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Associations of blood trihalomethanes with semen quality among 1199 healthy Chinese men screened as potential sperm donors

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ABSTRACT

Background: Trihalomethanes (THMs) have demonstrated adverse effects on male reproductive systems in experimental animals, but human evidence has been inconsistent. Prior researches have been limited by small sample sizes and inadequate exposure assessment.

Objectives: To investigate the association between blood THMs and repeated measurements of semen quality parameters among 1199 healthy men screened as potential sperm donors.

Methods: We recruited healthy men presenting to the Hubei Province Human Sperm Bank from April to December 2017. At study entry, each participant provided a spot blood sample which was used to quantify blood concentrations of four THMs: chloroform (TCM), bromodichloromethane (BDCM), dibromochloromethane (DBCM) and bromoform (TBM). The summary measures of exposure for brominated THMs (Br-THMs; molar sum of BDCM, DBCM and TBM) and total THMs (TTHMs; molar sum of TCM and Br-THMs) were also calculated. We used multivariable linear regression models to estimate the cross-sectional associations of tertiles of blood THM concentrations with semen quality parameters measured at study entry, and mixed-effect models to estimate the longitudinal associations accounting for repeated measures of semen quality, adjusting for relevant confounding factors.

Results: In the cross-sectional analysis, several inverse dose-response relationships were observed across tertiles of blood TCM concentrations and sperm count, total motility and progressive motility, and between blood DBCM, and Br-THMs, and TTHMs and sperm count and concentration. The inverse associations of blood TCM, DBCM, Br-THMs and TTHMs with sperm count were confirmed in the longitudinal, repeated measure analysis.

Conclusion: Our results suggest that exposure to THMs from drinking water may be related to decreased semen quality in young healthy men.

1. Introduction

Public water systems rely on disinfection to eliminate disease-causing microbes. While such treatment makes water safe for human consumption, these chemical processes result in the formation of disinfection by-products (DBPs) typically as a result of chlorine or chloride-based reactions with raw water matter. Among 700 identified DBPs, trihalomethanes (THMs), which include chloroform (TCM),

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bromoform (TBM), bromodichloromethane (BDCM) and dibromochloromethane (DBCM), are the most abundant by-products detected in disinfected water supplies. Exposure to THMs occurs mainly through dermal absorption and inhalation during routine water-use activities (e.g., showering/bathing and swimming), as well as through ingestion of drinking water (Nieuwenhuijsen et al., 2009a; Villanueva et al., 2011). The U.S. Environmental Protection Agency (USEPA) has regulated total THMs (TTHMs)—the sum of TCM, TBM, BDCM and DBCM—in public drinking water to 0.08 mg/L (USEPA, 2010), because of their potential cancer and developmental and reproductive health effects (Grellier et al., 2015; Nieuwenhuijsen et al., 2009b; Nieuwenhuijsen et al., 2009c; Richardson et al., 2007; Villanueva et al., 2015).

A growing body of evidence suggests that human semen quality is in decline (Auger et al., 1995; Huang et al., 2017; Levine et al., 2017). This decline is reported to have occurred over a relatively short period of time – a few decades –indicating important roles of environmental pollutants (Jurewicz et al., 2009). Toxicological studies have demonstrated that exposure to THMs caused testicular toxicity in male rats, manifested as delayed spermiation and distorted sperm motility and morphology (Klinefelter et al., 1995; Land et al., 1981; Narotsky et al., 2015), histopathologic changes in testis and epididymis (Linder et al., 1997), and decreased serum testosterone (Potter et al., 1996). To date, limited human studies have examined the associations between THM exposure and semen quality parameters, with largely inconsistent results. Fenster et al. (2003) reported an inverse association between estimated THM via tap water consumption and percent normal sperm among 157 healthy men. In a case study, Chang et al. (2001) observed a reduction of sperm motility in a laboratory worker exposed to high levels of chloroform. Zeng et al. (2014) reported dose-response relationships between TCM, Br-THM (sum of TBM, BDCM and DBCM), and TTHM uptake via ingestion with sperm concentration and total count among 324 Chinese men from an infertility clinic. However, others have reported no significant association between THM exposures and semen quality (Iszatt et al., 2013; Luben et al., 2007). All these studies used concentrations of THMs in tap water or additionally combined data on water-use activities to estimate individual exposure dose, which may have resulted in exposure misclassification due to spatial and temporal THM variability in water systems, as well as the variation of daily water-use activities within individuals (Lee et al., 2013; Savitz, 2012).

