Maternal urinary levels of trichloroacetic acid and association with adverse pregnancy outcomes
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ABSTRACT
The current study aimed to determine the association between trichloroacetic acid (TCAA) levels and adverse pregnancy outcomes among third-trimester pregnant women who were exposed to chlorinated drinking water. A total of 205 pregnant women who participated in the disinfection by-products exposure and adverse pregnancy outcome study in South Africa were randomly asked to participate in this study by providing their morning urine sample voids. Samples were analysed for urinary creatinine and TCAA. Furthermore, participants gave individual data using a structured questionnaire. The mean (median) concentration of creatinine-adjusted urinary TCAA was 2.34 (1.95) μg/g creatinine. Elevated levels of creatinine-adjusted TCAA concentrations showed an increased risk of premature birth, small for gestational age (SGA) and low birth weight. There was no significant statistical correlation observed between creatinine-adjusted TCAA concentrations and the total volume of cold water ingested among the study population. No statistically significant association was observed between creatinine-adjusted urinary TCAA and premature birth, SGA and low birth weight newborns among the study subjects. However, the urinary TCAA concentrations identified in this study suggest potential health risks towards women and foetus. Therefore, further studies are warranted to prevent further adverse pregnancy outcomes.

Key words | adverse pregnancy outcomes, creatinine-adjusted urinary trichloroacetic acid (TCAA), drinking water

INTRODUCTION
Disinfection by-products (DBPs) are formed when chlorine-based compounds react with natural organic matter present in water during the drinking water treatment process. Until now, over 700 DBPs have been identified (Richardson et al. 1999), some of which have been quantified and tested on toxicological experiments (Bull et al. 1995; Boorman 1999). The most commonly studied DBPs are trihalomethanes (THMs) and haloacetic acids (HAAs) as they occur in high concentrations in drinking water worldwide (Richardson et al. 1999), including South Africa (Ncube et al. 2012). The exposure to these DBPs varies according to the individual as they can occur through water-use activities such as the consumption of chlorinated drinking water, showering/bathing and swimming (Nieuwenhuijsen et al. 2009a, 2009b). There have been ongoing studies to determine whether DBPs pose a human health risk since their discovery in 1974 (Rook 1974). Studies have been focusing on the association of DBPs with the risk of cancers (Cantor et al. 1998; Villanueva et al. 2006; Bove et al. 2007), but recently there are several epidemiological studies addressing the possibility of adverse pregnancy outcomes (Grazuleviciene et al. 2011; Costet et al. 2011; Cao et al. 2016). THMs and HAAs are metabolised into...
mutagenic intermediates through conjugation with glutathione (St-Pierre et al. 2005), resulting in potentially adverse health effects.

Epidemiological studies have found inconsistent evidence on the association between DBPs and adverse pregnancy outcomes, such as small for gestational age (SGA) infant, preterm birth, low birth weight and foetal growth restriction (Grazuleviciene et al. 2011; Costet et al. 2011; Zhou et al. 2012; Cao et al. 2016). An assessment of exposure has become the main limitation in most epidemiological studies of DBPs exposure and human health (Arbuckle et al. 2013). Inappropriate exposure assessment may result in bias, the loss of study power and exposure misclassification (Cao et al. 2016).

