Environmental Research 126 (2013) 145–151

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Validation of trichloroacetic acid exposure via drinking water during pregnancy using a urinary TCAA biomarker

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ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form
24 December 2012
Accepted 13 May 2013
Available online 12 June 2013

Keywords:
Pregnancy
Biomarker
Disinfection by-products
Exposure assessment
Drinking water

ABSTRACT

Disinfection by-product (DBP) exposure during pregnancy may be related to reduced fetal growth, but the evidence is inconclusive and improved DBP exposure assessment is required. The authors conducted a nested exposure study on a subset ($n=39$) of pregnant women in the Born in Bradford cohort to assess validity of TCAA exposure assessment based on tap water sampling and self-reported water-use; water-use questionnaire validity; and use of a one-time urinary TCAA biomarker. TCAA levels in urine and home tap water supply were quantified, and water use was measured via a questionnaire and 7-day diary, at 28 weeks gestation. Diary and urine measures were repeated later in pregnancy ($n=14$). TCAA level in home tap water supply was not correlated with urinary TCAA (0.18, $P=0.29$). Cold unfiltered tap water intake at home measured by questionnaire was correlated with urinary TCAA (0.44, $P=0.007$), but correlation was stronger still for cold unfiltered tap water intake reported over the 3 days prior to urine sampling (0.60, $P<0.001$). For unemployed women TCAA ingestion at home, derived from tap water sampling and self-reported water-use, correlated strongly with urinary TCAA (0.78, $P<0.001$), but for employed women the correlation was weak (0.31, $P=0.20$). Results suggest individual tap water intake is most influential in determining TCAA exposure variability in this cohort, and that TCAA ingestion at home is a valid proxy for TCAA exposure for unemployed women but less satisfactory for employed women.

1. Introduction

During pregnancy exposure to disinfection by-products (DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs) in public water supplies may be related to reduced fetal growth (Grellier et al., 2010; Nieuwenhuijsen et al., 2009). However because exposure assessment is difficult and prone to measurement error, and the effects seen are likely to be small, the evidence is inconsistent and inconclusive. Improved DBP exposure assessment is required, particularly addressing non-THM classes of DBPs such as HAAs, and use of biomarkers may allow advancement in this respect. HAAs are non-volatile and the predominant route for exposure is ingestion; with no significant contribution from inhalation and dermal absorption (Xu et al., 2002; Xu and Weisel 2003). TCAA is one of the most common HAAs found in chlorinated drinking water, and urinary TCAA has been validated as a biomarker for measuring TCAA exposure by ingestion of drinking water (Zhang et al., 2009). Incorporation of biomarkers into exposure assessment in epidemiological studies examining DBPs and fetal growth outcomes has occurred only recently, with two studies using urinary TCAA biomarkers (Costet et al., 2012; Zhou et al., 2012). Whilst there are knowledge gaps to address before we can fully rely upon or interpret urinary TCAA as an exposure biomarker, e.g. required number of samples and effectiveness of TCAA as a proxy for other DBPs (Savitz, 2012), as an integrated objective measure of exposure urinary TCAA may be a useful validation tool in epidemiological studies using existing exposure assessment methods based on DBP concentrations at the tap and individual water use.

We are investigating DBPs and fetal growth in the Born in Bradford (BiB) birth cohort. Exposure assessment comprises modelled area-level DBP concentrations combined with individual water-use data to estimate 'semi-individual' DBP exposure metrics. This paper presents a nested exposure validation study which

Abbreviations: BiB, Born in Bradford; CI, Confidence Interval; DBP, Disinfection by-product; FMU, First morning urine; ICC, Intra-class correlation coefficient; HAA, Haloacetic acid; TCAA, Trichloroacetic acid; THM, Trihalomethane.

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0013-9351
http://dx.doi.org/10.1016/j.envres.2013.05.004

collected detailed individual-level exposure information, including urinary TCAA biomarkers and water use diaries as gold-standard measures, for a subset of women in the BiB cohort. The primary aim of this study was to evaluate the validity of TCAA exposure assessment, in order to improve interpretation of health-risk estimates in future epidemiological analyses. To achieve this we assessed correlation between urinary TCAA and TCAA exposure assessment based on tap water concentration and water-use questionnaire, and validity of a water-use questionnaire. The secondary aim was to assess correlation between repeated measures of both urinary TCAA and water use during the third trimester in order to evaluate exposure variability and adequacy of a one-time urinary TCAA biomarker to assess TCAA exposure during pregnancy.

2. Materials and methods

2.1. Recruitment

Born in Bradford (BiB) is a large, multi-ethnic birth cohort ($n=13,750$) in Bradford, UK, recruited 2007–2010. Pregnant women were recruited at 26–28 weeks gestation by BiB project workers, who informed them about the study, obtained written consent and conducted a baseline questionnaire including questions on tap water use, by interview (Raynor and Born in Bradford Collaborative Group, 2008). Recruitment for this nested study occurred in 2 phases, with 20 women recruited during February/March 2008, and 19 women during May 2008, and has been described in detail elsewhere (Smith et al., 2009). BiB and this nested study were reviewed and approved by the Bradford Research Ethics Committee. All subjects signed an informed consent form prior to participation.

