1 blood sample, and 137%–149% in 5 blood samples.

The LLME-SPME-GC-ECD allowed the analysis of DBP compounds in water and biological samples in a rapid manner with acceptable precision. This methodology could be used in a large cohort epidemiological study provided that the sampling schedule could spread the sample loading to the analytical laboratory over time.

6.5 Applicability in the General Population

The background levels of blood and urinary TCAA provided a basis for validity analysis after experimental water consumption. Understanding the baseline levels of TCAA in biological media is essential to evaluate variability and the relationship between TCAA ingestion and excretion.

The natural variability of a biomarker of exposure in humans is the result of environmental and genetic factors. Intraindividual and interindividual variability contributes to the background in biomonitoring and epidemiological studies (Schulte and Perera 1993). An understanding of the range of biomarker values in the general population and characterization of intraindividual and interindividual variability are essential before starting a large cohort study. In an epidemiological study, the background levels of a biomarker of exposure provide a baseline for classifying participants to the groups being compared (Schulte and Perera 1993). A study also should be conducted to examine whether changed levels of a biomarker of exposure are related to exposure gradients to xenobiotics in the environmental samples.

In our study, background levels of blood and urinary TCAA concentration were detected in participants (Figure 6-6). The median blood TCAA concentration was 8.7 μg/L. The median urinary TCAA concentration was 3.5 μg/L (2.2 μg/g Cr). The median urinary TCAA levels in our study were consistent with the

background levels of 3.3 μg/L (3.2 μg/g Cr) in an urban U.S. population (Calafat *et al.* 2003). The median TCAA level (6.9 μg/L) in tap water from City B was 8.5% of the median TCAA concentration of City A (78.6 μg/L), which was used for the exposure trial. The source of background TCAA was likely derived from ingestion of TCAA-containing tap water from City B. Changed levels of urinary TCAA were observed in exposure groups with exposure gradients to TCAA in tap water (Figure 6-7).

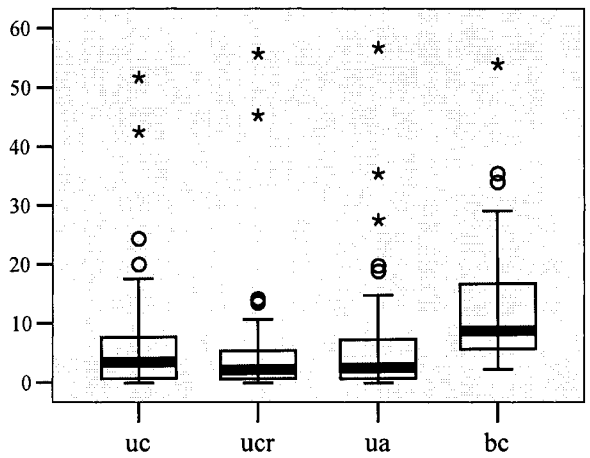

Figure 6-6 Background Levels of TCAA in Biological Samples

[uc = Urinary TCAA concentration μg/L; ucr = Cr-adj. TCAA concentration g/g Cr; ua = amount of urinary TCAA excretion μg/d; bc = blood TCAA concentration μg/L]

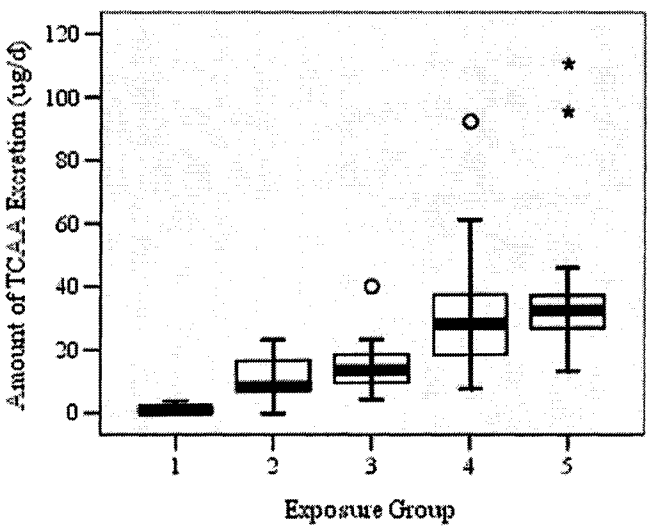

Figure 6-7 Changes of Urinary TCAA with Exposure Gradients in Tap Water

[1= Control, 2 – 5 = exposure to TCAA in tap water from low to high]

The results from our study demonstrated that the measurement of the TCAA biomarker in a larger cohort is possible. The baseline values could be used as a basis for classifying individuals into different exposure groups. Because TCAA levels in drinking water demonstrate spatial and seasonal variation, the background TCAA levels in a study population should be determined according to the study locations.

6.6 Confounding Factors

The confounding factors are often difficult to measure in exposure assessment. Such unmeasured confounders can cause measurement error. In our study, elevated background levels of TCAA were observed in five urine samples and three blood samples. Urinary TCAA concentrations ranged between 10 and 52 μg/L. In the Calafat *et al.* (2002) study, the 90th percentile concentration of urinary TCAA was 23 μg/L and higher urinary TCAA levels in some cases ranged from 50 to >100 μg/L. These higher values may result from other exposure sources.

In TCAA biomarker studies, sources of interindividual variability could arise from exposure to other compounds that may compete for the same biotransformation sites or metabolize to TCAA in the human body. CH is one of DBP compounds measured in drinking water. CH is also used in medicine. CH is rapidly metabolized in the liver and other tissues to trichloroethanol, trichloroethanol-glucuronide, and TCAA (Breimer *et al.* 1974; Marshall and Owens 1954; Reimche *et al.* 1989; Gorecki *et al.* 1990; Humbert *et al.* 1994). Thirty-five percent (a range of 5%–47%) of the initial dose of CH is converted to TCAA (Marshall and Owens 1954; Sellers *et al.* 1972; Allen and Fisher 1993; Humbert *et al.* 1994). The half-life of TCAA after ingestion of chloral hydrate ranged from 3 to 5 days (Breimer *et al.* 1974; Muller *et al.* 1974).

No participants selected for our study used any medications before or during the study. The median concentration of CH in tap water from City B was very low (0.55 μg/L) so it is unlikely that CH contributed to the higher background of TCAA in these individuals. In our study, CH was measurable in tap water from City A (Figure 6-8). The ratio of CH to TCAA intake among individuals was 0.22. Using a 35% conversion factor, through metabolism, CH may have contributed about 8% additional TCAA. Because there was no CH measurement in biological samples, the intraindividual and interindividual variability of additional TCAA cannot be assessed in our study.

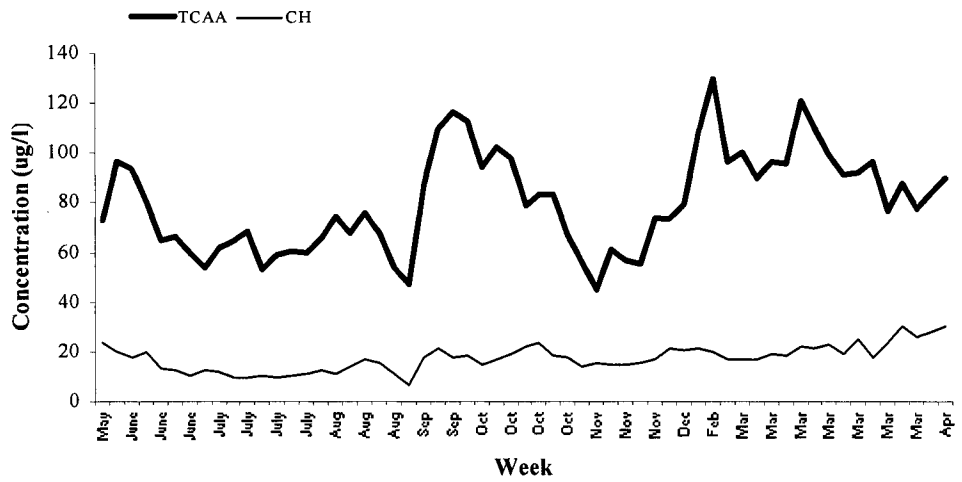

Figure 6-8 Variation of CH and TCAA in Tap Water

Non-ingestion sources for TCAA include showering, bathing, swimming, or dishwashing in TCAA-containing water, visiting dry-cleaning shops, or using chlorinated bleach, and other solvents. Some solvents such as TCE, tetrachloroethylene (PERC), trichloroethanol, trichloroethane (TRI,) and tetrachloroethane can be metabolized to TCAA in the human body (Nomiyama 1971; Monster 1979; Caperos *et al.* 1982; Bruckner *et al.* 1989; Inoue *et al.* 1989; Fisher *et al.* 1998, 2000; Volkel *et al.* 1998; Bloemen *et al.* 2001, Furuki *et al.* 2003). These solvents are used as vapour for metal degreasing or in cold cleaning agents, dry cleaning, printing, printing ink, and some consumer products such as typewriter correction fluid, paint remover, adhesive, stain remover, and rug-cleaning fluid (Aggazzotti *et al.* 1994a, b; WHO 1985; IARC 1995). In our study a questionnaire was administered to each individual to evaluate non-ingestion TCAA sources. The use of household cleaning solutions and products containing solvents was recorded by three participants. These solutions included liquid bleach (sodium hypochlorite), hydroponic solution (nutrients for soils), Ajax oxygen bleach cleanser (crystalline), nail polish (toluene, butyl acetate, ethyl acetate, and dibutyl phthalate), and contact lens cleaning solution (some chloride compounds). A clear pattern between urinary TCAA and solvent use was not observed.

TCAA was detected in food and beverages such as coffee, tea, fruit juice, and canned soups (Raymer *et al.* 2000). The influence of coffee and tea on the levels of urinary TCAA is not documented. In our study, one participant used supplied tap water to make coffee (500–700 mL/d) and tea (250–500 mL/d). Urinary TCAA concentrations were not detectable, but blood TCAA levels were detected. In the future it will be interesting to investigate the relationship between coffee/tea consumption and urinary TCAA levels.

HAAs including TCAA are formed in swimming pools (Aggazzotti *et al.* 1987, 1990, 1993, 1995, 1998; Aiking *et al.* 1994; Lindstrom *et al.* 1997; Kim and Weisel 1998; Fantuzzi *et al.* 2001; Kim *et al.* 2002). Because HAAs are non-volatile compounds, permeability through the skin is very low (Xu *et al.* 2002; Xu and Weisel 2003). The daily exposure dose resulting from showering, bathing, or swimming is insignificant for HAAs (Kim and Weisel 1998; Xu *et al.* 2002; Xu and Weisel 2003). For example, the proportion of absorbed doses of TCAA from daily bathing via dermal contact is about 0.005% to 0.5% of the daily ingestion doses of TCAA (Cleek and Bunge 1993; Xu *et al.* 2002). In our study, four participants swam during the study period. In one case, the urinary TCAA levels declined. In one case, the urinary TCAA level slightly increased on Urine Collection Day 8. In two cases, the urinary TCAA levels remained stable. In general, inhalation and dermal contact via showering, bathing, and swimming appear to be minor routes for TCAA exposure.

Genetic factors are an underlying confounder in individuals. The different rate of metabolism of a xenobiotic in humans is attributable to interindividual variability. The proposed pathways of TCAA metabolism are reductive dechlorination involving cytochrome P450 and the microsomal enzyme-mediated dehalogenation process (Larson and Bull 1992). Metabolism of TCAA generates free radicals and induces lipid peroxidation (Larson and Bull 1992; Ni *et al.* 1996). CYP2E1 is the major isozyme for the metabolic activation of TCAA. Three polymorphisms of CYP2E1 have been detected (Uematsu *et al.* 1991; Hu. *et al.*1997). Wide interindividual variability was found in CYP2E1 (Peter *et al.* 1990; Chang *et al.* 1993; Raucy *et al.* 1995; Lieber 1997; Clewell III *et al.* 2000; Pastino *et al.* 2000; Bebia *et al.* 2004). Theoretically, the variation in metabolisms related to cytochrome P450 activities could result in the variation of urinary TCAA excretion among individuals.

In our study, ethics approval to identify CYP2E1 genotyping in volunteers was obtained. Appropriate blood samples containing white blood cells were collected from 36 consenting participants and stored in the freezer (-72 °C). In the next phase of study, CYP2E1 genotying will be performed by PCR. The evidence from the literature does not indicate a high likelihood that the genotyping of a subset of the study cohort would explain much interindividual variability. However, that hypothesis will be tested in the proposed follow-up.

CYP2E1 is the major isozyme for the metabolic activation of ethanol and many small environmental contaminants. Alcohol consumption could be a confounding factor for urinary TCAA excretion in the body. In our study, six participants consumed beer during some days of the study period. No clear patterns between alcohol consumption and urinary TCAA excretion were observed for these participants.