Blood THM concentrations, which represent integrative measures of exposure from multiple routes, are sensitive biomarkers of low levels of THM exposure (Blount et al., 2011). Although the elimination half-lives of THMs range from minutes to hours, their concentrations in blood are thought to be relatively stable due to chronic exposure, and thus are increasingly used for evaluating internal exposure status in epidemiological studies (Cao et al., 2016; Min and Min, 2016; Riederer et al., 2014; Yang et al., 2017). In a previous cross-sectional study among 401 Chinese men seeking infertility investigation, we measured blood THM concentrations and reported inverse dose-response relationships between blood TCM and TTHMs and sperm concentrations (Zeng et al., 2013). However, this study was limited by a small sample size and a cross-sectional design using a single semen quality measurement. Moreover, results are likely only generalizable to subfertile men, given the infertility clinic study population (Carrell et al., 2002; Hofmann et al., 2014; Olshan et al., 2007).

In the present study, we measured blood THM concentrations in healthy men presenting to the Hubei Province Human Sperm Bank as potential donors. Our objective was to evaluate the association between blood THM concentrations and semen quality parameters, using both a cross-sectional and longitudinal, repeated measure design.

2. Materials and methods

2.1. Study design and participants

Study participants were healthy men between the ages of 22 and 45 years who self-identified as sperm donors at the Hubei Province Human Sperm Bank. Most participants lived in the same water supply district, which was served by a single large water treatment plant. The raw water source for the water treatment plant is surface water, and the disinfection method is chlorination. Participants were eligible if they fulfilled the following criteria: (a) had a no less than high school degree; (b) aged between 22 and 45 years; (c) no genetic or sexually transmitted diseases, such as HIV, Hepatitis, Syphilis, human T-lympohocyte virus, Gonorrhoea, Chlamydia, karyotype and thalassemia; and (d) without history of occupational exposure. Each volunteer completed a questionnaire (see Supplemental Material), underwent a physical examination, provided a semen sample, and had blood samples drawn at enrollment (in most cases within 1 h). The self-reported questionnaire collected information on demographic characteristics (e.g., age, educational and income levels), occupational exposures (e.g., chemical solvents, dye, disinfectant), medical and reproductive history (e.g., marital status and having ever fathered a pregnancy) and lifestyle factors (e.g., smoking status and alcohol consumption). We also collected detailed information on water-use activities and consumption. Specifically, we queried participants' water consumption sources, daily consumption of tap water, time interval since last showering/bathing, and swimming frequency in the past week. Physical examinations including height, waist circumference, hip circumference and weight were undertaken by trained research staff. Blood biochemistry detection was performed to confirm if the participants had sexually transmitted infections or genetic diseases.

Between April and December 2017, a total of 1341 men were recruited. All potential donors underwent an initial semen quality analysis at the recruitment. Those who met the donation criteria (semen volume $>$ 2 ml, concentration $>$ 60 $\times$ 10^6/ml, progressive motility rate $>$ 60% and normal sperm morphology rate $>$ 15%) were required to provide 8–15 additional semen samples at different time points over an approximately 3-month period; those who did not meet the criteria were asked to provide 2–4 samples for further screening. We excluded 79 men because of the following reasons: azoospermia (n = 1), sexually transmitted infections diagnosed at later examination (n = 21), and being afraid of or refusal of blood draw (n = 57). We further excluded 63 men due to insufficient blood volume for analysis. Thus, the final study sample included 1199 men. This research protocol was approved by the Ethics Committee of the Center for Reproductive Medicine, Tongji Medical College. All participants gave written informed consent before enrollment.