2.2. Exposure assessment

Participants were supplied with a 7-day water-use diary (Diary 1) based on a diary used by Kaur et al. (2004), which they completed over 7 consecutive days commencing on average one day after completing the BiB baseline questionnaire. Tap water was sampled at each participant's home by the researcher. Participants collected a first morning urine (FMU) sample (Urine 1) three days after tap sampling to account for approximate TCAA half-life when comparing tap water and urine TCAA concentrations. Urinary TCAA measured in FMU samples has been validated as a biomarker for TCAA exposure by ingestion of drinking water, in a controlled direct exposure experiment in humans which demonstrated urinary TCAA concentration reflected TCAA ingestion (μg/day) (Zhang et al., 2009). Diary 1 recorded the same water exposures as the baseline questionnaire (daily intake at home, work/study and elsewhere of tap water, bottled water, tea, coffee and squash/cordial measured in mugs/glasses; water filtering habits; and daily frequency and duration of showering, bathing and swimming), additional tap water intakes and water-related activities, and potential DBP exposure modifiers (e.g. ventilation during showering/bathing). To assess potential exposure to chlorinated solvents (some of which metabolise to TCAA) the diary included questions on visiting/working in particular industries (dry cleaners, metal manufacturers, auto parts, textile production, paint production, printers), household members working in dry cleaning, and usage of various household products (correction fluid, carpet cleaner, stain removers, paint/varnish, thinners, adhesives, pesticides and disinfectants). No participant visited/worked in the industries listed during the diary week, or had a household member working in dry cleaning. Of 39 household products reported used, ingredient lists could be identified for 26 and none contained chlorinated solvents of interest. Fourteen participants (36%) repeated the study, providing a second urine sample (Urine 2) and completing a second diary (Diary 2). Tap water samples were analysed for TCAA, and urine samples were analysed for TCAA and creatinine (to adjust for diuresis)—detailed sampling and analytical methods provided in Appendices A and B. (shown in Supplementary Materials)

Water intakes at home, work, elsewhere and across all locations were calculated in L/day from both the questionnaire and the diary. Weekly duration (minutes per week) of showering, bathing and swimming were calculated from the questionnaire and diary. TCAA ingestion at home (μg/day) was calculated by multiplying home tap water TCAA concentration (μg/L) with tap water intakes at home (L/day), incorporating a 32% reduction in TCAA concentration for boiling (average of 2 and 5 min boiling tests (Krasner and Wright 2005; Ma, 2008; Wu et al., 2001)) applied to tea/coffee intake, and 64% reduction for filtering (average of all new/used pitcher and tap-mounted tests (Egorov et al., 2003; Levesque et al., 2006; Ma, 2008; Weinberg et al., 2006) applied to cold filtered tap water intake. Four metrics of TCAA ingestion at home were calculated based on (a) tap water intake at home from the questionnaire, (b) average tap water intake at home over 7 days of Diary 1, (c) average tap water intake at home over the 3 days of Diary 1 preceding urine sample collection, and (d) average tap water intake at home across 5 weekdays of Diary 1. TCAA ingestion was calculated at home only because data on TCAA in workplace tap water supplies were not available.

2.3. Statistical analysis

Spearman's correlations were calculated between urinary TCAA, and tap water intakes, tap TCAA concentration, and TCAA ingestion at home. Correlations between urinary TCAA and showering, bathing and swimming were also calculated to determine whether the relationship between urinary TCAA and ingestion is specific and is not seen with other sources, as would be expected. Agreement between questionnaire and Diary 1 was examined by mean difference between methods, intra-class correlation coefficient (ICC) and Spearman's correlation coefficient. Analyses were stratified by employment status, because employed women are potentially exposed to DBPs in tap water at work and other sources of TCAA. A Z-test (following Fisher's r to Z transformation) was used to test difference in correlation coefficients for employed versus unemployed women. William's T2-test (Steiger, 1980) was used to test difference in correlation coefficients using unadjusted and creatinine-adjusted urinary TCAA measures. For repeated measures we calculated Spearman's correlation and ICC. All ICCs were calculated as ICC (type A,1)

Table 1
Summary statistics for (a) TCAA in home tap water supply and (b) urinary TCAA (Urine 1) for pregnant women in a nested exposure study, BiB cohort, Bradford, UK, 2008.