Other factors (age, sex, ethnicity, and lifestyle) may affect the measurement of a biomarker of exposure. The measured TCAA concentration in blood and urine

depends mainly on the TCAA concentration in water and the volume of water consumed by individuals. Water consumption patterns vary greatly in different age, gender, and ethnic groups, and in different geographic areas and time-activities within individuals. Children or youth aged 11 to 19 consumed higher volumes of community water than adults 20 years and older (EPA 2000; Raman *et al.* 2004). Men ingested more community water than women (EPA 2000; Raman *et al.* 2004). Significant ethnic variation in water intake was observed in terms of availability of water sources (Williams *et al.* 2001). Pregnant women ingested more tap water at home than at work and otherwise outside the home (Shimokura *et al.* 1998). Changes in ingestion of drinking water containing TCAA will be reflected in changes in blood or urinary TCAA, according to our findings. The quantity of a biomarker of exposure can change within an individual over time as a result of changes in diet, health status, and time-activities (Vineis 1997).

Confounding factors are not readily identified in individuals and not easily analyzed for their distinguishing effects. CH in drinking water is a major confounding factor when using TCAA as a biomarker of exposure in an epidemiological study. CH can be measured in water and biological samples. An average correction factor of 35% of CH intake dose can be used to estimate the influence of CH intake. Inhalation and dermal contact via showering, bathing, and swimming are minor routes for TCAA exposure. Other factors such as solvents, diet, and lifestyle are not well-documented.

6.7 Ethical Considerations

Ethical considerations in the use of biomarkers of exposure have received attention (Schulte 1992; Schulte and Sweeney 1995; Schulte *et al.* 1997; Schulte 2004). This complex issue is concerned about misinterpretation, misapplication, and inappropriate dissemination of biomarker information. The exposure information derived from exposure screening may be incorrectly interpreted as implying health risk rather than simply documenting exposure (DeCaprio 2000).

In our study, ethics approval was obtained before the study (Appendix VI). The information collected from each individual was confidential. After evaluating the benefits and risks of participation (Appendix V), participants signed an informed consent form. Participants could withdraw from the study at any time. During the entire study period, trained researchers communicated frequently with participants. Schedules and locations for sample collection were set up to be convenient for participants. This process improved compliance with water consumption (96%) and sample collection (90%). All participants completed the 15-day study.

6.8 Cost-Effectiveness Considerations

The selection of a biomarker of exposure requires consideration of the financial resources required. The use of a biomarker of exposure for an epidemiological study in the community can be very expensive. The estimated cost of a study with a population of 2000 couples using the TCAA biomarker would be more than $1.5 million over a 2-year period (Arbuckle *et al.* 2002). It is necessary to determine all the resources and associated costs required, thereby ensuring the study can be conducted. It is preferable to minimize the cost of sampling programs, while meeting the study objectives. Cost-effectiveness considerations include trade-offs between the loss of statistical power and the cost of data acquisition, such as the cost of the sampling numbers, repeated samples, collecting samples, data analysis, and reporting.

6.9 Summary of Feasibility

TCAA as a biomarker of exposure can indicate TCAA exposure itself or serve as a surrogate of DBP mixture exposure in drinking water. The incorporation of TCAA biomarker of exposure into appropriate studies requires an understanding of the purposes of those studies. Three types of applications are summarized in Figure 6-9.

First, the TCAA biomarker could be utilized to explore the relationship between exposure and biomarker responses in epidemiological studies pertaining to reproductive/developmental and cancer outcomes based on some support of biological relevance. The optimal study design with practical logistics of sampling is a prospective cohort to explore the relative risk of reproductive/developmental outcomes associated with DBP exposure in a group of pregnant women. Considering its representation of short-term exposure and the lack of logistics of sampling, the use of a TCAA biomarker of exposure in a cancer study is not feasible.

Second, the TCAA biomarker is useful in the public health field for long-term biomonitoring or health surveillance at the population level as well as at the individual level. The information from routine biomonitoring can be used to confirm exposure in individuals in communities, to characterize exposure status and patterns, to identify the relationship between exposure and susceptibility factors (e.g., high exposure group), and to provide the basis for prevention effort and policy making. The TCAA biomarker of exposure could serve as a surrogate for DBP mixtures because of its dominance and stability in those mixtures. The use of the TCAA biomarker as a surrogate of THMs depends on the nature of water treatment processes at specific sites. DCAA is not useful as a biomarker of exposure because of its rapid metabolism and very short elimination half-life in the human body.

Third, different measurements of the TCAA biomarker of exposure can be employed to explore the relationship between exposure and internal dose. Direct measurement of the TCAA biomarker allows confirmation of TCAA exposure from all routes in individuals. The limitation of this measure is intraindividual variability which can lead to non-differential misclassification. Environmental measurement provides information on major exposure sources and routes and the quantity of external exposure in population groups rather than individuals. Questionnaires allow collection of information on exposure patterns and confounding variables. Because of the strengths and limitations of each exposure measurement, it is best to combine the three exposure measurements in epidemiological studies to improve exposure assessment. The combined approach may not be practical in terms of cost-effectiveness.

The feasibility of recruitment and sampling is summarized in Figure 6-10. The selection of the candidate populations relies on the study question. General populations are eligible for public health surveillance. Pregnant women are suitable for reproductive and developmental studies.

Sampling strategies are developed based on knowledge of TCAA kinetics, availability of laboratory methodology, and reliability and validity analysis (Figure 6-9). Ideally all protocols should be tested in a pilot study for logistics and standardization. The determination of sample size is based on a trade-off between statistical power and cost. To achieve reliability of measurement, one-day blood sampling or two-day urine sampling in an individual after TCAA exposure over two weeks is recommended. Blood and urine samples can be used. First morning urine samples are suitable for public health surveillance and can be used in a large cohort study. The correction of the TCAA excretion for urinary creatinine improves reliability to a limited extent.

All tap water and urine collected for TCAA or DCAA analysis have to be transferred to a designated laboratory within 24 hours. Polycarbonate containers or bottles can be used for storing specimens. Storage at 4 °C for water and urine samples is preferred. TCAA and DCAA in tap water should be analyzed within three days after sample collection. TCAA and DCAA in urine should be analyzed within two weeks after sample collection.

Measurement of TCAA biomarker can be applied in a larger cohort (Figure 6-11). Baseline values can be used as a basis for classifying individuals into different exposure groups. Because TCAA levels in drinking water demonstrate spatial and seasonal variation, the background TCAA levels in a study population should be determined according to the study locations.

Confounding factors are not readily identified and their effects on individuals analyzed. CH in drinking water is a major confounding factor when using TCAA biomarker of exposure in an epidemiological study (Figure 6-11). CH can be measured in water and biological samples with an assumed correction factor of

35% of CH intake. Inhalation and dermal contact via showering, bathing, and swimming are minor routes for TCAA exposure. Other factors such as solvents, diet, and lifestyle are not well-documented.

Ethic approval must be obtained before the start of a study (Figure 6-11). Informed consent forms must be signed by participants. Frequent communication between participants and researchers improve compliance rates.

6.10 Conclusions

The selection of TCAA as a biomarker of exposure to TCAA or DBP mixture requires an understanding of the purposes of the study. It is feasible to employ TCAA as a biomarker in a prospective cohort to explore the relationship between DBP exposure and reproductive and developmental outcomes. It can be utilized for public health surveillance to screen DBP exposure in the general population or for validating various exposure assessment methods.

The selection of candidate populations depends on the purposes of the study. Pregnant women are eligible for reproductive and developmental outcome studies. The general population is eligible for public health surveillance. Laboratory methods are available for detecting the TCAA biomarker in environmental and biological samples. QA/QC should be performed for protocol development and laboratory analysis. The cost-effectiveness needs to be considered in a study design and sample collection. The determination of sample size depends on a balance between cost and statistical power or repeated sampling. In a steady-state of the TCAA biomarker in the human body (exposure over two weeks), one-day blood samples or two-day urine samples collected from an individual are sufficient to reduce intraindividual variability. Blood and urine samples can be used. First morning urine samples are suitable for large cohort studies. All types of samples should be transferred to laboratories within 24 hours and stored at 4 °C. The suggested maximum storage time is three days for water samples and two weeks for urine samples.

TCAA is applicable as a biomarker of exposure in the general population. In an epidemiological study, a major confounder – chloral hydrate in drinking water – should be identified and analyzed. Ethical and social issues must be considered.

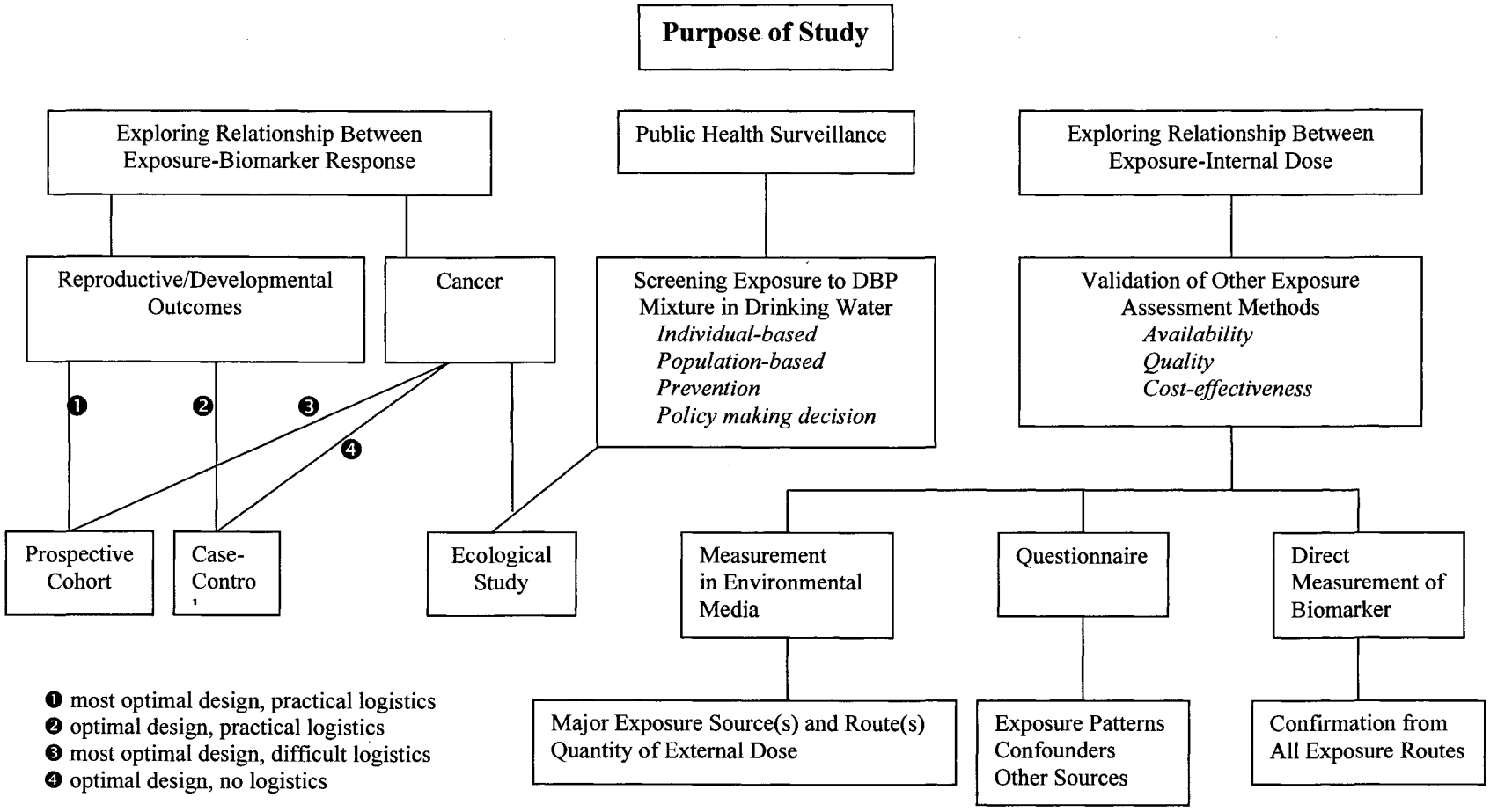

Figure 6-9 Feasibility of Selection of TCAA Biomarker of Exposure Based on Understanding of Purposes of Study