With this background, the use of biomarkers provides an alternative measurement to improve the assessment of exposure in order to compare with indirect measures of DBPs (Smith et al. 2015). There are two valid biomarkers for DBPs including blood THMs and urinary trichloroacetic acid (TCAA) (Froese et al. 2002; Bader et al. 2004). THMs are measured in blood as they are volatile compounds which are rapidly metabolised following ingestion, inhalation or dermal contact. However, it is difficult to use TMs as a biomarker in epidemiological studies, because the collection of blood is invasive and exhaled air also difficult to measure (Costet et al. 2011). Therefore, a TCAA biomarker has been deemed as a valid biomarker of chronic ingestion exposure to HAAs from chlorinated drinking water (Zhang et al. 2009; Smith et al. 2013), and urine samples are easy to collect in the field of survey. Several studies have found a significant correlation between TCAA concentrations in urine samples and ingestion exposure of TCAA from drinking water (Kim et al. 1999; Weisel et al. 1999; Zhang et al. 2009). TCAA is one of the significant HAAs which are non-volatile. Therefore, the main route of exposure is through the ingestion of chlorinated drinking water (Xu & Weisel 2005). The excretion half-life of urinary TCAA ranges between 2.1 and 6.3 days (Smith et al. 2013). Therefore, it has been suggested as a potential ‘gold standard’ for individual exposure assessment in chlorinated drinking water (Zhang et al. 2009; Smith et al. 2013), as compared to others. The use of urine samples represents valid biomarkers for recent exposure through ingestion, and it is easy to collect and analyse. Furthermore, the collection of urine samples is convenient since it is non-invasive and thus desirable in large-scale epidemiological studies (Zhang et al. 2009).

Investigations have been conducted in developed countries mostly in the USA, Europe and Australia, while in sub-Saharan African countries, there is limited evidence in this field. Exposure to DBPs may differ according to the geographical area and the levels of DBPs present in the chlorinated drinking water (Richardson et al. 2007). The concentration of TCAA in the urine of pregnant women is an indication of the exposure experienced by the developing foetus, which has been associated with adverse pregnancy outcomes (Costet et al. 2011; Cao et al. 2015). Therefore, it is vital to understand the biomarkers of prenatal exposure to DBPs among pregnant women.

In this cross-sectional study, we report the urinary TCAA concentration levels as a tool for assessing internal exposure to DBPs (especially HAAs) among the subset of pregnant women who participated in a cohort of prenatal exposure to drinking water DBPs and adverse pregnancy outcomes in South Africa. We assess any possible association between urinary TCAA concentrations in relation to adverse pregnancy outcomes. These results can provide ways of how to reduce DBPs exposure (especially HAAs) during pregnancy and further to reduce the risks of adverse pregnancy outcomes.

MATERIALS AND METHODS

Participation, recruitment and informed consent

The authors studied the associations between prenatal exposure to DBPs and adverse pregnancy outcomes in the prospective cohort, a South African study comprising 1167 pregnant women recruited between 2017 and 2018. Exposure assessments were determined by estimating levels of individual THM uptakes during pregnancy and by measuring maternal urinary levels of TCAA during third-trimester pregnancy in a cross-sectional design. In this study, all pregnant women who were recruited from the cohort while visiting for prenatal care between March and June 2017 between 24 and 36 weeks of pregnancy were invited to take part in the assessment.
The exclusion criteria during recruitment in the assessment were (1) women younger than 18 years, (2) women residing outside in one of the largest metropolitan districts Gauteng, South Africa and (3) those who did not understand or speak either local languages or English. For all participants, women read or were to read the written informed consent and agreed to participate. The initial population of women who expressed interest in this assessment was 250 pregnant women. Of these 250, 30 were not eligible and therefore excluded from the outset of the study. From the 220, 216 provided urine samples, of which 205 were deemed to be valid samples for this assessment. During the follow-up at delivery to collect infant measurements, seven participants were excluded or lost; therefore, the study included 198 mothers with live singleton infants (see Figure 1 for details).

Ethical consideration

The researcher obtained ethical approval from the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria, South Africa (reference 115/2016) and endorsement by the Department of Health, Gauteng, South Africa in 2017.

Research instrument

Previously validated questionnaires (Villanueva et al. 2006) were administered after the study participants signed the consent forms. The questionnaire included questions on demographics, pregnancy history, medical history, household exposure and water-use habits, including the use of tap water, the number and size of glasses/mugs of tap water consumed per day and the frequency and duration of bathing and showering. The above questions have been validated and used in this field of epidemiological studies (Barbone et al. 2002; Kaur et al. 2004).