		n[a]	Mean	95% CI for Mean	SD	Min	25th %ile	Median	75th %ile	Max
a) TCAA in home tap water supply (μg/L)	**All**	36[b]	11.3	10.1, 12.5	3.4	5.3	8.9	10.5	13.6	17.7
	Phase 1[c]	20	10.6	9.1, 12.1	3.2	5.3	8.5	10.2	12.8	17.5
	Phase 2[c]	16	12.2	10.3, 14.1	3.6	5.9	9.6	11.6	15.1	17.7
	Unemployed	17	11.4	9.5, 13.3	3.7	5.3	8.7	12.4	14.2	17.5
	Employed	19	11.2	9.6, 12.8	3.3	5.9	9.3	10.2	12.4	17.7
b) Urinary TCAA (nmol/L)	**All**	37[b]	37.1	28.9, 45.2	24.4	1.5	21.0	33.0	45.0	112.0
	Phase 1[c]	20	32.9	22.7, 43.2	21.9	1.5	18.3	31.0	41.3	86.0
	Phase 2[c]	17	42.0	28.1, 55.9	27.0	13.0	24.0	34.0	50.0	112.0
	Unemployed	17	38.5	27.3, 49.7	21.8	5.0	21.0	36.0	45.0	86.0
	Employed	20	35.9	23.2, 48.5	27.0	1.5	23.3	29.0	41.3	112.0
Creatinine-adjusted urinary TCAA (μmol/mol creatinine)	**All**	37[b]	4.9	3.4, 6.3	4.4	0.1	2.2	3.5	6.7	20.6
	Phase 1[c]	20	4.2	2.0, 6.3	4.6	0.1	2.1	2.6	4.2	20.6
	Phase 2[c]	17	5.7	3.6, 7.8	4.1	1.5	3.1	4.5	6.9	15.1
	Unemployed	17	3.7	2.5, 4.9	2.3	1.0	2.1	3.4	4.1	8.5
	Employed	20	5.9	3.3, 8.4	5.4	0.1	2.4	4.2	7.7	20.6

Abbreviations: BiB, Born in Bradford; CI, confidence interval; TCAA, trichloroacetic acid.

[a] *n* represents 1 sample, either (a) tap water or (b) urine, per woman enrolled in the study. For urinary TCAA, the statistics are for the initial Urine 1 sample only (Urine 2 samples are not included in this analysis).

[b] 37 Women had Urine 1 TCAA data, but only 36 women had data for TCAA in tap water.

[c] Recruitment and fieldwork for this nested validation study was carried out in 2 phases: during February/March 2008 (Phase 1), and during May 2008 (Phase 2). Statistics are calculated separately for each Phase to allow for any seasonal variation in TCAA in tap water.

using a two-way random effects model (McGraw and Wong, 1996) after square-root transformation to achieve approximate normality. For ICCs, negative lower bounds on confidence interval estimates were set to zero. 95% confidence intervals and two-sided *P*-values are reported. Statistical analysis was performed using R statistical software version 2.4.1 (R Development Core Team, 2008) and IBM SPSS Statistics 20 (IBM Corp., 2011).

3. Results

Characteristics and water use patterns for the 39 study participants, as recorded by the questionnaire, have been reported elsewhere (Smith et al., 2009). Mean total tap water intake was 1.8 L/day, and on average participants spent 74 min/week showering and 72 min/week bathing. Only 6 participants (15.4%) reported swimming at least once a week.

Table 1 presents summary statistics for concentration of TCAA in home tap water supply and in Urine 1. Variability of TCAA in tap water was low but urinary TCAA demonstrated greater variability.

Table 2 presents correlations between Urine 1 TCAA and tap water TCAA concentration at home, tap water intakes, and TCAA ingestion at home. Tap water TCAA concentration at home was not correlated with urinary TCAA (0.18, $P=0.29$). Cold unfiltered tap water intake at home measured by questionnaire was correlated with urinary TCAA (0.44, $P=0.007$), but stronger correlation was observed for cold tap water intake averaged over the 3 diary days preceding urine sampling (0.60, $P<0.001$). Correlations between TCAA ingestion at home and urinary TCAA (0.50, $P=0.002$) were of similar magnitude to those with cold unfiltered tap water intake at home. Stratifying by employment status we observed that correlations between urinary TCAA and both tap water intake and TCAA ingestion at home appeared stronger for unemployed women compared to employed women, e.g. for TCAA ingestion at home (0.78, $P<0.001$; and 0.31, $P=0.20$, respectively). Differences in correlation for unemployed and employed women were statistically significant for total tap water intake and borderline significant for TCAA ingestion at home. For unemployed women TCAA ingestion at home (based on tap concentration and self-reported water use questionnaire) explained 65% of total variability in urinary TCAA, but only 17% for employed women (Fig. 1). On average, cold unfiltered tap water constituted 61.2% and 40.9% of total tap water intake for unemployed and employed women respectively, and 83.6% and 70.7% of total tap water intake was consumed at home by unemployed and employed women respectively (shown in Supplementary Table A.1). Correlations between creatinine-adjusted urinary TCAA and tap water TCAA concentration at home, tap water intakes and TCAA ingestion at home tended overall to be weaker than those with unadjusted urinary TCAA, although for many of these comparisons the difference was

Table 2
Correlations between urinary TCAA (Urine 1) and TCAA in home tap water supply, tap water intakes and TCAA ingestion at home for pregnant women in a nested exposure study, BiB cohort, Bradford, UK, 2008.