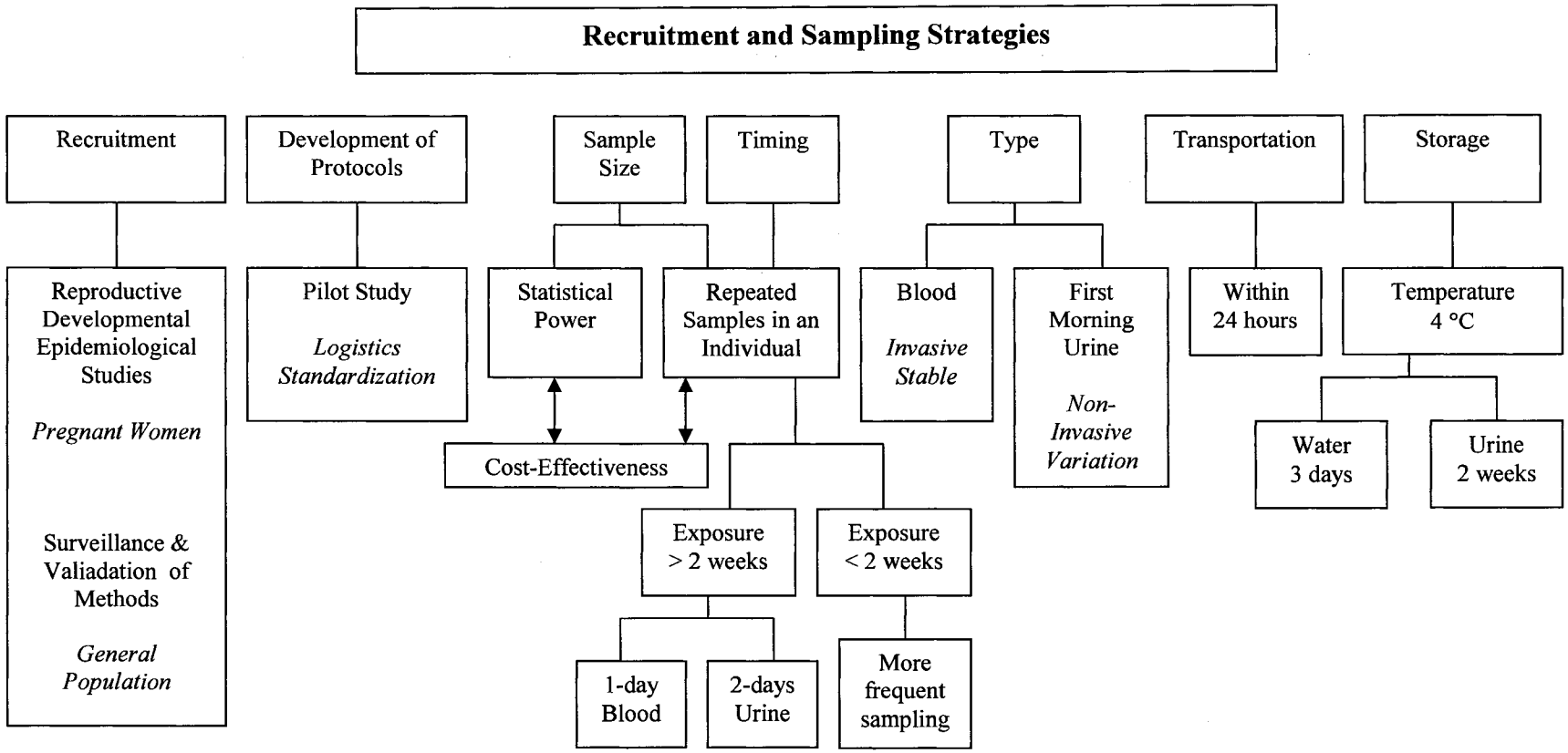

Figure 6-10 Feasibility of Recruitment and Sampling Strategies Based on Kinetics, Methodology, Reliability and Validity

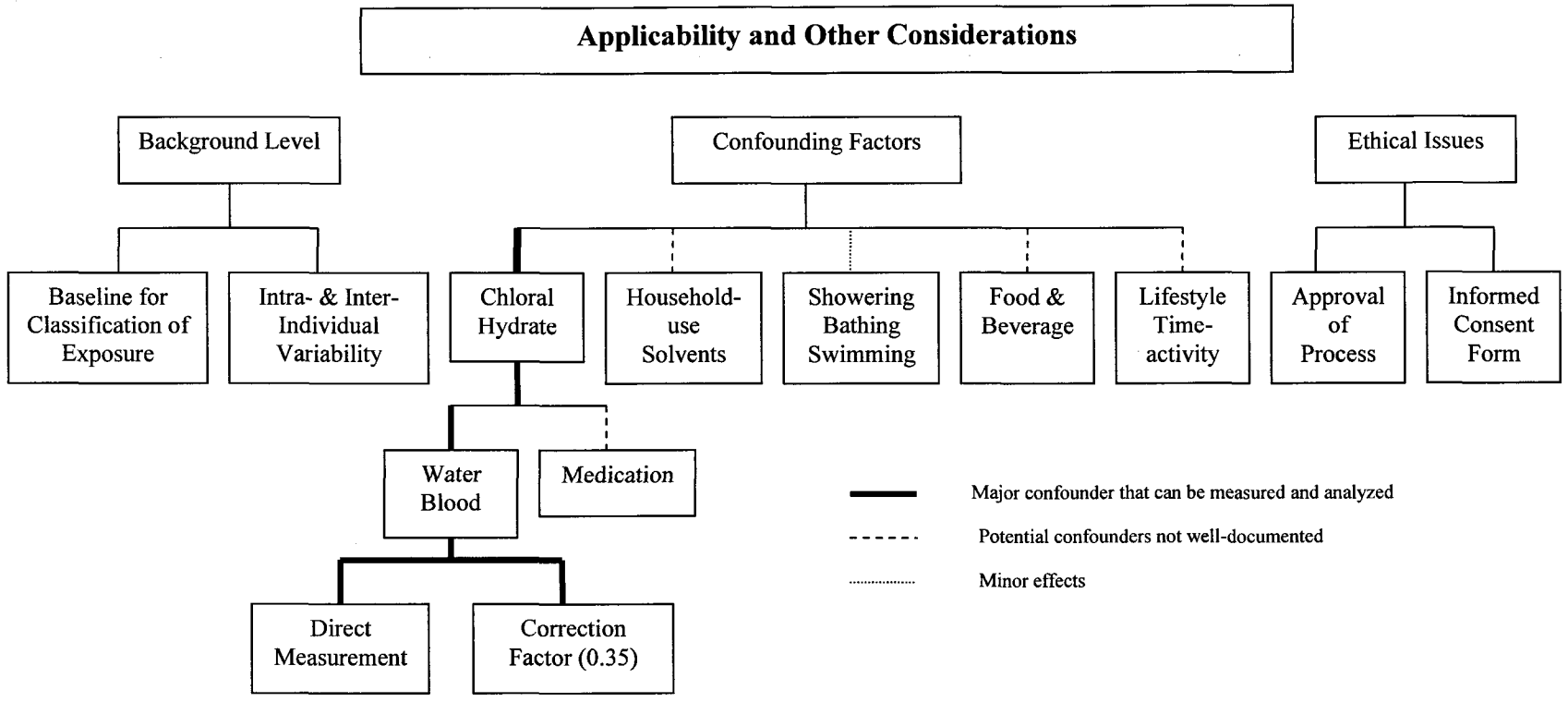

Figure 6-11 Feasibility Based on Applicability and Other Considerations

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CHAPTER 7 DISCUSSION AND CONCLUSIONS

Some epidemiological studies have reported weak associations between exposure to several DBPs and occurrence of carcinogenicity or adverse reproductive and developmental effects. THMs were generally selected as surrogates of DBP exposure in these epidemiological studies. In these studies, measurement errors occurred frequently in exposure assessment. The exposure assessment relied on various surrogates such as individual residence, water sources, routine THM monitoring in treatment plants or distribution systems, and volume of water intake. These surrogate measures were inadequate to classify an individual exposure during a specific time window. Exposure misclassification is a major obstacle to obtaining accurate rates of association between adverse health outcomes and exposure, and is usually expected to cause attenuation in health risk estimates if exposure misclassification is random and non-differential. In order to improve the accuracy of exposure assessment in reproductive and developmental studies, development of biomarkers of exposure is critical. A biomarker of exposure can provide both qualitative and quantitative information about external exposure at an individual level.

The study reported in this thesis explores the validity of urinary TCAA as a potential biomarker for DBP exposure from drinking water ingestion in a large cohort. Validation of TCAA biomarker of exposure results from an evaluation of the factors that influence the biomarker to predict exposure and allow it to be used in appropriate epidemiological studies. Validation processes include understanding of the purpose of using a biomarker, knowledge about the natural history of a biomarker and the relationship between a biomarker and exposure, toxicokinetics, temporal and spatial variability, persistence, laboratory methodology and types of specimens, determination of background level, evaluation of reliability, validity and relevant statistical analysis, selection of an appropriate biomarker, and distinguishing confounding factors and their effect on the marker.

Selection of an appropriate biomarker of exposure is the first step in validation processes. In this study, the relationship of TCAA with two major THM species, TCM and BDCM, in tap water was examined. THMs and HAAs are two predominant groups of DBPs in drinking water. Because THMs were routinely monitored at the water treatment plants, the concentrations of THMs have been used in the most of the recent reproductive and developmental studies in the past decades. It is important to examine the relationship between THMs and HAAs in drinking water in order to understand the ability and limitations of THMs and HAAs to represent other DBPs. In addition to variations in formation and stability of these two groups of DBPs, their respective physical/chemical properties also affect the relevance of different human exposure routes. Because THMs are much more volatile than HAAs, inhalation exposure in showering and bathing is a major human exposure route for THMs, but not for HAAs. Likewise, because

THMs are much more lipophilic than HAAs, dermal exposure is much more important for THMs than it is for HAAs. TCAA itself has a much longer elimination half-life in the human body than chloroform, the dominant THM. The relationship between TCAA ingestion and excretion in urine has been studied (Kim *et al.* 1999; Weisel *et al.* 1999; Bader *et al.* 2004; Froese *et al.* 2002; Calafat *et al.* 2003). TCAA was selected as a potential biomarker of exposure to DBPs in drinking water in this study. The utility of the TCAA as a biomarker of exposure to THMs in an epidemiological study was not always suitable. The findings from our study and other studies indicated that whether or not TCAA can be used as a surrogate for THM exposure in drinking water depends on the nature of both the water treatment and distribution systems, making the use of TCAA for this purpose very site-specific. Because THMs themselves are only surrogates for other DBPs, the inability of TCAA to predict THMs may not be important unless the unknown, causal DBPs have physical/chemical properties closer to THMs than to TCAA.

Characterization of TCAA exposure in human volunteers is a basic component in validation process. In order to explore the validity of relationship between TCAA ingestion and excretion and to control some confounding factors, the selection of population was restricted to healthy, non-pregnant, medicine-use free, solvent-use free women of reproductive age. It might have been ideal to recruit pregnant women as a "representative group" in the study. Instead, 52 non-pregnant women of reproductive age were recruited. The reasons for recruiting this specific group of population were to avoid exposure to any unknown risk factors for pregnant women, to recognize that the most likely use of TCAA biomarker of exposure for a prospective cohort study would be focused on adverse reproductive and developmental outcomes, and to select the target population who was certainly relevant to this application. The findings obtained using a restricted sub-population should not be generalized to the overall population.

Differences in physiological, biochemical, and anatomic changes exist between non-pregnant and pregnant women. In an epidemiological study related to reproductive and developmental outcomes, the alteration in teleology, metabolic function in the liver, and the renal function during pregnancy should be considered (Koos and Moore 2003). Amount of TCAA ingestion and urinary excretion could increase due to an increase of food and fluid intake, a decrease in production of the albumin protein, and an increase of urine excretion rate. In an epidemiological study, a population representative of the general population may not always be recruited. It would be important to evaluate characteristics of TCAA exposure in a given population at the beginning of the study. However, unless it was known that TCAA was promising from experiments such as those reported here, there would be little justification for risking a method which had no validation in a full study protocol.

The most significant contributions achieved from this study are insight about TCAA as a biomarker of exposure for DBP exposure in drinking water in a large

cohort to judge reliability, validity, and feasibility. This study provides a basis for a thorough and critical evaluation of both the utility and the limitations of using a urinary TCAA biomarker for future epidemiological studies and health surveillance of DBP exposure in drinking water in order to improve classification of exposure. This study also aimed to perform a practical evaluation of the feasibility of using biomarkers in a major epidemiologic study pertaining to DBPs exposure and adverse health outcomes.

Reliability analysis is a major component in the validation processes for a biomarker of exposure. Intraindividual variability is a characteristic of most biomarkers of exposure. It is related to laboratory errors, measurement process errors, exposure unique to an individual, and biological factors. Laboratory errors, personnel, methods, sample transport, and storage procedures may affect the reliability of a biomarker. Exposure misclassification occurs due to intraindividual variability if a measure from a single sample in an individual is used to estimate long-term exposure status and chronic health outcomes. In two previous human exposure pilot trials performed by the Environmental Health Sciences research group, larger intraindividual and interindividual variability of TCAA ingestion and excretion was observed in small cohorts.

The current study evaluated intraindividual and interindividual variability in a substantially larger cohort. Repeated samples in tap water, urine and blood within an individual were collected 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. In our study, the interindividual reliability was acceptable for measurements of TCAA ingestion (ICC: 0.69–0.95), loading in blood (ICC=0.90), and urinary excretion (ICC: 0.73–0.77). On the basis of this, TCAA is considered as a reliable biomarker of exposure.

Internal consistency of TCAA measures in tap water consumption, blood, and urine samples was acceptable (Cronbach's $\alpha>0.80$) for 2 to 4 days of sampling. The results indicated an increased reliability with repeated measures. For cost-effectiveness in sampling strategies, two-days urinary sampling was found to be sufficient for measuring volume of tap water consumption and TCAA in urine in an individual if a steady-state TCAA level in the body is reached and the exposure variability across the cohort is relatively high.