2.2. Blood collection and THM quantification

Blood samples were collected in anticoagulant tubes (potassium-ethylenediaminetetraacetic acid) that were specially treated to eliminate background THM contamination, as previously described in detail (Zeng et al., 2013). After collection, the samples were mildly shaken and immediately kept in a 4 °C refrigerator free of the light. We shipped the blood samples in an ice cooler to our laboratory (within the same day) and stored them in a 4 °C lucifugal refrigerator until THM determinations (within a maximum of 10 days from blood collection) (Bonin et al., 2005). Concentrations of TCM, BDCM, DBCM and TBM in blood were detected using our established headspace solid phase microextraction-gas chromatography (SPME-GC) method, as described previously (Chen et al., 2019). Briefly, a 3-mL whole blood was sealed in a headspace vial that contained a magnetic stirrer. The blood sample was heated (20 °C) and agitated (650 rpm) to facilitate extraction of THMs from the samples onto a SPME fiber (75-μm Carboxen/PDMS, Supelco, Bellefonte, PA). After extraction, we immediately inserted the

SPME fiber into the GC inlet and maintained 3 mins for desorption. The target analytes were separated from each other using a capillary column (Aglient 19091J-433 HP-5; 30.0 m × 250 μm × 0.25 μm) and then detected by an Agilent Technologies 6890 N gas chromatograph equipped with an electron capture detector (ECD) that is highly sensitive to THMs. We quantified each THM congener based on external standard calibration curves. Blank water samples were analyzed each day to assess possible laboratory contamination. To maintain extraction efficiency, each SPME fiber extracted < 200 samples. We validated the accuracy of methods by calculating the recovery of blood spiked with 1 and 5 ng/L of each compound. The spiked recoveries of THMs ranged from 80% to 120%; their coefficients of variation (within-day and between-day variation) were all < 10%. The limits of detection (LODs) of TCM, BDCM, DBCM and TBM were 1.9, 0.5, 0.7 and 2.0 ng/L, respectively. Values lower than the LODs were replaced by LOD/√2 before statistical analyses.

2.3. Semen collection and analysis

After an abstinence period of 2–7 days, semen samples were collected by masturbation into a sterile container at the sperm bank. Semen samples were stored at 37 °C for liquefaction and then analyzed by professional technicians at the Spermatology Laboratory of Hubei Province Human Sperm Bank, according to the World Health Organization (WHO) laboratory manual (WHO, 2010). Semen volume was measured by weighing using an electronic balance (HUAZHI Scientific Instrument Co., Ltd). Sperm concentration was determined using a counting chamber (Sefi Medical Instrument. Ltd). We calculated sperm count per ejaculate for each sample by multiplying semen volume by concentration. Sperm mobility parameters, including progressive motility (PR), non-progressive motility (NR) and total motility (PR + NR), were examined using a 10-μm deep slide by a system microscope (BX53, OLYMPUS). Sperm morphology was determined by the same high-power microscope on fixed and Papanicolaou stained smears (BX53, OLYMPUS). We determined sperm morphology only once for most of the participants because the measurement process was time-consuming. All technicians were professionally trained and certified. Quality control samples were measured every day to confirm if within-day and between-day variations were < 10%.

2.4. Statistical analysis

We characterized participants' demographics, lifestyle factors, routinely water-use activities, as well as their blood THM and semen-quality parameter distributions. The Kruskal–Wallis test was used to examine overall differences in the geometric mean of blood trihalomethane concentrations among the subgroups of participants stratified by their numbers of semen specimens (1, 2, 3, 4, and ≥ 5). The correlations between blood concentrations of THMs were assessed using Pearson correlations coefficients. We used multivariable linear regressions to examine the associations between concentrations of blood THMs and continuous measurements of semen quality parameters tested at baseline. To improve normality of data, we transformed sperm count, concentration, progressive motility, and total motility by $\log_{10}$-transformation and examined their distributions using Kolmogorov-Smimov test. The distribution of sperm morphology was close to normal and was thus analyzed using the untransformed value. We examined individual THMs concentrations as well as summary measures of exposure for brominated THMs (Br-THMs; molar sum of BDCM, DBCM and TBM) and total THMs (TTHMs; molar sum of TCM and Br-THMs). Blood concentrations of TCM, BDCM, Br-THMs and TTHMs were divided into tertiles. The detection rates of DBCM and TBM were 59.7% and 41.8%, respectively; we thus constructed a three-level categorical variable by classifying detected values below the LOD as the lowest exposure group, and then dividing the detectable values equally (at the median) as the middle- and high-exposure groups to represent a total of three exposure levels. Therefore, the cut-off percentages for the three levels were 40.3% (< LOD) and 70.1% for DBCM, and 58.2% (< LOD) and 79.1% for TBM. We further used mixed-effects models to assess the associations between blood THM concentrations tested at baseline and repeated measurements of semen quality parameters tested during a 3-month period. We treated THM exposure and covariates as fixed effects while identified random effect for the intercept to adjust for intra-individual correlation of semen sample measurements at multiple time points. Tests for trend were conducted by modeling blood THM tertiles (or categories for DBCM and TBM) as an ordinal level variable in multivariable regression models.