Exposure metric		Spearman's correlation with urinary TCAA concentration (Urine 1) (nmol/L)						Difference between rho for employed vs. unemployed
		All women ($n=37$)		Employed ($n=20$)		Unemployed ($n=17$)		
		rho	*P*-value	**rho**	*P*-value	**rho**	*P*-value	*P*-value
a) **TCAA in home tap water supply** (μg/L)[a]		**0.18**	0.29	**0.04**	0.89	**0.22**	0.39	0.60
b) **Total tap water intake**[b] **at Home** (L/day) calculated from:	Questionnaire	**0.31**	0.06	**0.01**	0.96	**0.72**	0.001	0.01
	Diary 1 (7 days)	**0.30**	0.08	**−0.07**	0.77	**0.75**	< 0.001	0.003
	Diary 1 (3 days pre urine)[c]	**0.40**	0.02	**0.16**	0.52	**0.76**	< 0.001	0.03
Total tap water intake[b] **at Work** (L/day) calculated from:	Questionnaire			**0.35**	0.13			
	Diary 1 (7 days)			**−0.19**	0.42			
	Diary 1 (3 days pre urine)[c]			**−0.32**	0.20			
c) **Cold unfiltered tap water intake**[d] **at Home** (L/day) calculated from:	Questionnaire	**0.44**	0.007	**0.29**	0.21	**0.58**	0.01	0.31
	Diary 1 (7 days)	**0.48**	0.002	**0.33**	0.16	**0.74**	0.001	0.09
	Diary 1 (3 days pre urine)[c]	**0.60**	< 0.001	**0.43**	0.07	**0.76**	< 0.001	0.15
Cold unfiltered tap water intake[d] **at Work** (L/day) calculated from:	Questionnaire			**0.20**	0.40			
	Diary 1 (7 days)			**0.06**	0.79			
	Diary 1 (3 days pre urine)[c]			**−0.28**	0.26			
d) **TCAA ingestion at Home**[e] (μg/day) calculated from:	Questionnaire [a]	**0.50**	0.002	**0.31**	0.20	**0.78**	< 0.001	0.05
	Diary 1 (7 days) [a]	**0.48**	0.003	**0.26**	0.28	**0.80**	< 0.001	0.03
	Diary 1 (3 days pre urine)[f]	**0.53**	0.001	**0.40**	0.11	**0.72**	0.001	0.21

Abbreviations: BiB, Born in Bradford; TCAA, trichloroacetic acid.

[a] For exposure metric a) and d) $n=36$ for all and $n=19$ for Employed because for one employed woman tap water sampling data was missing.

[b] Total tap water intake equals sum of tap water, tea, coffee, and squash intakes.

[c] $n=35$ for All and $n=18$ for Employed because for two employed women tap water intake in the 3 days immediately prior to urine collection could not be calculated (because one had collected the urine sample late, and one had started the diary a day late).

[d] Cold unfiltered tap water intake equals sum of unfiltered tap water and squash intakes.

[e] TCAA ingestion at Home was calculated by multiplying the volume of tap water consumed (either from Questionnaire or Diary) by the concentration of TCAA in tap water at the woman's Home. Reduction factors to account for the effect of boiling and filtering upon TCAA concentration in the tap water were applied when calculating TCAA ingestion.

[f] $n=34$ for All and $n=17$ for Employed because out of the 36 women who had tap water sampling data, for two employed women tap water intake in the 3 days immediately prior to urine collection could not be calculated.

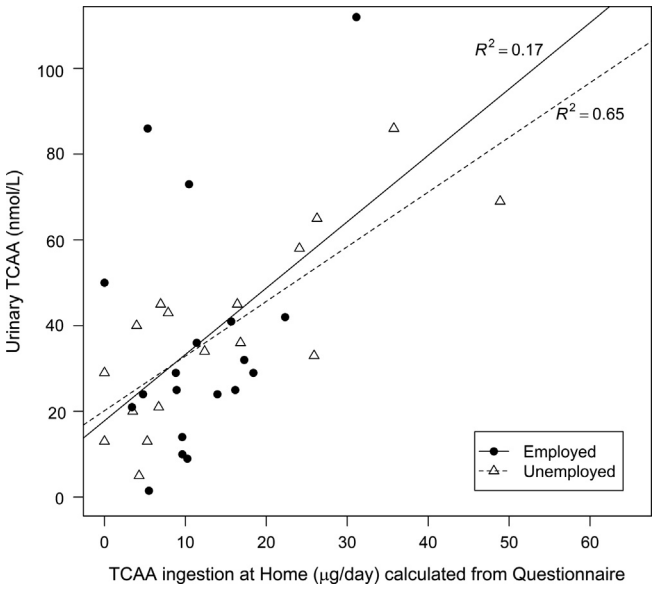

Fig. 1. Urinary TCAA (nmol/L) vs. TCAA ingestion at Home (μg/day) calculated using self-reported water consumption from Questionnaire.

not statistically significant (shown in Supplementary Table A.2 and Table A.3). Weekly duration of showering, bathing or swimming was not correlated with urinary TCAA, with the exception of a weak correlation with bathing recorded in Diary 1 (rho=0.33, P=0.05) (not shown).