Validity analysis is the most important component in validation process. Two earlier studies reported the relationship between TCAA ingestion and urinary excretion in a group of women of reproductive age (Kim *et al.* 1999; Weisel *et al.* 1999). As compared to these earlier studies, the current study involved design and data collection improvements that would have been expected to yield substantially improved correlation of TCAA urinary excretion with TCAA exposure via ingesting tap water. The improvements from our study included using a wide range of exposure levels assigned to participants to overcome the effect of a

restricted exposure range for statistical analysis to improve the ability to detect correlation between ingestion and excretion, the direct daily measurement of volume of tap water intake, repeated sampling in an individual during 15 days to allow assessment of reliability of measures, and the log-transformed analysis for the skewed data.

In the earlier studies by Kim and Weisel (Kim *et al.* 1999, Weisel *et al.* 1999), the expected significant prediction of urinary excretion measures with the concentration of TCAA in tap water was not found to be significant. This may result from the effect of restriction of range of exposure because the TCAA concentrations in tap water measured in a single day were clustered at the low end and the sample size was relatively small. However, these studies did not find a substantially improved prediction of the measures of TCAA excretion using amount of TCAA ingested (vs. concentration) as might have been expected. The latter result is not convincing given the limitations of the methods used (e.g. single samples, dependence on questionnaire data for consumption volume) and their use of linear, rather than log-transformed analyses for their skewed data. Given these deficiencies, we expected a greater degree of prediction with our current study compared to their studies.

The utility of three TCAA urinary measurements, urinary concentration, Cr-Adj. concentration and amount of urinary excretion, were evaluated in the current study. The results suggested that all three measurements were considered to be similar for measuring TCAA in urine. This finding is a little surprising because the excretion amount should be related with ingestion amount better than excretion concentration would be related to excretion concentration. Some of the lower than expected prediction (for amount vs. concentration) may relate to uncertainty added by extrapolating FMU excretion to a 24 h excretion amount. FMU was likely a variable proportion of the 24 h urinary volume among participants.

Type of sample is another important component in validation processes. Two types of specimen, blood and urine, were assessed in the current study. From reliability perspective, the observed intraindividual variability was smaller using blood TCAA measurement compared to urinary TCAA measurement. From a validity perspective, the correlation of a single day sample for TCAA concentrations in blood with either ingested TCAA tap water concentration (r: 0.77–0.82) or ingested TCAA amount (r: 0.78–0.82) was higher than any single day urinary excretion number (r: 0.57–0.77). If 2 or more days of urinary excretion measures were combined, correlations as high as those observed for blood were observed (r: 0.77–0.83). This may be due to different source errors in measurements of TCAA loading in blood and urinary excretion. TCAA was absorbed and entered the blood via the liver pathway. The elimination of TCAA from the body via urine was affected by various biological factors (rate of plasma binding, blood flow rate through kidney, pH in urine, and excretion rate) and measurement errors (interval time for collecting FMU and volume of FMU).

Although the use of a blood TCAA biomarker is better than the use of urinary TCAA biomarker, urinary TCAA biomarker is recommended to use in an epidemiological study with a larger study population because of the invasive nature of blood sampling.

Although TCAA is a reliable, valid biomarker of exposure, the application of TCAA biomarker of exposure needs to be carefully considered at a practical level. The TCAA biomarker could be utilized to explore the relationship between exposure and biomarker responses in epidemiological studies pertaining to reproductive/developmental outcomes, but this biomarker offers much less promise for cancer outcomes.

The TCAA biomarker of exposure could serve as a surrogate for ingestion of non-volatile DBP mixtures because of its dominance and stability in those mixtures. The use of the TCAA biomarker as a surrogate of THMs depends on the nature of water treatment processes at specific sites.

Direct measurement of the TCAA biomarker allows confirmation of TCAA exposure from all routes in individuals. The limitation of this measure is intraindividual variability which can lead to non-differential misclassification. Environmental measurement and questionnaires provide additional information on major exposure sources and routes, the quantity of external exposure in population groups, exposure patterns, and confounding variables. Because of the strengths and limitations of each exposure measurement, it is best to combine the three exposure measurements in epidemiological studies to improve exposure assessment. The combined approach may not be practical in terms of cost-effectiveness.

Some other limitations of this study are that: some factors (e.g., time-activity and total fluid intake patterns) were not controlled to avoid the experimental design from becoming too demanding for participants, chloral hydrate as an important confounder in biological samples was not analyzed, the variants of CYP2E1 was not analyzed, and quantitative assessment about the cost-benefit of this biomarker approach for improving exposure assessment was not performed.

This study makes several findings that are important factors in considering the utility of TCAA as a biomarker of exposure to chlorinated disinfection by-products in drinking water.

1. Spatial and temporal variability occurs in measurements of TCAA concentrations in tap water making suspect any assumptions about the validity of results from a single sample, as has been used in earlier studies;
2. Whether TCAA can be used as a surrogate of THMs depends on the nature of water treatment and storage for each system, and this makes the possibility of TCAA being an effective surrogate for THMs very site-specific. There are cases where THMs correlate very well with TCAA and others where

correlation is poor. In any case, TCAA could only serve as a surrogate for ingestion exposure to THMs because the semi-volatile character of TCAA precludes it from representing inhalation exposure and others have shown that dermal uptake of TCAA is also limited.

3. Where a given study population is exposed to a low levels of TCAA mainly from ingestion of tap water from municipal water systems, such exposure provides background TCAA levels in the body. This reality means that pilot investigations to determine background TCAA levels in a prospective study population are necessary to develop an effective study design.
4. DCAA is not a useful biomarker of exposure because it is so readily metabolized. This finding is not surprising given expectations from the literature, but the data collected provided an excellent data base to demonstrate how ineffective a substance such as DCAA, which is readily metabolized, will be as a biomarker.
5. Increased urinary and blood TCAA levels mainly arise from the ingestion of TCAA-containing tap water supplied for the study. The finding indicates that a major source for TCAA exposure is ingestion of tap water rather than non-ingestion sources such as solvent use, bathing, showering, or swimming.
6. Because of the relatively high interindividual reliability observed in all measurements of TCAA ingestion, loading in blood, and urinary excretion, these measurements using our protocol are reliable for use in epidemiological studies.
7. Because variation of laboratory analysis contributes only slightly to intraindividual variability and biological factors are not easily controlled within an individual, the effort to minimize intraindividual variability should focus on reduction of variation from sampling, sample transport, sample storage, and data manipulation.
8. TCAA ingestion is proportional to urinary TCAA excretion, and TCAA doses ingested from tap water can predict urinary TCAA levels. The prediction model in the current study is not provided because it may not be generalizeable to other sub-population from this human experimental cohort.
9. To a limited extent, the correction of urinary excretion to urinary creatinine improves the result of intraindividual variability, but the benefit is marginal at best.
10. One-day blood sampling and two-day urine sampling are sufficient to achieve reliability in a study if a steady-state TCAA level in the body is reached and exposure variability across the sample population is relatively high. The result provides a practical basis for management of the financial resources required for an epidemiological study. Cost-effectiveness considerations should focus on the balance between the cost of data acquisition and achievement of study objectives.
11. The use of TCAA biomarker of exposure is feasible under field conditions. Laboratory analysis methodology was developed in this study to improve speed and trace sample volume for analysis. Sampling protocols were tested to provide practical evaluation on type of samples, timing of sampling, number

of repeated samples, sample handling and transport, storage methods, and duration of storages.

Within the constraints imposed by the limitations of this study, the data obtained from a substantive human cohort participating in a major exposure experiment suggest that TCAA can be a reliable, valid biomarker for measuring exposure to TCAA in a DBP mixture present in drinking water.

Recommendations for future studies include:

1. TCAA should be used as a biomarker of exposure in an appropriate prospective cohort epidemiological study to improve exposure assessment for ingestion exposure to non-volatile DBPs in drinking water.
2. Study design and determination of sample size will need to consider the trade-off between study power and cost-effectiveness.
3. Comparative research to evaluate different exposure assessment methods to achieve reasonable accuracy of exposure measurements and cost benefit would be useful.

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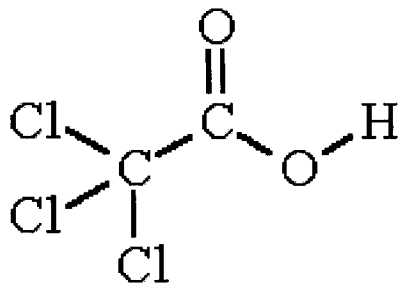
APPENDIX I IDENTITY OF TRICHLOROACETIC ACID

TRICHLOROACETIC ACID (TCAA)



TCAA: $C_2HCl_3O_2/CCl_3COOH$

Molecular Mass: 163.39

ICSC No. 0586

CAS Registry No.: 76-03-9

Synonyms: Trichloroethanoic Acid, Trichloromethane Carboxylic Acid, and Aceto-Caustin

Physical State: Colourless hygroscopic crystals with pungent odour

Melting point of the solid: 58 °C (α-form) or 49.6 °C (β-form)

Boiling point of the liquid: 197.5 °C

Density: 1.6 g/cm^3

Solubility in water: very good

Vapour pressure: Pa at 51 °C : 133

Relative vapour density (air = 1): 5.6

Octanol/water partition coefficient at log Pow: 1.7

Source: International Chemical Safety Cards (http://www.cdc.gov/niosh/ipcsneng/neng0586.html)

APPENDIX II CONCENTRATIONS OF SEVEN DBPS IN TAP WATER

Table II-1 Concentrations of Measured DBPs in Tap Water

Concentration (μg/L)	Mean	SD	Min.	Max.	95% CI for Mean	
					Lower	Upper
City A (N=59)						
TCAA	80.4	20.0	44.8	130	75.2	85.7
DCAA	71.0	24.9	16.2	110	64.5	77.4
CH	17.4	5.3	6.6	30.0	16.0	18.8
TCM	132	23.0	77.2	168	126	138
BDCM	8.5	1.8	5.0	11.8	8.0	8.9
DCAN	11.1	2.8	6.8	16.0	10.4	11.9
TCP	7.8	2.7	1.1	12.8	7.1	8.5
City B (N=28)						
TCAA	6.7	2.4	2.1	11.9	5.7	7.6
DCAA	7.1	5.7	nd	19.9	4.9	9.4
CH	0.5	0.3	0.01	1.0	0.4	0.6
TCM	19.1	5.0	14.7	39.4	17.1	21.1
BDCM	1.2	0.7	0.1	2.1	1.0	1.5
DCAN	1.3	0.8	0.05	2.3	1.0	1.6
TCP	1.1	0.5	0.5	1.8	0.9	1.3

Note: The ranges of some DBPs in drinking water from the Canadian National Survey (Health Canada 1995) are: TCAA 4.1–56.7 μg/L, DCAA 4.6–63.6 μg/L, CH 2.2–22.5 μg/L, DCAN 0.8–16.3 μg/L, TCP 0.9–10 μg/L, and TTHM 6.8–6.7 μg/L.

Table II-2 Correlations Among Seven DBPs in Tap Water

		TCAA	CH	DCAA	TCM	BDCM	DCAN
City A	CH	0.57					
	DCAA	0.54	0.65				
	TCM	0.57	0.42	0.60			
	BDCM	0.58	0.48	0.73	0.85		
	DCAN	0.58	0.75	0.80	0.59	0.72	
	TCPP	0.62	0.76	0.76	0.45	0.68	0.76
City B	CH	-0.1					
	DCAA	-0.25	0.43*				
	TCM	0.02	0.01	0.11			
	BDCM	-0.33	0.55*	0.85*	0.26		
	DCAN	-0.24	0.64*	0.79*	0.10	0.94*	
	TCPP	-0.27	0.63*	0.61*	0.09	0.78*	0.82*

r values: $p < 0.001$ for all values in City A; * $p < 0.01$ in City B.