Birth outcome assessment

All measurements of the newborn babies were done at the clinic of childbirth according to the Department of Health’s Guidelines for maternity care in South Africa, 2015 (SADoH 2015). The clinician took the measurements and recorded in both individual childbirth card and clinics log register. In this study, information on newborn babies was collected using individual child clinic cards for live births through self-reporting interviews conducted by the research team via telephone. The WHO guidelines for anthropometric measurements were used, which include variables on infant date of birth, birth weight, length, sex, birth rank, any disabilities observed by mother on a child, gestational age at birth and method of delivery. The adverse pregnancy outcomes were assessed using standard definitions. Premature or preterm were defined as live births with a gestational age of <37 weeks. Gestational age was estimated using the duration of pregnancy in completed weeks from the first day of the last menstrual period. The clinical file was visited to record this information.

Full-term birth analyses were restricted to infants born ≥37 weeks completed gestational age, while post-term birth were infants born ≥42 weeks completed gestational age. An SGA infant was defined as an infant with a birth weight below the 10th percentile for his or her gestational age (raw, squared and cubed) at birth, sex, maternal pregnancy weight and height, and parity (raw, squared and cubed) (SADoH 2015). The 10th percentile cut-point values were obtained from standardised birth weight curves. Birth weight was coded as continuous in grams. Low birth weight was defined as weight at birth of less than 2,500 g (WHO 2008). Adverse birth outcomes were analysed first according to the above definitions and then coded as binary variables (1 = case; 0 = non-case).

Sample collection and analysis

Maternal urine collection

The procedures of the collection, handling and transportation of samples were discussed with the participating laboratory, Lancet in Pretoria. In brief, a 20-mL sterile conical polyethylene container was used to collect urine samples. Each participant collected their sample after receiving the instruction from the research team on how to collect it. The collection of samples was done on a day of antenatal visits. Samples were labelled with a unique participant’s identification number and placed in a self-sealed plastic bag and handed over to the research team member.
Figure 1 | Flow diagram of screening and responses among the study subjects.

Total study population invited

N = 250

Eligible participants

N = 220

Volunteered and provided urine samples

N = 216

Face-to-face interviews

N = 210

Valid urine samples

N = 205

Study subjects with live singleton

N = 198

Participants excluded with the following reason:

(N = 30) were not willing to participate

Did not participate in interviews

N = 6

(N = 4) Samples had less than 0.3 g/L or more than 3.0 g/L creatinine or subjects indicated occupational exposure.

(N = 1) Did not provide enough sample.

N = 7; participants excluded or lost because of the following reasons:

- (N = 2) refused to give child birth information.
- (N = 1) had missing contacts.
- (N = 4) had miscarriages
All samples collected were then placed in a cooler bag with ice packs inside to maintain an ambient temperature of ±4°C. Such samples were transported by the principal investigator to the laboratory at the ambient temperature and then stored in the laboratory until analysis within 14 days.

**Determination of TCAA in maternal urine**

Lancet laboratory, toxicology department in South Africa carried out the urine samples analysis. The laboratory is the South African National Standards (SANAS) accredited to conduct the analysis. The urinary TCAA concentrations were measured according to the standard method described in detail in a previous study (Zeng et al. 2014). In brief, a 10-mL urine sample was extracted using methyl-tert-butyl-ether which contained the internal standard 1,2-dipropyl bromide. After centrifugation, TCAA extraction was converted to its methyl ester by the addition of acidic methanol. The target analyte was analysed using 6890N gas chromatography (G1530N) coupled with an electron capture detector (G2397A). The column used for the analysis is the DB 17MS 30 m × 0.25 mm × 0.15 μm (part no. 122-4731). One blank and two quality control samples were also analysed along with each analysis run. The limit of detection (LOD) for TCAA was 2 μg/L for this study. Urinary creatinine was determined by the picric acid assay using commercial test kits (Alinity c Creatinine (Enzymatic) reagent kit 08P01, Abbott Laboratories, Abbott Park, IL 60064, USA) to adjust for the variation in urine diluteness. The creatinine-adjusted TCAA concentration was expressed as μg/g creatinine using the TCAA value divided by the creatinine value (Zeng et al. 2014).