Potential confounders were retained in the multivariable models based on biological/causal models and statistical considerations. Covariates with a p-value < 0.2 in their relationship with at least one outcome or exposure measure in bivariate analyses were included in a "full" multivariable model; covariates with a *p*-value > 0.15 in full models for all tested semen quality parameters were further removed from the "final" multivariable models (Meeker et al., 2011). Finally, BMI (kg/m^2), age (year), abstinence time (days), and time interval since last showering/bathing (hour) were included as continuous variables; education (high school, bachelor degree, or above bachelor degree), smoking status (never, former, or current), alcohol use (never, former, or current), incomes (≤4000, 4001–8000, or ≥ 8001 Yuan per month) and seasons [spring (March–May), summer (June–August), fall (September–November), or winter (December–February)] were included as categorical variables in all final multivariable models. The correlation between selected covariates and outcomes and exposures based on bivariate analyses were shown in the Supplemental Material (Table S5 and S6).

Several sensitivity analyses were conducted. First, given that adjusting for strong predictors of an exposure may lead to bias amplification (Nuckols et al., 2005), we excluded the time interval since last showering/bathing (hours) in the final multivariable models. Second, we re-analyzed the mixed-effect models by excluding the semen samples that were collected at baseline to assess if the association between THMs and semen quality weakened due to longer interval between the determination of exposures and outcomes. Third, we calculated the mean levels of semen quality parameters collected from each participant, and assessed the associations between blood THM concentrations and average semen-quality parameters. Fourth, we excluded men who had extremely high THM values (above 99% percentile of the whole population) in the multivariable models. Fifth, for dependent variables that did not meet the criterion of normal distribution in the multivariable models, we re-analyzed the data by using generalized linear regression models or generalized linear mixed models where appropriate, which allow a non-normal distribution of dependent variables. Analyses were performed using SPSS 23.0 (SPSS Statistics for Windows, IBM, Armonk, NY, USA) or SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

3.1. Characteristics of the study population

The characteristics of the study population are summarized in Table 1. In total, 1199 men provided 5213 semen samples during the study period. Most of the participants (75.1%) provided repeated semen samples (mean: 4.4 ± 3.8 samples per subject). The mean (SD) age of the participants was 28.0 (5.2) years and the mean (SD) BMI was 22.8 (3.2) kg/m^2. Of these 1199 participants, 780 men (65.1%) had less than bachelor degree, 826 (69%) had an income of < 8000 Yuan/month, and 472 (39.4%) were self-reported current smokers. The mean time interval since last showering/bathing was 18.7 (± 16.8) hours. Most participants (85.2%) used tap water, but only half (52.1%) drank it every day; few participants (2.8%) swam in a public pool in the past week.

Table 1
Characteristics of 1199 study participants who contributed 5213 semen samples during the study period.

Participants characteristics	Total Study Sample: N = 1199 [a] n(%) or mean±SD
Number of semen samples per subject	
1	299 (24.9%)
2	217 (18.1%)
3	176 (14.7%)
4	104 (8.7%)
≥ 5	403 (33.6%)
Age (years)	28.0 ± 5.2
BMI (kg/m^2)	22.8 ± 3.2
Marital status	
Unmarried	790 (65.9%)
Married	374 (31.2%)
Divorce/separation	35 (2.9%)
Having ever fathered a pregnancy	
Yes	331 (27.7%)
No	864 (72.3%)
Education (%)	
High school	781 (65.1%)
Bachelor degree	343 (28.6%)
Above bachelor degree	75 (6.3%)
Income (Yuan/month) (%)	
≤ 4000	374 (31.2%)
4001 – 8000	452 (37.8%)
≥ 8001	371 (31.0%)
Smoking status (%)	
Never	647 (54.0%)
Former	80 (6.7%)
Current	472 (39.3%)
Alcohol use	
Never	1024 (85.4%)
Former	11 (0.9%)
Current	164 (13.7%)
Season of semen collection	
Spring (March-May)	730 (14.0%)
Summer (June-August)	1995(38.3%)
Autumn (September-November)	1698 (32.5%)
Winter (December-February)	790 (15.2%)
Abstinence time (day)	
≤ 2	130 (2.5%)
3 – 7	4485 (86.0%)
≥ 8	598 (11.5%)
Water Exposure Source Characteristics	
Time interval since last shower/bath (hours)	18.7 ± 16.8
Source of domestic water	
Tap water	1021 (85.2%)
Others	178 (14.8%)
Source of drinking water	
Tap water	625 (52.1%)
Others	574 (47.9%)
Tap water consumption (ml/day)	
0	574 (47.9%)
< 1000	171 (14.3%)
≥ 1000	454 (37.8%)
Swimming during the last week	
Yes	33 (2.8%)
No	1160 (97.2%)
Duration of showering (minutes/week)	
< 60	316 (26.4%)
≥ 60	877 (73.1%)