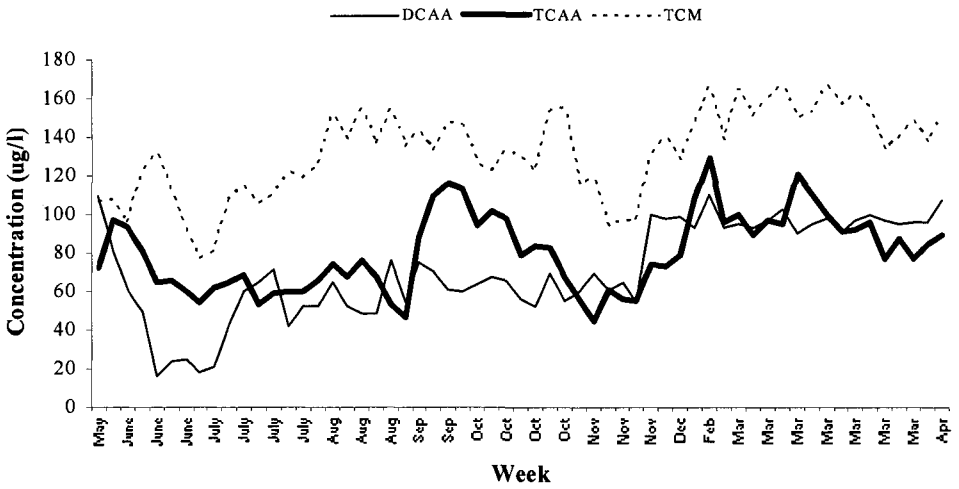


Figure II-1 Variation of Concentrations of TCAA, DCAA and TCM (City A)

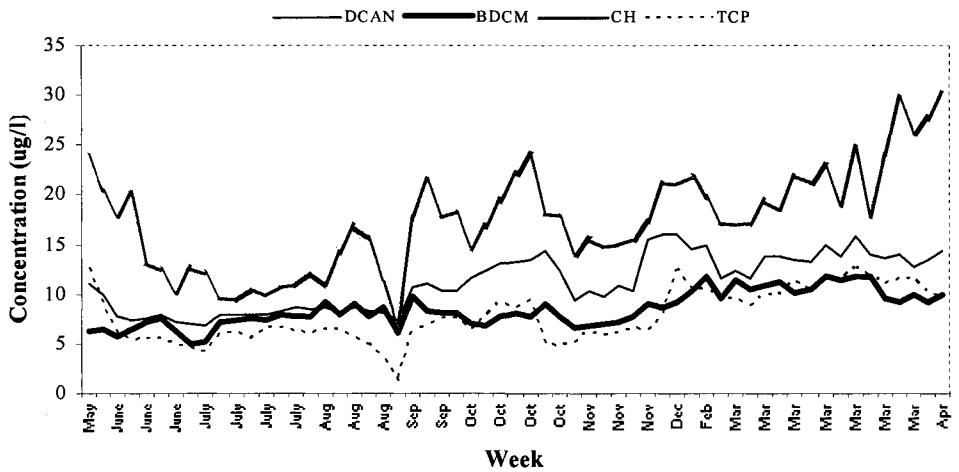


Figure II-2 Variation of Concentrations of Other DBPs (City A)

APPENDIX III COEFFICIENTS OF VARIATION

Table III-1 Coefficient of Variation (%) of Multiplicate Analysis in Urine Samples

ID	Duplicate (N)	CV	Triplicate (N)	CV	Quadruplicate (N)	CV
1	5	4.2–15	2	0		
2	7	1.4–20				
3	7	0				
4	7	6.8–28				
5	7	0				
6	7	0–20				
7	7	1–20				
8	6	1.9–19				
9	7	9–36				
10	2	0–24	4	5.5–12	1	14
11	7	0–23				
12	5	5.5–17	1	17	1	23
13	5	2.3–18	2	13–17		
14	6	0.33–28	1	12		
15	3	9–21	3	7–19		
16	6	5–13	1	46		
17	6	3–20	1	0		
18	4	1. 3–19	2	16–19	1	16
19	7	0.05–19				
20	6	0.2–19			1	16
21	1	5.6	2	10–16	3	10–20
22	3	2–4	1	19	2	4.6–11
23	7	5–20				
24	1	21	6	2.5–19		
25	2	17–39	3	4–12	2	14–20
26	5	2.5–14	2	10–17		
27	7	1.4–20				
28	6	0.5–19	1	12		
29	5	2.6–21	1	17		
30	7	4.2–24				
31	3	5.3–19	4	14–30		
32	4	0–19	2	20	1	16
33	3	4–18	4	12–17		
34	7	0.3–19				
35	3	0–7	4	4–25		
36	5	3.5–20			1	9
37	4	2–16	3	5.4–16		
38	5	6–18	1	14	1	22
39	6	0.5–19	1	2.5		
40	3	3.6–27	4	1.7–9		
41	7	6–28				
42	5	0.9–21	2	10–16		
43	6	0.2–13	1	22		
44	5	0.3–15	2	3.4–15		
45	7	3.2–27				
46	4	5.8–22	2	12–15	1	4
47	3	16–19	4	9.5–25		
48	7	1.4–16				
49	7	0–21				
50	3	1.3–20	3	5–11	1	20
51	5	3.8–20	1	13	1	12
52	3	10–19	4	7–22		

APPENDIX IV CORRELATION COEFFICIENTS

Table IV-1 The r Values of Urinary TCAA Excretion vs. TCAA Ingestion

	inc_12	inc_13	inc_14	inc_15	ina_13	inc_14	inc_15	inc_16
uc_13	.77	.77	.77	.77	.77	.77	.77	.77
uc_14	.62	.62	.62	.62	.63	.63	.62	.63
uc_15	.63	.63	.63	.63	.62	.62	.62	.62
uc_16	.57	.57	.57	.57	.57	.57	.57	.57
ucr_13	.77	.77	.77	.77	.77	.77	.77	.77
ucr_14	.63	.63	.63	.63	.63	.63	.62	.63
ucr_15	.61	.61	.61	.61	.61	.60	.60	.60
ucr_16	.56	.56	.56	.56	.55	.55	.55	.55
ua_13	.77	.77	.77	.77	.77	.77	.76	.77
ua_14	.62	.62	.62	.62	.62	.62	.61	.62
ua_15	.61	.61	.61	.61	.61	.60	.60	.60
ua_16	.62	.62	.62	.62	.62	.62	.62	.62

Note: all log transformed values: $p < 0.001$
inc = TCAA concentration in tap water (μg/L)
ina = amount of TCAA ingestion (μg/d)
uc = urinary TCAA concentration (μg/L)
ucr = creatinine-adjusted urinary TCAA concentration (μg/g Cr)
ua = amount of urinary TCAA excretion (μg/d)
Numbers in row: day of tap water consumption
Number in column: day of urine collection

Table IV-2 The r Values of Blood TCAA Concentration vs. TCAA Ingestion or Urinary TCAA Excretion

	inc_13	inc_14	ina_13	ina_14	uc_14	uc_15	ua_14	ua_15
bc_14	.77	.77	.78	.78	.58		.56	
bc_15	.82	.82	.82	.82		.75		.73

Note: all log transformed values: $p<0.001$
inc = TCAA concentration in tap water (μg/L)
ina = amount of TCAA ingestion (μg/d)
uc = urinary TCAA concentration (μg/L)
ua = amount of urinary TCAA excretion (μg/d)
bc = blood TCAA concentration (μg/L)
Numbers in row: day of tap water consumption
Number in column: day of blood collection

APPENDIX V PROTOCOLS

A

RECRUITMENT PROTOCOL

1. Criteria for Recruitment

- Female
- Aged between 18 to 45 years old
- Healthy (no chronic disease)
- Non-pregnant
- Living in Edmonton between July 2002 and December 2003
- Drinking tap water
- Literacy in English

2. Preparation

- Recruitment e-mail message to the Graduate Students' Association:

"Researchers at the University of Alberta are conducting in collaboration with Alberta Health and Wellness a research study concerning our drinking water. Drinking water is treated with chlorine to prevent disease. Chlorine reacts with natural matter to form so-called "Disinfection By-Products" (DBPs). Some research suggests that DBPs might affect pregnancy outcomes. Because we do not know the amount of DBPs individual women have consumed in drinking water, we are very uncertain about whether DBPs cause any health problems.

To improve our understanding, we invite you to participate in this study. If you agree to take part, you will be asked to do the following:

Two interviews: an initial 10-min telephone interview and a 20-min person-to-person interview. For 15 days, you will drink tap water shipped from another major city in Canada. During these 15 days of tapwater consumption and on the day following the tapwater consumption period, you will provide first morning urine samples six times (on days 1, 2, 8, 14, 15 and on the day ,16, immediately after the tapwater period). You will be asked to provide small blood samples four times (on days 1, 8, 14 and 15), but this is optional. You will be asked to maintain a brief daily diary.

Trained personnel will be available to help you with sample handling or other study requirements. The samples will be sent to the laboratory at the University of Alberta. They will be analyzed for DBPs such as trichloroacetic acid in urine and blood. An enzyme activity related to metabolism of some DBPs compounds, called CYP2E1, will be analyzed in blood sample by genotyping methods. This test is only to determine how easily your body is able to metabolize DBPs. All stored blood samples will be anonymous.

All your samples will be destroyed any time that you request but otherwise they will be stored up to 5 years before being destroyed. The results will be entered to a database for statistical and data analysis purpose. You can request the results and study report via a request form that we will provide.

If you wish to participate in this study or would like to know more details about this study, please phone staff at xxx-xxxx. You may also contact the undersigned to explain any matter that may concern you. Finally, you are completely free to decide about participating in this study. Even after starting, you will be free to withdraw at any time from the study without giving any reasons.

At the end of the study, you will get 15 x 1L Nalgene water bottles (at value of $12/bottle)."

- Consent Form, Questionnaires, sample collection protocols, dairy booklet, labels for tap water, bottles and sampling bottles and sample collection recording sheets.

2. Procedures

1. Send the Recruitment E-mail Massage to the Graduate Students' Association.
2. Publish the message in the GAS newsletters every two weeks.
3. Receive initial e-mail responses from potential participants.
4. Provide detailed study information to all respondents via e-mail.
5. Set up schedules of telephone interviews for people who are willing to be interviewed.
6. Conduct telephone interview:
   - Oral consent for a 10-min telephone interview
   - Answer a few questions from the initial questionnaire
   - Set up an appointment for the person-to-person interview.
7. E-mail a consent form and information sheet to potential eligible participants.
8. Have a research staff member visit home or workplace for the person-to-person interview:
   - Sign the consent form with witness
   - Answer a few questions from the initial questionnaire
   - Receive a protocol and schedule for water delivery and consumption
   - Set up time and pick-up location for water delivery
   - Receive a protocol and schedule for urine collection
   - Set up a specific time and pick-up location for urine collection
   - Receive a protocol and schedule for blood collection (optional)
   - Set up time and location for blood collection
   - Receive the information about daily diary booklet
   - Receive a schedule sheet of water delivery, water consumption, urine collection device kit delivery, urine collection, and blood collection
9. Provide instruction for water consumption, and urine & blood sample collection to participants (Section C).

B.

QUESTIONNAIRES

1. Interview Protocol

- Conduct initial telephone interview for about 10 minutes to identify eligible participants:
  - Obtain volunteer's oral consent
  - Confirm name, address
  - Conduct an initial questionnaire
  - Inform the participant about the process that will follow
  - Make an appointment for the person-to-person interview
- Prepare consent form and information sheet.
- Mailing out consent form to the eligible participants.
- Conduct the person-to-person interview for about 30 minutes:
  - Visit volunteers' home
  - Sign consent form: participant signature and witness signature
  - Conduct a second questionnaire
  - Provide and explain the protocols and schedules of water delivery, water consumption, daily diary, urine collection and blood collection
  - Set up a schedule for water delivery, water consumption, urine collection and blood collection
    - Keep all original consent forms and interview sheets in a secured place.
    - Enter the information to an Access database.

2. The Initial Questionnaire - Telephone Interview

VALIDATING URINARY TRICHLOROACETIC ACID AS A BIOMARKER OF EXPOSURE FOR DISINFECTION BY-PRODUCTS IN DRINKING WATER

Participant ID # _____

Date _____

The Initial Questionnaire

Hello, _____, my name is _____. I am calling you on behalf of the Department of Public Health Sciences at the University of Alberta. We have been funded by Alberta Health and Wellness to undertake research on drinking water quality. We received your e-mail message to indicate that you are willing to participate in the study. We very much appreciate this.

As you are probably already aware, the goal of this study called "the validating urinary trichloroacetic acid as a biomarker of exposure for disinfection by-products in drinking water" is to assess levels of people's exposure to disinfection by-products from drinking water in the human body. The purpose of this telephone call is to obtain information about your water consumption patterns, dietary habits, physical activity, and health.

Do you have 10 minutes to discuss this with me? I will answer any questions you may have and I will need to ask you a few questions.

[No. I do not have time.] May I call later?
[No. I am not interested in this study]. Okay, Thank you for your time.

[Yes, could you call me back at _____ time]. Thanks, I will call you back at _____time.

[Yes, I have time to answer a few question]

Thanks, may I confirm your name and home address?

Your name is _____

Your address is _____

Do you have any questions about this study?

[Yes.]

Questions and Answer sheet: backgrounder, purpose, process, benefit and risk and privacy and confidential policies.

May I ask you a few questions?

[Yes.]

The information recorded in this interview will be held in strict confidence and will be used solely for research into the effects of environmental factors on population health. We are asking the same questions of each participant in the study. All results will be summarized for groups of people; no information about any individual person will be released without the consent of the individual. You are not obliged to respond, but we are seeking your cooperation to make the results of this study comprehensive, accurate, and timely. This questionnaire will take approximately 15 min to complete. Please answer all questions as accurately as possible and feel free to ask any questions you have about this questionnaire or express any other concerns about the study.

1. What is your date of birth?_____/_____/_____
   Month Day Year

2. What is the number of school years which you completed? (Please **circle one** only. If you are currently in school, indicate your current year.)