**Statistical analysis**

The TCAA values were positively skewed; therefore, the base-10 logarithm of TCAA concentrations (log₁₀-transformed TCAA concentrations) was done as recommended by previous studies (Calafat et al. 2005; Zeng et al. 2014). Spearman correlation was used to examine the correlations between log₁₀TCAA creatinine-adjusted and the total volume of ingested cold water (square root-transformed). Logistic regression was applied to determine the association between creatinine-adjusted urinary TCAA concentrations (log-transformed) and risks of premature, low birth weight and SGA. Creatinine-adjusted urinary TCAA was included as categorical using quartiles as cut points. Multiple logistic regression models were performed after adjusting the effect of significant covariates that changed the adjusted odds ratio (OR) for creatinine-adjusted urinary TCAA concentrations by 10% or more.

The potential confounders for adverse pregnancy outcomes were based on biological and statistical consideration. Risks factors associated with adverse pregnancy outcomes are well known such as maternal age, educational background, prenatal body mass index (BMI), race, marital status, employment, household income and chronic disease (Grazuleviciene et al. 2011; Horton et al. 2011). In this study, the covariables included maternal age (continuous), prenatal BMI (continuous), season, marital status, household income, educational background, alcohol consumption, maternal smoking, passive smoking, and infant sex and birth year. Maternal health characteristics included body mass index (BMI = weight/height²), high blood pressure, asthma, HIV status and diabetes. The BMI was based on participants’ medical records measured during pregnancy; this was referred to as prenatal BMI. The probability of exposure given the outcomes (OR) was used to present the results in this study. To indicate the precision of the effect, 95% CIs were calculated. Analyses were performed using Stata/IC version 14.1 (Stata Corp., USA).

**RESULTS**

**Demographic characteristics of study subjects**

Table 1 shows the distribution characteristics of the study subjects (mothers and infants) in the study. Thus, maternal age ranged from 18 to 40 years, with a mean value of 27 years of age. The majority (65%) were single at the time of the study, with approximately 74% of unemployed women. Most (67%) of women had high (secondary) school level of education. It is worth noting that 13% had previous adverse pregnancy outcomes mainly spontaneous abortion. There was a 16% prevalence of HIV-positive women in this study. The women who gave up taking alcohol during...
TABLE 1 | Distribution characteristics among the mothers and infants in the study (N = 203)

<table>
<thead>
<tr>
<th>Variables</th>
<th>n</th>
<th>%</th>
<th>Mean (SD)</th>
<th>Range</th>
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<td>Maternal characteristics</td>
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<td>24–27</td>
<td>52</td>
<td>26.26</td>
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<td>28–30</td>
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<td>≥31</td>
<td>43</td>
<td>21.72</td>
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<td>Marital status</td>
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<td>≥30.9</td>
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<tr>
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<td>166</td>
<td>83.84</td>
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(continued)
pregnancy were more (30%) than those that were still consuming alcohol (7%) at the later stage of pregnancy. More (61%) women indicated staying at home with someone who smokes a cigarette, which contributes to passive smoking.

There were 19 (10%) LBW, 19 (10%) premature birth and 26 (15%) SGA among newborns of the subjects. The birth weight ranged from 1,400 to 4,900 g. Up to 44% of infants were the second borns of the subjects (see Table 1 for details).

### Distribution of urinary TCAA concentrations (N = 198)

Valid samples (198) were used in this analysis. The urinary TCAA concentrations ranged from 2 to 817 μg/L, with the mean (median) of 205.6 (201) μg/L. Since TCAA values had positive-skewed distributions, base-10 logarithm of TCAA concentration (log₁₀-transformed TCAA concentrations) values were used in our statistical analysis. The log-transformed creatinine-adjusted TCAA values ranged from 0.76 to 3.26 μg/g of creatinine (see Table 2 for details).