[a] Missing data on characteristics: 1 man had missing information on BMI; 4 men on having ever fathered a pregnancy; 6 men on swimming during the last week; 2 men on monthly income; 6 men on duration of showering.

3.2. Distribution of semen quality parameters and blood THM concentrations

The distribution of semen quality parameters is shown in the Table 2. The distributions of most semen quality parameters (i.e., volume, total sperm motility, progressive motility and non-progressive motility) detected at baseline were similar to that tested throughout the study period, except for sperm concentration and count that were lower at baseline. The distribution of blood THM concentrations is presented in the Table 3. TCM and BDCM were detected in 98.3% and 86.5% of the blood specimens, respectively; DBCM and TBM were both detected in < 60% of the samples. Geometric means (95% CIs) of blood THMs concentrations (ng/L) stratified by number of semen samples collected are shown in the Table S1, and no significant differences were found. Most of the compounds were positively correlated with each other (Table S2).

3.3. Cross-sectional associations between blood THMs and semen quality parameters tested at baseline

The cross-sectional associations between blood THM concentrations and semen quality parameters tested at baseline are shown in the Table 4. After adjusting for relevant confounders, inverse dose-response relationships were observed across blood TCM tertiles and sperm count, total motility and progressive motility (p for trends = 0.004, 0.03 and 0.03, respectively), across blood Br-THMs tertiles and sperm count (p for trends = 0.01), and across blood TTHMs tertiles and sperm count and concentration (p for trends = 0.004 and 0.03, respectively). We also found suggestive inverse associations between blood DBCM categories and sperm count and concentration, and across blood Br-THMs tertiles in relation to sperm concentration (all p for trends < 0.10).

3.4. Associations between blood THMs and repeated measures of semen quality parameters

Consistent with the trends observed in the cross-sectional analysis, elevated blood TCM, DBCM, Br-THM and TTHM concentrations were associated with lower sperm count, although we noted some attenuation over time in this repeated-measures analysis (p for trend = 0.004, 0.04, 0.09 and 0.06, respectively; Table 5). Compared with men in the lowest tertiles, those in the highest tertiles of TCM, DBCM, Br-THM and TTHM had lower sperm count of 15.3% (95% CI: −24.3, −5.2%), 10.3% (95% CI: −19.7, 0.5%), 9.2% (95% CI: −18.9, 1.6%) and 10.1% (95% CI: −19.7, 0.7%), respectively. Although there was no evidence of a trend across tertile of blood TTHMs with sperm concentration (p for trend = 0.17), men in the middle tertile of TTHMs had lower sperm concentrations [8.6% (95% CI: −16.3, −0.2%)], compared with those in the lowest exposure tertile.

3.5. Sensitivity analysis

Our results in the multivariable models were robust based on generalized linear or mixed models, and were consistent whether we excluded the time interval since last showering/bathing or those who had extremely high THMs (above 99% percentile of the whole population) (data and quantitative results not shown). We re-analyzed the above-mentioned mixed-effects regression models, excluding semen samples that were collected at baseline, and only confirmed the inverse dose-response relationship between blood TCM and sperm count observed in the whole samples (see Supplemental Material, Table S3). We also assessed the associations of blood THM concentrations and the within-subject average semen-quality parameters; the inverse dose-response relationships of TCM and Br-THMs with sperm count remained robust (see Supplemental Material, Table S4).