College/Tech School	1	2	3	4	5	6+
University	1	2	3	4	5	6+

3. To what race do you belong?

☐ Caucasian
☐ First Nations
☐ Métis
☐ African-American
☐ Asian
☐ east Indian
☐ Other (please specify) _____

4. How tall are you? _____ feet _____ inches / _____centimeters

5. How much do you weigh? _____ pounds / _____ kilograms

6. What is the normal source of the water that you drink?

☐ Hot Edmonton tap water ☐ Cold Edmonton tap water
☐ Bottled water ☐

☐ Well ☐ Other (specify) _____

7. Approximately how much **liquid** do you drink **each day**? (tap water, bottled water, coffee, tea, juice) per day (1 cup=250 ml)?

☐ Less than 2 cups ☐ 3 – 4 cups
☐ 5 – 6 cups ☐ 7 – 8 cups

☐ Greater than 8 cups ☐ Other (specify) _____

8. When you drink water from the tap, do you run the water for a period of time before filling your glass?

☐ No ☐ Yes ☐ Sometimes

9. Do you have a filter on your water tap or any other type of filter that purifies the water?

☐ No ☐ Yes; What type? _____

10. Do yo u use bottled water?

☐ No Yes ☐ Sometimes

▸ **If yes or sometimes**, for what purposes do you use bottled water? Check (✓) **all** that apply.

☐ All drinking ☐ Cooking
Other (specify)
_____

11. Do yo u use tap water for making beverages (that is, for coffee, tea, mixing juice concentrate, etc.)?

☐ No ☐ Yes

12. Ho w long do you shower or bath?

☐ less than 15 min. ☐ 15 – 30 min.
☐ greater than 30 min.

13. Do yo u swim in a swimming pool?

☐ No ☐ Yes (go to question 14)

14. Ho w often do you go swimming in a swimming pool?

☐ ___ times per day ☐ ___ times per week
☐ ___ times per month

15. Ho w long do you go swimming each time?

☐ less than 15 min. ☐ 15 – 30 min.
☐ greater than 30 min

16. Do yo u use a hot tub (e.g. a spa or Jacuzzi)?

☐ No ☐ Yes (go to question 17)

17. Ho w often do you use a hot tub?

☐ _ times per day ☐ _ times per week
☐ __ times per month

18. Ho w long do you use hot tub every time?

☐ less than 15 min. ☐ 15 – 30 min.
☐ greater than 30 min.

19. Durin g the **past year** have you taken any **prescription** medications?

☐ No ☐ Yes (see below)

[**If yes**, please list **all** that she has taken below. Please note if she is **currently** taking this prescription medication by checking (✓) the "Current" column.]

Prescription Name	**Dosage**	**Frequency (#/day, week, etc.)**	**Current**

20. We would like to know if you have any long-term health conditions (that is, conditions that have lasted or are expected to last 6 months or more) that have been diagnosed by a health care professional.

[Below is a list of chronic health conditions. Please indicate by checking (✓) the appropriate box if she has ever been diagnosed by a health care professional for any of the following conditions.]

☐ Chronic bronchitis or emphysema
☐ Diabetes
☐ High blood pressure
☐ Heart Disease
☐ Effects of stroke
☐ Cancer – what type of cancer? _____
☐ Alcoholism
☐ Kidney failure or kidney disease
☐ Liver problems

☐ Any other long term condition (please specify)

_____

☐ None

21. Do yo u expect to become pregnant in the next 6 months?

Okay, these are all questions. Thank you very much for taking time to answer the questions.

If you are still willing to participate in this study, I will mail an information letter and consent form to you tomorrow. I will visit your home about 1 or 2 weeks late to pick up your consent form, ask a few more detailed questions, explain how to do study and set up study schedule with you. Is it OK?

[Yes.] May I set up time to visit your home?

[Yes.] Date _____, Time_____

Location _____

Thanks, if you have any change, please give me a call at _____.

[No. I am not interested in participating in this study in the future.] Okay, thank you for your time.

3. The Second Questionnaire – Person-to-Person Interview

VALIDATING URINARY TRICHLOROACETIC ACIDAS A BIOMARKER OF EXPOSURE FOR DISINFECTION BY-PRODUCTS IN DRINKING WATER

Participant ID #

_____

Date _____

The Second Questionnaire

1) Please think back over the last year when you answer these questions. Identify the fluid you can recall drinking **during the last year** and estimate the amount you usually drinking.

   First, indicate (by checking the appropriate box) whether your **usual** serving size of a particular fluid is small (S), medium (M) or large (L). Each item contains an example of a medium serving size. If you portion is similar to that listed, place a check mark (✓) in the medium (M) column. If you typically drink larger servings, place a check mark (✓) in the large (L) column. If you drink less than the medium serving size shown, place a check mark (✓) in the small column.

   Then, put a NUMBER in the most appropriate column to indicate *HOW OFTEN*, on the average, you drink the fluid. For example, you may drink beer twice a week, in which case you would put a "2" in the "week" column. If you never drink beer, you would place a check mark (✓) in the appropriate box in the "Rarely/Never" column. Please DO NOT SKIP items, and please BE CAREFUL which column you put your answer in. Each fluid category contains "other" spaces for you to add fluid that are not listed. Write the amount you normally drink beside the item in the "medium serving" column.

   Please look at the example below:

	Medium Serving	Your Serving Size			How often?				
		S	M	L	Day	Week	Month	Year	Rarely/ Never
Cantaloupe	¼ melon (½ cup)		✓			1			
Grapefruit	½		✓				2		
Sweet Potatoes, yams	½ cup (125 ml)	✓						3	
Ice Cream	1 cup (250 ml)		✓			3			
Squash, Yellow	½ cup (125 ml)								✓

This person:

1) eats a medium serving of cantaloupe once a week;
2) has ½ grapefruit about twice a month;
3) has a small serving of sweet potatoes about three times a year;
4) has a bowl of ice cream about three times a week; and
5) never eats squash.

MILK PRODUCTS	Medium Serving	Your Serving Size			How often?				
		S	M	L	Day	Week	Month	Year	Rarely/Never
Fluid milk: (including in coffee, tea, or on cereal)									
Homogenized or whole	1 cup (250 ml)								
2%	1 cup (250 ml)								
1%	1 cup (250 ml)								
Skim	1 cup (250 ml)								
Dry skim milk powder	1-2 tbsp								
Evaporated milk:									
Whole	½ cup (125 ml)								
2%	½ cup (125 ml)								
Skim	½ cup (125 ml)								
Other milk products:									

FRUITS AND VEGETABLES: FRUITS	Medium Serving	Your Serving Size			How often?				
		S	M	L	Day	Week	Month	Year	Rarely/Never
Apples or Applesauce	1 or ½ cup								
Apricots (not dried)	2-3								
Banana	1 med. (½ cup)								
Berries (saskatoons, raspberries, strawberries, etc.)	½ cup (125 ml)								
Cantaloupe	¼ melon								
Cherries	½ cup (125 ml)								
Grapefruit	½ or ½ cup								
Grapes	½ cup (125 ml)								
Nectarines	1 medium								
Oranges	1 med. (½ cup)								
Peaches	1 med. (½ cup)								
Pears	1 med. (½ cup)								
Pineapple	½ cup (125 ml)								
Plums	2-3 medium								
Pumpkin	½ cup (125 ml)								
Rhubarb	½ cup (125 ml)								
Tangerines	1 medium								
Watermelon	1 medium wedge								
Dried fruit (e.g. raisins, prunes, apricots, etc.)	2 Tbsp.								
Fruit Juices – all types (not crystals or fruit flavoured drinks (e.g., not Kool-Aid or Crystal Light)	½ cup (125 ml)								
Other Fruits:									

FRUITS AND VEGETABLES: VEGETABLES	Medium Serving	Your Serving Size			How often?				
		S	M	L	Day	Week	Month	Year	Rarely/ Never
Beans, green or yellow	½ cup (125 ml)								
Beets	½ cup (125 ml)								
Broccoli	2 stalks or ½ cup (125 ml)								
Brussel sprouts	½ cup (125 ml)								
Cabbage, coleslaw, sauerkraut	½ cup (125 ml)								
Carrots	½ cup (125 ml)								
Cauliflower	½ cup (125 ml)								
Celery	½ cup (125 ml)								
Corn	½ cup (125 ml)								
Cucumber	½ cup (125 ml)								
Garlic, fresh	1 Tsp. (minced or crushed)								
Kohlrabi, parsnips, and turnips	½ cup (125 ml)								
Lettuce salad	1 cup (250 ml)								
Mushrooms	½ cup (125 ml)								
Mustard greens, turnip greens, collards	½ cup (125 ml)								
Onions	¼ cup (75 ml)								
Peas	½ cup (125 ml)								
Peppers sweet (e.g., green, yellow, red); not hot	½ cup (125 ml)								
Potatoes (boiled, baked, potato salad, mashed)	1 med. or ½ cup (125 ml)								
Potatoes (fried, French fries, hash browns)	¾ cup								
Spinach, Swiss chard	½ cup (125 ml)								
Squash, yellow	½ cup (125 ml)								
Sweet potatoes, yams	½ cup (125 ml)								
Tomato, raw	1 med. (½ cup)								
Tomato sauce	¼ - ½ cup (75 – 125 ml)								
Tomato, canned	½ cup (125 ml)								
Zucchini	½ cup (125 ml)								
Mixed, assorted, or frozen vegetables	½ cup (125 ml)								
Vegetable soups, such as tomato	1 cup (250 ml)								
Vegetable drinks(e.g. tomato juice,Clamto,V-8)	½ cup (125 ml)								
Soy milk	1 cup (250ml)								
Other vegetables:									

In the example below, this person has indicated that they drink nine (9) cups of coffee per day. Please a check in the rarely/never column if never.

BEVERAGES	Medium Serving	How often?					Where?		
		Day	Week	Month	Year	Rarely/ Never	Home	Work	Other
Coffee, regular (not decaffeinated)	**1 cup (250 ml)**	**9**					**3**	**6**	
Coffee, regular (not decaffeinated)	1 cup (250 ml)								
Tea (not herbal)	1 cup (250 ml)								
Cola type drinks (all pops, except diet)	1 can (355 ml)								
Cola type drinks (diet only)	1 can (355 ml)								
Powdered drinks (sweetened) (e.g., Kool-Aid, Crystal Lite, etc.)	1 cup (250 ml) (reconstituted)								
Beer	1 can (350 ml)								
Wine	4 oz (125 ml)								
Other Liquor	1oz,1shot (30 ml)								
Other beverages (not fruit or vegetable drinks):									

2) Have you done any of the following during the past year? (Mark ALL that apply)

Type of Activity	✓	About how much time did you usually spend on each occasion?
Walking for exercise (indoor or outdoor)		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Hiking or snowshoeing		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Jogging/running (indoor or outdoor)		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Biking (any type, including stationary)		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Ice hockey		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Skating		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Cross-country skiing		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Downhill skiing		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Weight training		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Exercise class/ aerobics		☐ 1 to 15 minutes ☐ more than one hour

		☐ 16 to 30 minutes ☐ 31 to 60 minutes
Baseball/softball		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Basketball		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Bowling		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Football		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Golfing		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Tennis, racquetball, squash		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Volleyball		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Popular or social dancing		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Swimming (in pool or open water)		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Yoga or Tai-chi		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Fishing or hunting		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Gardening, cutting grass, other yard work		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Other (specify)		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Other (specify)		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
Other (specify)		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes
None		☐ 1 to 15 minutes ☐ more than one hour ☐ 16 to 30 minutes ☐ 31 to 60 minutes

3. Which day you started your last period? _____

4. Which day you completed your last period? _____

5. How many days your period usually last? _____ days

6. How many days between the last cycle and next cycle? _____ days

4. Information Letter and Consent Form

Information Letter

Title of Project: **Validating Urinary Trichloroacetic Acid as a Biomarker of Exposure for Disinfection By-Products (DBPs) in Drinking Water**

Principal Investigator(s): Dr. Steve Hrudey, Department of Public Health Sciences, University of Alberta
Co-Investigator(s): Weiping Zhang, Health Surveillance, Alberta Health and Wellness

The University of Alberta is funded by Alberta Health and Wellness to evaluate people's exposure to disinfection by-products (DBPs). Drinking water is treated with chlorine to prevent disease. Chlorine reacts with natural matter to form so-called "Disinfection By-Products" (DBPs). Some research suggests that DBPs might affect pregnancy outcomes. Because we do not know the amount of DBPs individual women have consumed in drinking water, we are very uncertain about whether DBPs cause any health problems. This survey is being conducted in order to measure the blood and urine levels of the selected substances such as trichloroacetic acid etc and *CYP2E1* enzyme activity by genotyping, and is limited to the purpose stated.