Table 3 presents validation of the water use questionnaire and shows good agreement between the questionnaire and Diary 1 for total tap water intake at home (ICC=0.64; 95% CI: 0.40, 0.80), showering (ICC=0.78; 95% CI:0.61, 0.88) and bathing (ICC=0.60; 95% CI: 0.34, 0.77). Agreement between questionnaire and Diary 1 was weak for tap water intake at work (ICC=0.34; 95% CI: 0, 0.66) and non-existent for tap water intake elsewhere (not shown). The questionnaire overestimated tap water intake by 0.41 L/day (95% CI: 0.13, 0.69) and bathing by 28.3 min/week (95% CI: 7.4, 49.2). Too few participants reported swimming in the questionnaire (n=6) or Diary 1 (n=4), to allow meaningful analysis of questionnaire validity for this activity. Agreement between questionnaire and Diary 1 appeared stronger for unemployed women (0.73≤ICC≤0.90) compared to employed women (0.34≤ICC≤0.49). Differences in correlation by employment status were not statistically significant, but the consistency of the pattern across all water use variables suggests the possibility of poorer accuracy in reporting by employed women in the questionnaire and/or Diary 1 should be considered. Tap water intakes captured by Diary 1 but not questionnaire constituted only 2.1% of all Diary 1 tap water intake on average, but considerable additional water-related activity is shown in Supplementary Table A.4.

The average gap between initial and repeat studies was 9 weeks 3 days, with most completing Diary 2/Urine 2 around 35–37 weeks gestation. TCAA in Urine 1 and Urine 2 demonstrated little correlation (0.26, P=0.39) and an ICC of 0.23 indicates within-subject variability exceeded between-subject variability (ICC reflects the proportion of total variance attributable to between-subject variability) (Table 4). Diary 1 and Diary 2 were strongly correlated for total tap water intake across all locations (0.74, P=0.005) with between-subject variability explaining a high proportion of total variability in tap water intake (ICC=0.80). There was no change in absolute volume of tap water intake between Diary 1 and Diary 2 (mean difference=−0.01 L/day; 95% CI: −0.30, 0.27), but results were suggestive of small increases in proportion of tap water being consumed at home, and corresponding decreases in that consumed outside the home over the third trimester, although these changes did not reach statistical significance (shown in Supplementary Table A.5).

4. Discussion

This study evaluated correlation between urinary TCAA and TCAA exposure based on tap water concentration and water-use questionnaire data, water-use questionnaire validity, and adequacy of a one-time urinary TCAA biomarker for assessing TCAA exposure during pregnancy. Urinary TCAA levels observed in this study (mean 37.1 nmol/L≈6.1 μg/L) were comparable with levels in a US general population sample (median 3.3 μg/L)(Calafat et al., 2003) and a Chinese sample of pregnant women (mean 7.7 μg/L) (Zhou et al., 2012). Comparison with urinary TCAA levels observed by Costet et al. (2012) is not meaningful because the analytical limit-of-detection (10 μg/L) was 20 times higher than in the present study.

In this study tap water TCAA concentration at home was not correlated with urinary TCAA, which is consistent with lack of correlation observed in a previous study (Weisel et al., 1999). We observed volume of tap water intake at home to be better correlated with urinary TCAA, particularly cold unfiltered tap water intake which is expected given boiling and filtering reduce TCAA concentrations (Levesque et al., 2006). We found correlations between TCAA ingestion at home (an exposure metric incorporating both volume of tap water intake and tap water TCAA concentration at home) and urinary TCAA were not greatly improved over correlations with cold unfiltered tap water intake at home. This suggests individual water consumption is the most influential determinant of TCAA exposure variability, whilst tap water TCAA concentration has relatively little influence in our study, probably due to low variability in tap water TCAA in the confined geographical study location. Urinary TCAA was largely unresponsive to duration of showering, bathing or swimming, which was as expected. We did observe a weak correlation between urinary TCAA and bathing recorded in Diary 1, but this appears to be explained by bathing correlating with cold unfiltered tap water intake for unemployed women, the latter correlating strongly with urinary TCAA, and both relating to employment status with unemployed women consuming more cold unfiltered tap water and spending longer bathing.

We found urinary TCAA was highly correlated with both volume of tap water intake and TCAA ingestion at home for unemployed women, but poorly correlated for employed women although differences did not reach significance in all comparisons possibly due to small numbers. This pattern is consistent with a previous study which found urinary TCAA excretion rate and TCAA ingestion (calculated using home tap water TCAA concentration) were more strongly associated for subjects not working outside the home (r^2=0.655) vs. all subjects (r^2=0.53)(Weisel et al., 1999).