Table 2
The distribution of semen quality parameters for 1199 men who contributed 5213 semen samples during the study follow-up period.

Sperm quality parameters	Measured at baseline				Measured during the study follow-up period			
	Percentile			Mean±SD	Percentile			Mean±SD
	25%	50%	75%		25%	50%	75%	
Volume (mL)	2.0	2.3	3.5	2.7 ± 1.4	2.0	2.6	4.0	3.0 ± 1.4
Concentration (10^6 per mL)	24	44	63	45 ± 25	40	60	68	56 ± 23
total count (10^6 per ejaculate)	47	116	163	123 ± 97	108	156	218	167 ± 94
Progressive motility (PR, %)	53	62	67	59 ± 13	51	60	65	57 ± 12
Non-progressive motility (NR, %)	2	2.0	4.0	3.1 ± 2.5	2.0	3.0	3.0	3.1 ± 2.4
Total motility (PR + NR, %)	57	65	70	62 ± 13	54	64	67	61 ± 12
Normal morphology (%)[a]	8.5	14	18	14 ± 6.7	–	–	–	–

[a]A total of 21 men had missing information on normal morphology; the other 1178 participants contributed 1693 measures.

Table 3
Distribution of trihalomethane concentrations (ng/L) in blood collected from 1199 participants at baseline.

Trihalomethane [c]	% above limit of detection	Minimum	Percentile			Maximum	Mean±SD (ng/L)	Geometric mean (ng/L)
			25%	50%	75%			
TCM	98.3%	< LOD	10.4	15.5	21.6	2286.4	21.0 ± 72.7	14.6
BDCM	86.5%	< LOD	0.6	0.9	1.2	7.8	1.0 ± 0.8	0.9
DBCM	59.7%	< LOD	< LOD	0.8	1.2	12.9	1.0 ± 0.7	0.8
TBM	41.8%	< LOD	< LOD	< LOD	54.2	1112.6	41.8 ± 82.3	6.8
Br-THMs [a]	–	2.3	2.9	4.0	57.3	1123.4	43.8 ± 82.6	11.1
TTHMs [b]	–	3.6	16.4	26.7	80.1	2355.3	64.8 ± 110.6	34.9

Abbreviations: TCM (chloroform); BDCM (bromodichloromethane); DBCM (dibromochloromethane); TBM (bromoform); Br-THMs (bromo-trihalomethanes); TTHMs (total THMs).
[a] Br-THMs is the sum of BDCM, DBCM and TBM.
[b] TTHMs is the sum of Br-THMs and TCM.
[c] The limits of detection (LODs) of TCM, BDCM, DBCM and TBM were 1.9, 0.5, 0.7 and 2.0 ng/L respectively.

4. Discussion

In this cohort of 1199 Chinese men screened as potential sperm donors, blood THMs were positively correlated with each other, which was expected given the simultaneous generation of by-products during water treatment processing. The geometric mean concentrations of blood TCM (14.6 ng/L), BDCM (0.9 ng/L), and DBCM (0.8 ng/L) in the present cohort were similar to that of United States (U.S.) adults from the National Health and Nutrition Examination Survey (NHANES) 1999–2006 (12.9, 1.5, and 0.6 ng/L, respectively) (Riederer et al., 2014). However, our geometric mean concentrations of TBM were much higher than that reported among U.S. adults (6.8 vs. 0.8 ng/L). This may be explained by geographical differences and variations in water-use activities and exposure metabolism across populations. In the cross-sectional analysis, we found inverse dose-response relationships between blood TCM concentrations and sperm count, motility, and progressive motility, and between blood DBCM, Br-THMs and TTHMs and sperm count and concentration. In the longitudinal analysis, the inverse associations between blood TCM, DBCM, Br-THMs and TTHMs with sperm count remained, albeit were attenuated in magnitude.