If you agree to participate, you will be asked to do the following things:

- You will be asked to consent to your participation in the study and you will need to confirm your consent by signing the consent form that will be provided at the in-person interview.
- You will take part in a 10 min telephone interview to determine whether you are eligible. If so, we will make an appointment for a subsequent 20 min person-to-person interview when we will bring the consent form for you to sign if you are willing to participate further.
- You will be asked a series of questions related to the project.
- You will be asked to maintain a brief written daily diary during the study. This will be collected at the end of study.
- For 15 days, you will drink tap water shipped from another major city in Canada (called Tap Water A). This will be diluted with bottled water in proportions from 0 to 100%. Tap water from this city contains higher DBP levels than tap water in Edmonton and this allows detecting DBPs in urine much easier. Residents living in this city drink this tap water every day. We expect no significant health risks to you from drinking this tap water.
- You will be randomly assigned to a water consumption group. You may be assigned to drink: no Tap Water A, 250 mL, 500 mL, 1 L or 2 L Tap water A every day. The rest of your water consumption every day will be bottled water that will be provided, and other beverages as you choose. We will encourage you to limit your consumption of other beverages during these 15 days, but as long as you accurately record your other beverage consumption in your daily diary you may consume beverages other than the water provided.
- The water bottles will be delivered and picked up every day except days that you request otherwise and weekends. All unused water should be returned.
- During these 15 days of tapwater consumption and on the day following the tapwater consumption period, you will provide first morning urine samples six times (on days 1, 2, 8, 14, 15 and on the day 16, immediately after the tapwater consumption period).

- During the 16 day study period, if you choose to, you will provide blood samples at the 1st, 8th, 15th and 16th day of your participation in the study. Provision of blood samples is optional. 17 mL blood samples will be taken in the lab of a designated hospital or clinic.
- All collection and pick-up process will be set up to be as convenient as possible.

Trained personnel will be available to help you with sample handling or other study requirements. The samples will be sent to our laboratory at the University of Alberta. They will be analyzed for DBPs such as trichloroacetic acid in urine and blood. An enzyme activity related to metabolism of some DBPs compounds, called *CYP2E1*, will be analyzed in blood sample by genotyping methods. This test is only to determine how easily your body is able to metabolize DBPs. Serum and urine creatinine will be measured to adjust amount of urinary DBPs.
Blood and urine samples will only be used for the evaluation of disinfection by-products and genetic-related enzymes, which determine your enzyme capacity for metabolizing various DBPs and for no other purposes. Serum hormone levels will also be determined to assess variability in our study population. All blood and urine samples will be coded to be anonymous and only the study team will have the code. The creatinine and hormone analyses will be done by a commercial laboratory on the anonymously coded samples. Your blood and urine samples may be kept in storage and used at a later date for confirmation of study results for up to five years. All your samples can be destroyed anytime sooner if you request.

All questionnaires from the two interviews will be stored in a secured place. Your name will be kept confidential and will not be disclosed, except to the principal investigator and co-investigators in this study. Your test results will not be referred to in any way except in statistical and summary formats required by the study. Any report published as a result of this study will not identify you by name.

You may receive no direct benefits from this study, other than the results of your own blood and urine test which you will receive after the University of Alberta verifies the accuracy of all test results. The results will be entered to a database for statistical and data analysis purpose. You can request your own results and the published study report by returning the attached request card or by telephone (xxx-xxxx), fax (xxx-xxxx).

It has been explained to you that there are no significant health risks to you from participation in this study. You will be free to ask any questions concerning the study or results of the study, subject always to the confidentiality rights of the other participants. You will be free to withdraw at any time without giving any reasons. If you have any further questions about the study, please contact:

Dr. Nicola Cherry, Chair,
Department of Public Health Sciences, 13-103 Clinical Sciences Building
University of Alberta, Edmonton, AB T6G2G3

Participant Consent Form

Title of Project: **Validating Urinary Trichloroacetic Acid as a Biomarker of Exposure for Disinfection By-Products in Drinking Water**

Principal Investigator(s): Dr. Steve Hrudey, Department of Public Health Sciences, University of Alberta
Co-Investigator(s): Weiping Zhang, Health Surveillance, Alberta Health and Wellness

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and received a copy of the attached Information Sheet?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No

Have you had an opportunity to ask questions and discuss this study? Yes No

Do you understand that you are free to refuse to participate or withdraw from the study at any time? You do not have to give a reason and it will not affect your future medical care. Yes No

Has the issue of confidentiality been explained to you? Do you understand who will have access to your records? Yes No

Do you want the investigator(s) to inform your family doctor that you are participating in this research study? Yes No

If so, please provide your doctor's name: _____

Who explained this study to you? _____

I agree to take part in this study. Yes ☐ No ☐

_____
Signature of Research Participant Date Printed Name

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____
Witness Date Printed Name

_____ _____
Signature of Investigator or Designee Date

C

PROTOCOLS OF SAMPLE COLLECTION

1. Tap Water Shipment Protocol

- Ship one (two) empty 20-L Nalgene bottles packed in a box every Wednesday, including two return address sheets, and shipping account number from City A to City B.
- Make sure that two bottles arrive in City A every Friday.
- Use tap water at the sampling location to rinse each bottle twice.
- Fill each Nalgene bottle at the Lab Tap Water location with tap water on Monday.
- Put cap tightly and seal the cap with tape.
- Pack the bottle into the same box.
- Seal the box with tape.
- Paste return-address sheets on outside the box.
- Call Purolator Courier Company at x-xxx-xxx-xxxx to pick up the bottle before 12:00 pm on Monday by overnight service.
- Advise Purolator Courier Company to deliver the bottle to City B by noon on Tuesday, that is, via over-night courier.
- Ship one (two) Nalgene bottles from City A to City B.
- All Nalgene bottles are kept in a cold, clean room (4 °C) in the Provincial Public Health Lab in City B.

2. Protocols of Sample Distribution, Storage and Transportation

- Clean-up of all types of water bottles will be carried out in the Environmental Health Sciences Laboratory (EHS Lab), University of Alberta.
- Clean bottles using dishwasher detergent; rinse with tap water first then a final rinse with Milli-Q water. Plastic bottles air dry and glass bottles are dried in an oven.
- Culligan Spring Bottled water will be delivered on delivery schedule.
- Tap water from City A will be shipped every Monday and arrive every Tuesday
- Storage and division of tap and bottled water will be carried out in the Provincial Public Health Laboratory – Microbiology.
- All 20-L polycarboated bottles and 20-L Culligan Spring bottles are kept in a clean cool room (4°C) in the Provincial Public Health Laboratory – Microbiology.
- Put the tap water bottle on the stand (table) and attach the Nalgene bottle spigot onto the carboy following the instruction.
- Disassemble spigot until the expiry date (next Wednesday) and put it in a safe clean place for reassembly when the new tap water arrives.
- Put the Culligan Spring bottled water on the water cooler.
- Each new time period, discard all leftover water in the bottles and run half of a new spring bottle through water cooler and half through carboy spigot.
- Use Aluminum paper to cover graduation bottles when unused. Receive all cleaned polycarbonate or glass bottles from EHS lab every day or two days.
- Run a little bit each time when opening the new tap or spring water bottles.
- **Important notes:** in order to protect from external contamination as much as possible, the following instructions should be followed for water distribution:
  - Wash hands each time before water distribution.
  - Use an alcohol-wipe to clean Nalgene spigot every time when removing from or placing onto the carboy; then allow to dry.
  - Use an alcohol-wipe to clean the spigot and allow to dry before distribution.
  - Don't touch the inside of bottles and caps.
- Label the bottle when each new bottle is opened (source code for tap water starting from 001 and for spring water starting from 201 on the information sheet).
- Measure the exact amount of water into the following bottles by using glass graduation bottle. (Separate one for tap water and another one for drinking water.)
- Label all drinking bottles.
- Labeling should include participant's ID, date (MM/DD/YY), week, and number of bottle.

For example,

Participant ID ED0001 10/10/02, Thursday	Participant ID ED0004 10/10/02, Thursday	Participant ID ED0010 10/10/02, Thursday

- Group I: **Red label** (0%) 1, 2, 3 = 1-L bottled water per bottle
- Group II: **Yellow label** (12.5%) 1 = 125 mL tap water + 875 mL bottled water, 2 = 125 mL tap water + 875 mL bottled water, 3 = 125 mL tap water + 875 mL bottled water
- Group III: **Blue label** (25%) 1 = 250 mL tap water + 750 mL bottled water, 2 = 250 mL tap water + 750 mL bottled water, 3 = 250 mL tap water + 750 mL bottled water
- Group IV: **Green label** (50%) 1 = 500 mL tap water + 500 mL bottled water, 2 = 500 mL tap water + 500 mL bottled water, 3 = 500 mL tap water + 500 mL bottled water
- Group V: **Orange label** (100%) 1 = 1 L tap water, 2 = 1 L tap water, 3 = 1 L tap water

- Fill up water bottles in the cool room
  - Fill up tap water from a 20-L polycarbonated bottle into a 1-L polycarbonate bottle (s) in a clean room in the afternoon before the delivery day. The amount of tap water will be coded in the "Labeling" from Monday to Friday.
  - Fill up bottled water from a 20-L Culligan Spring bottle into a 1-L polycarbonated bottle (s) in a clean room. The amount of bottled water will display in the "Labeling."
  - A total of three bottles will be delivered to each participant every day from M-F. If not practical or at weekends, a total of 9 bottles will be delivered to each participant for three-day use. The detailed schedule for water delivery will be modified based on the participants' convenience.
- Fill up water for TCAA testing in the cool room

Tap Water

- Every Monday and Thursday, send 250mL tap water for TCAA analysis
- Prepare 250mL clean brown glass bottles
- Label the bottle with collection date, drinking date, and source water number
- Fill up 250mL tap water from 20-L polycarbonate bottle to the brown glass bottle
- Cap it tightly and keep it in the cool room
- Bring it to the EHS lab for TCAA analysis at the same day

Spring bottled water

- Every Wednesday send 250mL spring bottled water for TCAA analysis
- Prepare 250mL clean brown glass bottles
- Label the bottle with collection date, drinking date and source water number
- Fill up 250mL tap water from 20-L polycarbonate bottle to the brown glass bottle
- Cap it tightly and keep it in the cool room
- Bring it to the EHS lab for TCAA analysis at the same day

- Filling water for E. Coli testing in the cool room
  - Prepare 150mL sterilized plastic bottles
  - Label the 150mL glass bottle for E.Coli testing with collection date, source bottle umber
  - Shake 20-L polycarbonate tap water bottle several times

- Every M-F dispense 100 mL tap water into a sterilized glass bottle up to the filling line prior to filling the water into 1-L polycarbonate bottle except weekends and holidays
- Shake 20-L Culligan spring bottled water for several times
- Every M-F except weekends and holidays dispense 100mL spring water into 150 sterilized glass bottle up to the filling line prior to filling the water into polycarbonate bottle
- Detach the label from requisition and stick on each bottle
- Fill in the collection time on the label and attach the requisition
- Send both bottles with label & requisition to the EHS lab immediately after distribution on M-Thursday before 3:00PM and Friday before 2:00PM

- Filling up water for trace metal testing in the lab room
  - Dispense 200mL tap water into clean plastic bottle for testing trace metals in drinking water in the lab room (not in the cool room) on every Wednesday and shipped to the Centre for Toxicology. (Sample collection procedure details see attached sheet)
  - Ship to the Centre for Toxicology.
- Samples for urinary TCAA analysis
  - Study day1, 2, 8, 13, 14, 15, and 16 will receive urine sample from each participant
  - Record the amount of FMU
  - Discard the urine bottle appropriately after complete the analysis
- Sample for blood TCAA analysis
  - Pick up blood sample collected by DKML at U of A hospital base lab on study day 1, 8, 14, and 15 for each participant.
  - Send one of them to provincial lab and dispense about 3ml blood for DNA storage (only the first time of blood sample of each participant)
  - EHS will receive blood samples for TCAA analysis
- Samples for urine creatinine analysis
  - Pour the urine from 1-L bottle to 5mL labeled tube weekly and send them to the DKML lab with completed requisition
- Schedules for Sample Transportation

Items	Sampling Date	Sampling Location	Pick-up by	Pick-up time & place	Shipment location & date
3x 1L Drinking Water Bottles	M-F (Thursday prepare for the following 3 days)	Cool room		Cool room	The next morning to volunteer's designated place
Tap Waters Sample Bottle for TCAA Testing	Thursday and Monday	Cool room		Cool room	Environmental Health Science Lab within the same day
Bottled Water Sample Bottle for TCAA Testing	Wednesday	Cool room		Cool room	Environmental Health Science Lab within the same day
Tap & Bottled Water Sample Bottle for Water Quality Testing	M-F	Cool room			Immediately send to 1B3.29

Tap & Bottled Water Sample Bottle for Trace Metal Testing	Wednesday	Lab room		Lab room	Send to the center of Toxicology in the receiving room