### Correlation between creatinine-adjusted urinary TCAA (log-transformed) and the total volume of ingested cold (square root-transformed) water

Spearman correlation coefficients between the creatinine-adjusted urinary TCAA (log-transformed) concentrations and the total volume of ingested cold (square root-transformed) water were none (Spearman’s ρ = −0.0242, p = 0.7355) (Figure 2). Therefore, the two variables are independent.

### Association between creatinine-adjusted urinary TCAA concentrations and risks of premature birth, low birth weight and SGA delivery

Table 3 presents the association between creatinine-adjusted TCAA concentrations and premature birth, low birth weight and SGA. The results showed that creatinine-adjusted TCAA concentrations were not significantly associated with premature birth, low birth weight and SGA newborns among the study subjects. However, both crude and adjusted results showed that an increase in creatinine-adjusted TCAA concentrations increased the risks of premature delivery, low birth weight and SGA infants. The adjusted odds ratios are reported in detail in Table 3.

### DISCUSSION

The study measured urinary TCAA concentrations among pregnant women in a South African epidemiological cohort. Urinary TCAA was detected in more than 98% of the urine samples of the study subjects. The study showed high TCAA concentration levels among the study population as compared to previous studies (Costet et al. 2011;
The median urinary TCAA concentration before adjusting creatinine concentrations was 201 μg/L, with a maximum value of 817 μg/L. Low TCAA concentrations were observed among 41 samples, with a median concentration of 30 μg/L (20 μg/g creatinine) and a maximum value of 630 μg/L. Another study (Zhou et al. 2012) observed a lower TCAA concentration ranging from less than the LOD to 57.7 μg/L with mean concentrations of 7.7 μg/L.

The TCAA values obtained in this study were very skewed. Like in other previous studies (Calafat et al. 2003; Zeng et al. 2014), log base 10-logarithms were used to...
transform the TCAA values. The transformation was done to obtain the robustness of the results for this assessment. After this transformation, creatinine-adjusted TCAA concentration levels ranged from 0.76 to 3.26 μg/g creatinine, with the mean (median) of 2.17 (2.20) μg/g creatinine. Similar results were obtained in the previous study (Calafat et al. 2003), where urban-residing women had mean TCAA concentrations of 2.9 μg/L, while creatinine-adjusted TCAA mean was 2.8 μg/g creatinine. The median creatinine-adjusted urinary TCAA concentration of 5.29 is found by a previous study (Zeng et al. 2010), where urban-residing women had mean TCAA concentrations of 2.9 μg/L, while creatinine-adjusted TCAA mean was 2.8 μg/g creatinine. The median creatinine-adjusted urinary TCAA concentration of 5.29 μg/g creatinine was found by a previous study (Zeng et al. 2014).

In drinking water, the TCAA exposure is mainly through ingestion (Weisel et al. 1999). In this study, the association between Cr-adjusted TCAA concentrations and the total volume of daily drinking cold tap water was assessed. Spearman correlations between creatinine-adjusted log_{10}TCAA and the total volume of ingested cold water were none (Spearman’s rho = -0.0242, p = 0.7355). The results suggest that there was no linear relationship between the two variables from the data obtained. A weak correlation between Cr-adjusted TCAA concentrations and the ingestion of water was previously observed with Pearson’s correlation coefficient = 0.15, p = 0.05 (Zhou et al. 2012). TCAA concentration in tap water occurs in lower concentrations. South African drinking water guidelines (SANS 241) does not include HAAs; therefore, water utilities are not mandated to report on TCAA concentrations. To our knowledge, there is less or no available data found in the study area. The study has suggested that HAA concentrations are predictors of the urinary biomarker level (Rivera-Nunez et al. 2012). However, tap TCAA concentrations are less correlated with TCAA in the urine sample (Zhang et al. 2009). Instead, water-use activity habits contribute considerably to the urinary TCAA concentration levels (Smith et al. 2005).