Only the inverse associations of blood TCM, DBCM, Br-THMs and TTHMs with sperm count were consistently observed in both our cross-sectional and longitudinal analyses. This is not surprising given that men who provided repeated semen specimens were more likely to be selected as eligible sperm donors. Thus, this selection process resulted in a sample of men with higher sperm count and concentration contributing to the longitudinal follow-up data. To assess the potential selection bias resulting from this changing population composition over time, we evaluated the associations of blood THM concentrations with the within-subject average semen-quality parameters. In this sensitivity analysis, we found that the associations with TCM and Br-THM remained significant. There was also no overall difference in the geometric mean of THM concentrations among the subgroups of participants who provided different numbers of semen specimens, suggesting that semen quality selection factors did not differ by exposure levels. The inconsistent results in our cross-sectional and longitudinal analyses for sperm concentration, count, and motility may also be explained by exposure measurement errors. We measured blood THMs at a single time point at baseline. Although blood THM concentrations are thought to be relatively stable due to high frequency of exposure (e.g., daily) (Blount et al., 2011), they are also likely to vary across months as a result of changes in diet, lifestyle factors, and external exposure magnitude. In additional sensitivity analyses, we re-analyzed the mixed-effects models, excluding the semen samples that were collected at baseline and only confirmed the inverse dose-response relationship between blood TCM and sperm count observed in the whole samples. Meanwhile, it should be noted that our cross-sectional analysis only used a single measurement of semen quality, which may also result in measurement error due to the high within-subject variations in sperm parameters (Carrell et al., 2002; Hofmann et al., 2014).

Previous epidemiological studies assessing the associations between THM exposure and semen quality have mostly relied on regulatory monitoring data of water systems to assign individual exposure dose, and the results were largely inconsistent. In support of our findings, Fenster et al. (2003) reported an inverse association between BDCM ingestion and linearity of sperm motion among 157 healthy men from couples without known risk factors for infertility; in a case study, Chang et al. (2001) observed a reduction of sperm motility in a male worker exposed to high levels of chloroform; in a cross-sectional study among 324 Chinese men from an infertility clinic, Zeng et al. (2014) revealed dose-response relationships of TCM, Br-THM and TTHM uptakes via ingestion with sperm concentration and count. However, some studies failed to observe significant association between THM exposure and semen quality parameters (Iszatt et al., 2013; Luben et al., 2007). None of these studies directly measured the internal dose of THMs and findings may be biased as a result of exposure misclassification because

Table 4
The percent change [%Δ (95% CI)] of semen quality parameters measured at baseline (n = 1196) in relation to blood trihalomethane concentrations [a].

Trihalomethane (ng/L)	%Δ (95% CI)[b]				
	Total count	Concentration	Total motility	Progressive motility	Normal morphology
TCM[d]					
T1 (< 12.3)	0	0	0	0	0
T2 (12.3–19.0)	−17.4 (−28.9, −4.1)	−9.2 (−18.7, 1.4)	−0.7 (−4.9, 4.0)	−0.5 (−5.2, 4.2)	−0.06 (−1.0, 0.9)
T3 (> 19.0)	−20.2 (−31.6, −6.9)	−7.3 (−17.2, 3.5)	−4.9 (−9.2, −0.5)	−5.2 (−9.6, −0.5)	0.2 (−0.8, 1.1)
P for trend[c]	0.004	0.18	0.03	0.03	0.76
BDCM[d]					
T1 (< 0.71)	0	0	0	0	0
T2 (0.71–1.0)	3.8 (−10.7, 20.5)	7.2 (−3.8, 19.4)	−0.5 (−4.7, 4.2)	−0.7 (-5.2, 4.2)	−0.05 (−1.0, 0.9)
T3 (> 1.0)	2.6 (−12.3, 20.2)	7.9 (−3.6, 21.1)	−1.4 (−5.8, 3.3)	−2.1 (−6.7, 3.0)	0.07 (−0.9, 1.1)
P for trend[c]	0.74	0.18	0.57	0.43	0.89
DBCM[d]					
< LOD (< 0.7)	0	0	0	0	0
LOD-70TH (0.7–1.1)	−18.5 (−29.7, −5.6)	−6.5 (−16.1, 4.2)	0.2 (−4.1, 4.7)	0.2 (−4.3, 5.0)	−0.4 (−1.3, 0.6)
> 70TH (> 1.1)	−11.5 (−23.6, 2.6)	−9.4 (−18.7, 0.9)	0.7 (−3.6, 5.2)	0.7 (−4.1, 5.4)	−0.08 (−1.0, 0.9)
P for trend[c]	0.07	0.07	0.76	0.80	0.83
TBM[d]					
< LOD (< 2.0)	0	0	0	0	0
LOD-79TH (2.0–70.9)	−16.4 (−28.6, −2.1)	−9.8 (-19.7, 1.2)	−3.0 (−7.5, 1.6)	−3.0 (−7.5, 2.1)	−0.4 (−1.4, 0.6)
> 79TH (> 70.9)	−3.8 (−18.0, 12.5)	−4.1 (−14.5, 7.7)	−0.2 (−4.9, 4.5)	0.5 (−4.3, 5.4)	−0.3 (−1.3, 0.7)
P for trend[c]	0.34	0.30	0.68	0.93	0.46
Br-THMs[d]					
T1 (< 3.1)	0	0	0	0	0
T2 (3.1–29.2)	−12.1 (−24.8, 2.8)	−10.3 (−19.8, 0.7)	−3.6 (−8.2, 0.9)	−4.1 (-8.6, 0.7)	−0.6 (−1.6, 0.4)
T3 (> 29.2)	−17.96 (−29.9, −4.1)	−10.5 (−20.2, 0.2)	−1.1 (−5.6, 3.5)	−0.7 (−5.6, 4.2)	−0.5 (−1.4, 0.5)
P for trend[c]	0.01	0.06	0.71	0.81	0.39
TTHMs[d]					
T1 (< 19.2)	0	0	0	0	0
T2 (19.2–51.9)	−20.4 (−31.9, −6.7)	−12.9 (−22.4, −2.3)	−2.1 (−6.5, 2.8)	−1.8 (−6.5, 3.3)	0.4 (−0.6, 1.4)
T3 (> 51.9)	−21.3 (−32.9, −8.0)	−12.5 (−22.0, −2.1)	−2.1 (−6.5, 2.8)	−1.4 (−6.0, 3.8)	0.1 (−0.9, 1.1)
P for trend[c]	0.004	0.03	0.43	0.62	0.87