3. Instruction of Sample Collection for Participants

1. A period of the study is 15 days
2. Call research staff anytime for changing delivery schedule and location
3. The first water delivery day will be the first Wednesday after your last menstruation (0 day of exposure)
4. Three 1L bottles will be provided every morning one day before drinking at the designated location
5. Keep three bottles in room temperature or a refrigerator (preferable) overnight
6. Drinking delivered water starting from next morning to night (before bedtime)
7. Drinking water ordering from 1st bottle, 2nd bottle to 3rd bottle **(very important)**
8. Take the bottles with you during the day and drink as much as you can
9. Leave all left-over water in original bottle(s) **(Do not empty bottles)**
10. Lea ve all three bottles (including unused water) at the designated location for pick up in the next morning
11. Usin g City B tap water for cooking
12. Usin g City B tap water or bottle (preferable) for making coffee or tea
13. Drink ing other beverage as usual but please record all beverage consumption accurately in the daily diary.
14. Please li mit your exposure to the solvents (e.g. lens cleaning, dry cleaning, paper whit-out, bleacher and such). If so, record the relative information in the daily diary book and specify the name of the solvent you deal with and how long for each time.
15. Receive a dail y diary booklet at the 0 day of the study. (Wednesday)
16. Record the in formation to the booklet every day for 15 days.
17. Receive a urine collection device kit in a small cooler with one ice-pack at the 0,1st, 7th, 12th, 13th, 14th and 15th day of the study.
18. Collect the first morning urine sample at 1st, 2nd, 8th, 13th, 14th, 15th and 16th day of the study:
    - Set up time and location with the research staff for picking up the urine collection bottles
    - Call the staff anytime for changing time and location of collection
    - Read the instruction of urine collection
    - Collect the first morning urine according to the instruction
    - Leave the urine collection bottle at the designated location for pick-up

18. Blood collection (optional):
    - Select the blood collection site and make sure to check the clinic schedule before you go
    - Receive Lab requisition from the research staff for blood collection for the day of 0, 7th, 13th, and 14th day of the study
    - Confirm the name and Study ID.
    - Go to a designated clinical lab and present requisition to the Lab staff at the day of 1st, 8th, 14th, 15th.
    - Donate about 12 ml blood sample

Daily Diary Booklet

Participant ID # _____
Date: From_____
To_____

Project: Validating Urinary Trichloroacetic Acid as a biomarker of exposure for disinfection by-products in drinking water
Contact: xxx-xxxx
****

Side 1

Day 1 Thursday ☐ Rain/Snow ☐ Sunny _____°C

Beverage consumption

Item	Amount (whole day)	Use of Tap Water	Use of bottled water
Coffee	Cup (s)		
Tea	Cup (s)		
Milk	Cup (s)	-	-
Soft drinks	ml or Can (s)	-	-
Beer	ml or Can (s)	-	-
Wine	ml	-	-
Other. Specify:		-	-

Side 2

Physical Activities

Type	Times per day	Minutes per time	
Bathing			
Showering			
Sauna			
Hot-tub			
Swimming			
Dish washing			
Jogging			
Biking			
Others. Specify:			

4. Instruction of the First Morning Urine Collection

You have been asked to collect the first morning urine (FMU) sample at 1st, 2nd, 8th, 13, 14th, 15th, and 16th day of the study. For accurate results, it is important to follow the collection procedure. If you have questions about the procedure, please free feel to ask the research staff.

- You will receive FMU collection kit on the day before each collection day. Each kit contains:
  - One urine collection funnel
  - One 1-litre clean urine collection container
  - Container label with your study ID and the date of collection
  - One small cooler with one ice-pack
  - One Ziploc plastic bag
- Make sure the correct study ID on each label. If not labeled properly, you may call the collection staff to correct it.
- Keep a clean ice-pack to the freezer overnight
- Do not collect urine if you are ill (e.g.with flu) or have signs of a urinary tract infection (needing to pass urine frequently with burning or pain).
- Empty your bladder as completely as you can before going to bed and discard the urine at night prior to collection day
- Avoid drinking water during sleeping
- On the collection day, urinate and void into the collection container through the funnel when you get out of the bed in the morning.
- Collect the whole amount of FMU within 30 minutes before you start drinking anything.
- Screw the lid of container tightly to prevent leaks.
- Complete the last urination time and FMU collection time on the label
- Place the urinated container into a plastic bag and zip it.
- Place a plastic bag into a cooler
- Keep the urine container inside the cooler with a frozen ice-pack.
- Leave the cooler at the designed location for pick-up.

5. Urine Collection Protocol

- Explain to each participant about the urine collection protocol and collection schedule during the person-to-person interview.
- Set up a collection schedule during the person-to-person interview.
- Provide the urine collection schedule sheet and protocol to each participant during the person-to-person interview.
- Prepare a package of the urine collection supplies:
  - One clean urine collection funnel
  - 1L clean, proof-leak, Nalgene HDPE, white bottle labeled with participant's ID and date for collection
  - One small cooler with one ice-pack and labeled
- Prepare 100ml clean brown glass bottle labeled with participant's ID and date for TCAA collection by the EHS Lab.
- Obtain the following urine creatinine testing supplies by DKML
  - Urine creatinine testing requisitions (fill in the requisitions before the participation day of each volunteer)
  - 5ml- plastic tubes
  - Hazardous plastic bags
- Deliver one package of the supplies to each participant's home in the morning of 0, 1st, 7th, 12th, 13th, 14th and15th day of exposure period, respectively.
- Collect the first morning urine sample from each participant' home in the morning of the 1st, 2nd, 8th, 13th, 14th, 15th, and 16th day of exposure period, respectively.
- Deliver the urine sample to the EHS Lab as soon as possible but within 4-6 hours after collection.

- Keep the sample at a 4 °C refrigerator immediately.
- Measure the volume of urine of each urine container at EHS lab and enter the information to Access database
- Pour 50ml of each sample into 100ml brown glass bottle at ESH lab for TCAA analysis

6. Blood Sample Collection

- Prepare a list of blood collection sites in City B provided by DKML
- Select a collection site based on each participant's convenient location during the person to person interview
- Schedule for blood sample collection with nearest location during the person-to-person interview.
- Obtain the sampling and testing processes and protocols from DKML.
- Prepare blood sample collection supplies at each designated collection site by DKML as follows:
    - Gold Top serum tube, volume 6ML for Creatinine test
    - Gray Top whole blood tube, volume 6ML for TCAA collection
- Explain the blood collection protocol during the person-to-person interview.
- Provide the blood collection schedule and location sheet during the interview.
- Provide a collection requisition to each participant for blood collection at the 0st, 7^{th}, 13^{th}, and 14^{th} day of exposure. (prepared by DKML) and collect the blood sample at day 1, 8, 14 and 15.
- For serum creatinine test, get the test results within in 48 hours after samples are collected from DKML and enter results into Access database.
- For TCAA blood sample, DKML arranges delivering them to the UAH lab within 4-6 hours after collection and keep the sample at a 4° refrigerator
- Pick up TCAA blood samples at UAH lab at the same collection day
- For the first time blood sample of each participant, send to Dr. Pang and transfer 200ul whole blood sample to the DNA-free straw and keep the straw at -70 degree for further CYP2E1 analysis.
- The rest of them will be sent to EHS lab immediately.
- Get TCAA results from EHS Lab and enter the results to Access database
- Type of blood test
    - Serum creatinine test at 1^{st}, 8^{th}, 14^{th} and 15^{th} day
    - Whole blood TCAA test at days 1, 8, 14 and 15
    - CYP2E1 at the first time of each participant's blood sample

D

PROTOCOL OF CHEMICAL ANALYSIS

1. Analysis of HAAs in Water, Urine and Blood

Liquid-liquid microextraction (LLME) headspace solid phase microextraction (SPME) gas chromatography (GC) electron capture detector (ECD) method for the analysis of haloacetic acids (HAAs)

Gas chromatography system

Varian CP 3800 GC-ECD
Column: DB-1 20m×0.18mm×0.4μm
Column Temp: 40°C 0min, to 70°C at 10°C/min, hold 4min, to 205°C at 15°C/min, hold 2min
Inj. Temp: 200°C, Det. Temp: 260°C. Initial split off, 2 min split on, ratio 100
Flow rate: 0.8ml/min (He), make up gas: 25ml/min (Ar + 4% Methane)
100μm Polydimethylsilonxane (PDMS) coated fiber, absorb 10min, desorb 2min.

Sample preparation and extraction

1) 0.1 mL sample was placed in a 1.5mL microcentrifuge vial in fume hood
2) 10 μL 2,3-dichloropropionic acid was added
3) 0.2 mL 0.1 M acetate buffer (pH5.2) was added
4) Sample was acidified with 25μL 50% sulfuric acid
5) HAAs were extracted with 0.6 mL MTBE and vortex mixed for 5 min
6) After extracted, organic layer (MTBE) was removed and placed in a 2 mL GC vial, the remaining aqueous layer was discarded as organic waste
7) Evaporated MTBE to dryness under a gently stream of pure nitrogen
8) 0.1 g sodium sulfate and 10μL methanol and 10μL sulfuric acid were added to the dried residue in the vial, then the vial was sealed
9) Vial was heated 20 min at 80 °C in fume hood
10) Headspace was sampled using solid phase microextraction fiber and analyzed in GC-ECD

Detection limit of LLME-SPME-GC-ECD method (S/N=3)

- 2,3-dichloropropionic acid was used as the internal standard
- DCAA 0.5 μg/L; TCAA 0.2 μg/L

A1 Procedure

1. Water, urine (0.1 mL) or blood (25-50 μL) and 0.1 M acetate buffer (0.2 mL, pH 5.2) were combined and vortex mixed in a 1.5-mL polypropylene microcentrifuge vial.
2. 10 μL of 2,3-DCPA were added as internal standard.
3. The solution was acidified with 25 μL of 50% sulfuric acid and HAAs were extracted with 0.6 mL MTBE.
4. MTBE was placed in a 2 mL autosampler vial and evaporated just to dryness under a gentle stream of N_2 (99.999% pure).
5. Sodium sulfate (0.10 g), methanol (10 μL) and sulfuric acid (10 μL) were added to the dried residue in the vial after which the vial was sealed using a Teflon lined crimp-cap. The solution was vortex mixed and the HAAs were derivatized at 80°C for 20 min.
6. After derivatization, the sample was cooled down to room temperature. The sample components were absorbed from the headspace by the 100 μm PDMS fiber for 10 min at room temperature (25°C), desorbed for 2 min in the injection port of the GC and detected with ECD detector.

A2 GC-ECD condition

1. Instrument: Varian CP3800 GC-ECD with 8200 autosampler or HP 5890 GC-ECD with HP 7683 autosampler
2. Column: DB-1 20m×0.18mm×0.4μm

3. Column Temperature: 40°C to 70°C at 10°C /min, hold 4min, to 205°C at 15°C /min hold 2min
4. Injector & Detector Temperature: 200°C, 260°C
5. Flow rate (Helium): 0.8ml/min
6. Make up gas (Ar+5%methane): 60ml/min for HP6890, 25ml/min for CP3800
7. Injection mode: splitless 2min

A3 Reference

Wu, F.W., W. Gabryelski, and K. Froese, *Improved gas chromatography methods for micro-volume analysis of haloacetic acids in water and biological matrices.* Analyst, 2002. **127**(10): p. 1318-1323.

2. Analysis of Other DBP Compounds

Liquid-liquid extraction (LLE) GC ECD method for the analysis of THMs (trihalomethane), HANs (haloacetonitrile), HKs (haloketone), CH (chloral hydrate), CP (chloropicrin or trichloronitromethane)

B1 Procedure

1. 20ml of water sample was added to a 30ml vial with screw cap.
2. 2ml of MTBE, and 6.6g Na_2SO_4 was added to the sample vial and the vial was shaken immediately in order to prevent the solidification of undissolved salt at the bottom.
3. Recap and extract Na_2SO_4/MTBE/sample mixture by vigorously and consistently shaking the vial by hand for 4min.
4. Invert the vial and allow the water and MTBE phases to separate. Transfer 1.0 ml MTBE to the 2ml vial, add 10µl 4-bromofluorobenzene as internal standard, cap the vial and vortex it.
5. Analyze it with GC-ECD.

B2 GC-ECD condition

1. Instrument: Varian CP3800 GC-ECD with 8200 autosampler or HP 5890 GC-ECD with HP 7683 autosampler
2. Column: DB-1 20m×0.18mm×0.4µm
3. Column Temperature: 30°C hold 11min, to 150°C at 10°C /min hold 2min
4. Injector & Detector Temperature: 200°C, 260°C
5. Flow rate (Helium): 0.8ml/min
6. Make up gas (Ar+5%methane): 60ml/min for HP6890, 25ml/min for CP3800
7. Injection mode: splitless 0.2min
8. Injection volume: 2µL

B3 Reference

Munch, D. and D. Hautman, *Method 551.1 Revision 1.0: Determination of chlorination disinfection by-products, chlorinated solvents, and halogenated pesticides/herbicides in drinking water by liquid-liquid extraction and gas chromatography with electron capture detection.* 1995, US Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory: Cincinnati, OH.

APPENDIX VI ETHICS APPROVAL

ETHICS APPROVAL FORM

Date: November 2002

Name(s) of Principal Investigator(s): Dr. Stephen Hrudey

Department: Public Health Sciences

Title: Validating urinary trichloroacetic acid as a biomarker of exposure for dissinfection by-products in drinking water

The Health Research Ethics Board (Biomedical Panel) has reviewed the protocol involved in this project which has been found to be acceptable within the limitations of human experimentation. The REB has also reviewed and approved the subject information material and consent form.

Specific Comments: See accompanying letter for final approval date.

D.W. Morrish, M.D.
Chairman of Health Research Ethics Board
Biomedical Panel

This approval is valid for one year

Issue: #4257