Using a urinary TCAA biomarker we demonstrated that TCAA ingestion at home (derived from tap water concentration and self-reported water consumption) provides a valid proxy for TCAA exposure for unemployed women in our study, explaining a large proportion of total variability in their urinary TCAA. The same measure does not satisfactorily explain variability in urinary TCAA for employed women. This could reflect a possible difference in error in tap water intakes reported by employed women, and our inability to account fully for TCAA ingestion at work. Whilst exposure to other sources of TCAA could plausibly differ by employment status, we ruled out occupational exposures to chlorinated solvents which could metabolise to TCAA.

We observed that creatinine-adjusted urinary TCAA generally demonstrated weaker correlation with volume of tap water intake

Table 3
Comparison of questionnaire and Diary 1 responses for tap water intake, showering, bathing and swimming for pregnant women in a nested exposure study, BiB cohort, Bradford, UK, 2008.

	n	Employment Status	ICC	95% CI for ICC	Mean difference (Q-D)[a]	95% CI for Mean difference	Spearman's correlation coefficient		Difference between rho for employed vs. Unemployed
							rho	*P*-value	*P*-value
Total Tap water intake[b] (L/day) at Home	39	All women	**0.64**	0.40, 0.80	**0.40**	0.18, 0.62	**0.66**	< 0.001	0.12
	21	Employed	**0.41**	0.02, 0.71	**0.42**	0.06, 0.78	**0.51**	0.019	
	18	Unemployed	**0.82**	0.56, 0.93	**0.37**	0.08, 0.66	**0.81**	< 0.001	
Total Tap water intake[b] (L/day) at Work	21	Employed	**0.34**	0, 0.66	**0.29**	0.06, 0.53	**0.26**	0.25	
Total Tap water intake[b] (L/day) at All locations	39	All women	**0.67**	0.44, 0.82	**0.41**	0.13, 0.69	**0.70**	< 0.001	0.29
	21	Employed	**0.47**	0.07, 0.75	**0.59**	0.12, 1.06	**0.60**	0.004	
	18	Unemployed	**0.81**	0.56, 0.93	**0.19**	−0.11, 0.49	**0.78**	< 0.001	
Showering (min/week)	39	All women	**0.78**	0.61, 0.88	**3.0**	−15.3, 21.3	**0.71**	< 0.001	0.10
	21	Employed	**0.49**	0.07, 0.76	**2.3**	−27.5, 32.1	**0.55**	0.010	
	18	Unemployed	**0.90**	0.75, 0.96	**3.9**	−18.8, 26.6	**0.83**	< 0.001	
Bathing (min/week)	39	All women	**0.60**	0.34, 0.77	**28.3**	7.4, 49.2	**0.65**	< 0.001	0.24
	21	Employed	**0.42**	0, 0.72	**38.9**	−36.2, 113.9	**0.59**	0.005	
	18	Unemployed	**0.73**	0.41, 0.89	**16.0**	−17.2, 49.3	**0.80**	< 0.001	
Swimming (min/week)	39	All women	**0.28**	0, 0.54	**6.5**	−2.0, 15.1	**0.32**	0.049	

Abbreviations: BiB, Born in Bradford; CI, confidence interval.

[a] Q-D mean difference between questionnaire and Diary 1, when the Diary 1 value is subtracted from the questionnaire value.

[b] Total tap water intake equals sum of tap water, tea, coffee, and squash intakes.

Table 4
Correlations within and between initial and repeat studies for urinary TCAA and diary tap water intakes, for a subset[a] of participants in a nested exposure study, BiB cohort, Bradford, UK, 2008.

				n	Spearman's correlation		ICC (95% CI)
					rho	*P*-value	
Within initial study	**Urinary TCAA 1** (nmol/L)	vs.	**Total tap water intake**[b] **at Home** (L/day) from **Diary 1** (7 day average)	13	**0.12**	0.69	
			Total tap water intake[b] **at Work** (L/day) from **Diary 1** (7 day average)	7[c]	**0.13**	0.79	
			Total tap water intake[b] **at All Locations** (L/day) from **Diary 1** (7 day average)	13	**0.37**	0.22	
Within repeat study	**Urinary TCAA 2** (nmol/L)	vs.	**Total tap water intake**[b] **at Home** (L/day) from **Diary 2** (7 day average)	13	**0.24**	0.43	
			Total tap water intake[b] **at Work** (L/day) from **Diary 2** (7 day average)	7[c]	**-0.16**	0.73	
			Total tap water intake[b] **at All Locations** (L/day) from **Diary 2** (7 day average)	13	**0.32**	0.29	
Between initial and repeat study	**Urinary TCAA 1** (nmol/L) vs. **Urinary TCAA 2** (nmol/L)			13	**0.26**	0.39	0.23 (0, 0.69)
	Diary 1 (7 day average) vs. **Diary 2** (7 day average), for:						
			Total tap water intake[b] **at home** (L/day)	13	**0.51**	0.08	0.69 (0.24, 0.89)
			Total tap water intake[b] **at work** (L/day)	7[c]	**0.41**	0.36	0.48 (0, 0.89)
			Total tap water intake[b] **all locations** (L/day)	13	**0.74**	0.005	0.80 (0.46, 0.93)

Abbreviations: BiB, Born in Bradford; TCAA, trichloroacetic acid.