In this study, the focus was on the later stage of the third trimester for the associations between Cr-adjusted TCAA concentrations and adverse pregnancy outcomes. The results showed that an elevated level of Cr-adjusted urinary TCAA concentrations increased the risks of delivering premature, low birth weight and SGA infants. However, the results were not statistically significant with p > 0.05 among the quantiles. It has been suggested that high levels of urinary TCAA cause a decrease in birth weight; however, the results from that study were also not statistically significant (Zhou et al. 2012).

The use of urinary TCAA as a biomarker still has some challenges. The use of a one-time urine sample as a biomarker of exposure is not valid to represent the average TCAA exposure during the entire pregnancy (Zhang et al. 2009). A previous study (Smith et al. 2015) has recommended taking 2-day urine samples. However, this becomes practically challenging, because the participation rate always decreases if we ask for two or more samples. Secondly, because the analysis of urinary TCAA is expensive, it is difficult to collect many samples. The other challenge of using TCAA as a biomarker is that it reflects the TCAA ingested only. TCAA is non-volatile, and another exposure route is minimal (Weisel et al. 1999). Therefore, other DBPs cannot be represented using TCAA only. In addition, the levels of TCAA in urine have been used as a biomarker for occupational or unintentional exposure to trichloroethylene (TCE), 1,1,1-trichloroethane (TRI), tetrachloroethylene [perchloroethylene (PERC)] and chloral hydrate, which are compounds that metabolise to TCAA in humans (Fisher et al. 1998; Bloemen et al. 2001; Raaschou-Nielsen et al. 2009). TCE, TRI and PERC are chemicals that occur in industrial chemicals. Industries, such as painting, textures, dry cleaning and auto parts, are common. Household products, such as glue and aerosol sprays, can contain TRI (ATSDR 1995), while typewriter correction fluid and paint removers contain TCE (ATSDR 1997). A previous study found no significant correlation between the levels of urinary TCAA and blood PERC (Calafat et al. 2003). It has been found that only 1–3% of the absorbed PERC is metabolised to TCAA by humans (ATSDR 1996). In contrast, TRI was found to correlate (Pearson correlation = 0.32, p = 0.0059) with the levels of urinary creatinine-adjusted TCAA and blood TRI levels (Calafat et al. 2005). In that study, it was also found that samples with high levels of TCAA in urine also had high levels of blood TCE, with a statistically significant correlation between the two (Pearson correlation = 0.43, p = 0.0001). These correlations suggest that to a certain extent (20–40%), the absorbed TCE and TRI are metabolised to TCAA in humans (ATSDR 1997).

**LIMITATIONS**

The possible interference of household products to these chemicals was not eliminated in this study. Therefore,
high levels of TCAA concentrations in this study might also have resulted from the use of household products, and it should be considered when interpreting the results of the current study. It should also be considered, when interpreting the current study results, that this study measured an exposure biomarker at that time. Thus, the temporal relationship could not be established.

CONCLUSIONS

This study provides the levels of TCAA in pregnant women at the later stage of gestation age. There were no statistically significant associations between Cr-adjusted urinary TCAA concentrations and the delivery of premature, low birth weight and SGA infants. Despite the small sample size, the high urinary TCAA concentrations observed in this study provide evidence of acute exposure in the study population of Tshwane district, South Africa. The present study also highlighted the usefulness of urinary TCAA as a biomarker in epidemiological studies on adverse reproductive effects of exposure to DBPs. This evidence cannot be generalised within the South African population; thus, a more significant number of samples (2-day collection samples) is needed to produce more robust results. The measurement of HAAs in chlorinated drinking water in order to correlate with urinary TCAA concentrations in the study population is necessary, because HAAs are not monitored and regulated by authorities in the studied area. DBPs exposure differs according to the geographical area and levels of DBPs present in the chlorinated water. Therefore, future studies which involve different groups (urban vs. rural areas) must be considered. Other epidemiological study designs, such as case-control study design to determine any possible health effects of TCAA exposure on adverse birth outcomes, should also be considered in Southern Africa.

CONFLICT OF INTEREST

None declared.

DISCLAIMER

The views expressed in this article are those of the authors and do not reflect the views of the South African National Research Foundation (NRF).

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