[a] $n = 13$, which is the number of women who undertook both the initial and repeat studies and who had a complete set of initial and repeat diaries plus initial and repeat urinary TCAA data.

[b] Total tap water intake equals sum of tap water, tea, coffee, and squash intakes.

[c] Employed women only.

and TCAA ingestion than (unadjusted) urinary TCAA, although differences were often not statistically significant. This differs from the study by Zhang et al. which found similar correlations between TCAA ingestion and both creatinine-adjusted and unadjusted urinary TCAA (Zhang et al., 2009). The utility of using creatinine to account for diuresis depends on the analyte of interest being excreted in the same way as creatinine and for highly water soluble compounds like TCAA this is not always the case. Additionally, subjects in previous urinary TCAA biomarker exposure studies using creatinine adjustment have been female but not pregnant (Kim et al., 1999; Weisel et al., 1999; Zhang et al., 2009), and phthalate biomarker studies suggest creatinine adjustment may not be the most appropriate method of normalising urinary biomarker concentrations for pregnant women, as physiological changes may make urinary creatinine unusually diluted or concentrated during pregnancy (Adibi et al., 2008; Huang et al., 2007).

Finding an appropriate method to adjust urinary TCAA for diuresis in pregnant women may be important in developing urinary TCAA as a biomarker for exposure during pregnancy.

In this study we evaluated the validity of a water-use questionnaire, as any error in self-reported water use would contribute to TCAA exposure measurement error, and weaken the correlation between TCAA ingestion and urinary TCAA. Against a 7-day diary, the questionnaire demonstrated strong agreement, and thus acceptable validity for measuring tap water intake, showering and bathing for unemployed women. However, weaker agreement with the diary suggested the questionnaire was a less satisfactory measure of water use for employed women. In general our results are consistent with previous studies which have observed overestimation of tap water intake by questionnaires, and high questionnaire–diary correlation for water intakes at home (≥0.75) but weaker correlation for intakes outside the home (≤0.43) (Barbone et al., 2002; Kaur et al., 2004; Shimokura et al., 1998). Whilst differences in questionnaire–diary agreement according to employment status did not reach statistical significance (perhaps due to small sample size), the consistency of the pattern across water intakes and activities is suggestive of a possible difference in questionnaire validity by employment status, which may partially explain observed differences in correlation between TCAA ingestion/tap water intakes and urinary TCAA by employment status. A previous study found no difference in water-use questionnaire vs. diary correlation according to employment status, but diaries were completed later in pregnancy (≥36 weeks) when few women were still working, and questionnaires were completed after delivery (Barbone et al., 2002). In our study, only 1 out of 21 employed women reported being on sick/maternity leave when completing Diary 1 (≈28 weeks). Employed women may have greater difficulty estimating water use in a "typical" day/week in a questionnaire, if patterns of behaviour differ across working/non-working days and activity occurs at multiple locations. If employed women complete a diary at home after work it may be subject to recall error, and attendance and water intake at work may vary week-to-week as pregnancy progresses such that employed women are more likely to complete the diary on a non-representative week, leading to poor agreement with the questionnaire.

Finally, we assessed correlation between repeated measures of urinary TCAA and water use to evaluate exposure variability and adequacy of a one-time TCAA biomarker as a measure of TCAA exposure during pregnancy. Ideally, urinary TCAA would reflect exposure over a sufficient time period to be relevant to critical exposure windows during pregnancy, e.g. third trimester for fetal growth. We found little correlation between initial and repeat urinary TCAA measurements (collected at ≈28 and ≈37 weeks gestation), and within-subject variability exceeded between-subject variability. This suggests intra-individual variability of factors influencing exposure to, and uptake or excretion of, TCAA during the third trimester. We could not assess if tap water TCAA levels at home changed between the two time-points. However, as tap water TCAA contributed little to urinary TCAA variability in the initial study, poor correlation between initial and repeat urinary TCAA is unlikely to be explained solely by changes in tap water TCAA levels. Additionally, area-level tap water sampling we have conducted in the BiB study area reveals no clear temporal patterns in HAA variability which would explain this (Edwards S, Imperial College London, personal communication, 2011). We observed little change in overall volume of tap water intake or tap water handling behaviour during the third trimester which would explain weak correlation between initial and repeat urinary TCAA. This in itself is important as it is commonly assumed that consumption of water/fluids increases as pregnancy progresses. Changes in tap water intake during early to mid-pregnancy have previously been observed, with increased intake being more likely than decreased intake (Forssen et al., 2008; Windham et al., 1992), however, we found no evidence of systematic change during the third trimester. Variability in absorption, metabolism and excretion of TCAA in the human body may result in different urinary TCAA concentrations for the same exposure, and could vary with gestation as factors such as physical activity and body mass index influence the absorption and excretion of chemicals (Manini et al., 2007). Whilst variability in biological sample collection, storage and handling could introduce error into urinary TCAA measurements (due to analyte loss/degradation), because TCAA is a reasonably stable, non-volatile metabolite this is not thought to be significant. Day-to-day variation in urinary TCAA could partly explain the failure of urinary TCAA to correlate across time periods, and this could be addressed by pooling biomarker specimens to derive an improved exposure estimate that smooths out this variation. These sources of inter- and intra-individual variability in urinary TCAA could not be accounted for, but may weaken correlations between repeated urinary TCAA measures and between urinary TCAA and TCAA ingestion estimates. Our repeated biomarker analysis suggests reliance on a one-time urinary TCAA biomarker may not be sufficient to fully characterise exposure to TCAA during pregnancy. However, this is based on a small sample size and should be investigated further in a larger sample.

It is encouraging that in an uncontrolled setting we have observed urinary TCAA to be responsive to volume of cold unfiltered water intake and an exposure index combining volume of water intake and tap water concentration, and to be largely unresponsive to bathing, showering or swimming. This suggests that urinary TCAA is doing what we require of it as a biomarker – i.e. reflecting relevant external exposure determinants and pathways which we traditionally assess via questionnaire and tap water sampling. However, we are not yet ready to consider relying solely upon urinary TCAA to assess exposure. Our repeated biomarker analysis suggests that further development of the urinary TCAA biomarker in terms of its ability to assess longer term exposure (e.g. at least over a whole trimester) is needed. In addition, TCAA is not the primary or only DBP of concern – it is the focus here because urinary TCAA is the most convenient and well-developed biomarker available – but ideally we would like a biomarker which represents a range of DBPs. Any inferences we can make about other DBPs based solely on TCAA are currently limited by the knowledge gap regarding the effectiveness of TCAA as a proxy for other DBPs. This knowledge gap needs to be addressed before we can fully interpret urinary TCAA as an exposure biomarker and fully exploit its potential in DBP exposure assessment.

To our knowledge, this is the first epidemiological cohort examining DBPs and fetal growth to incorporate a nested exposure validation study. It incorporates a full suite of individual-level exposure data (water-use, biomarker and tap water DBP measurements at maternal residence) and use of a biomarker allowed validation against an objective measure. However, individual data collection was burdensome and self-selection may have biased the study sample. Non-English speakers could not be recruited to our nested study so complexities in questionnaire reporting due to foreign language completion could not be assessed, and may limit generalisability of results to the non-English speakers in the cohort. This study was limited by small numbers when analysing employment status and repeat study subsets, resulting in uncertainty surrounding parameter estimates.

5. Conclusions

In conclusion, individual volume of tap water intake is the most influential determinant of TCAA exposure variability, when considering a localised area with low TCAA variability in tap water supplies. Resources should be focussed on improving individual water use assessment in order to reduce DBP exposure measurement error. Employment status appears to be important in self-reporting of water use, and may result in differential exposure

measurement error. Use of biomarkers may therefore be particularly important for improving DBP exposure assessment for employed women. DBP exposure assessment is more complex and challenging for employed women, but such challenges are unlikely to be limited to DBPs, and environmental exposure validation studies should consider the potential for differential error by employment status in order that measurement error is properly understood and health-risk estimates can be interpreted appropriately. Whilst we are not ready to consider relying on it solely to assess exposure, urinary TCAA could be incorporated into epidemiological studies alongside traditional exposure assessment methods to simultaneously enhance exposure assessment, e.g. for employed women, and further develop it as a biomarker.

Funding sources

This research was funded by The Joint Environment & Human Health Programme [NERC grant NE/E008844/1], HiWATE [EU 6th Framework Programme Contract no. Food-CT-2006-036224] and by an ESRC studentship [award no. PTA-031-2006-00544]. Funding sources had no involvement in study design, collection, analysis and interpretation of data, writing the report or decision to submit the paper for publication.

Competing interests

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

Ethics committee approval

The study was reviewed and approved, prior to its conduct, by the Bradford Research Ethics Committee.

Acknowledgments

We are grateful to all the families who took part in this study, to Bradford's midwives, paediatricians and health visitors and to the Born in Bradford team which includes interviewers, data managers, laboratory staff, clerical workers, research scientists, volunteers and managers. We are grateful to David Briggs, Sirinath Jamieson, Nina Iszatt, Adeleh Shirangi and Anna Ma at Imperial College for assisting with fieldwork.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2013.05.004.

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