Abbreviations: LOD (limits of detection); TCM (chloroform); BDCM (bromodichloromethane); DBCM (dibromochloromethane); TBM (bromoform); Br-THMs (bromotrihalomethanes); TTHMs (total THMs).

[a] The number of semen samples was reduced from 1199 to 1196 by excluding 3 men who had missing information on BMI or monthly income. Models were adjusted for BMI, age, education, monthly income, time interval since last showering/bathing, smoking status, alcohol use, season, and abstinence time.

[b] Log_{10}-transformed values that were back-transformed $\{100\times[10^{beta}-1]\}$ to obtain percent change.

[c] P values for trend were estimated by modeling blood trihalomethane tertiles as an ordinal level variable in multivariable regression models.

[d] Blood concentrations of TCM, BDCM, Br-THMs and TTHMs were divided into tertiles (33.33%, 66.66%). DBCM (< LOD, 70%) and TBM (< LOD, 79%) were constructed as a three-level categorical variable by classifying detected values below the LOD as the lowest exposure group, and dividing the detectable values equally (at the median) as the middle- and high-exposure groups.

blood THMs may be a better and relatively consistent surrogate compared to external measurement (Savitz, 2012). In our previous research, we used blood THM concentrations as internal exposure biomarkers and found suggestive dose–response relationships between elevated blood concentrations of TCM and TTHMs and lower sperm concentrations among 401 men seeking semen examination from an infertility clinic (Zeng et al., 2013), which is in support of our current findings from the cross-sectional and longitudinal analysis in healthy men. In our present study, we also found inverse associations between blood DBCM and Br-THMs and sperm count. The discrepancy between studies may be due to the differences in sample size (1199 vs. 401 men), and, perhaps more importantly, the composition of study population (healthy men vs. men from an infertility clinic).

Animal studies have shown that THMs can cause testicular toxicity, manifested as delayed spermiation and impaired semen quality (Klinefelter et al., 1995; Land et al., 1981; Narotsky et al., 2015), probably by inducing oxidative stress (Gemma et al., 2003; Tomasi et al., 1985) and inhibiting testosterone secretion (Nickmilder and Bernard, 2011; Potter et al., 1996). Toxicological studies showed that DBPs, including THMs, induced oxidative stress (Wright et al., 2014), which damaged sperm DNA integrity and disrupted lipids and proteins in the sperm plasma membrane (Aitken et al., 2012). Epidemiological studies also revealed higher levels of oxidative stress markers in the blood and exhaled breath condensate of swimmers exposed to chlorinated water (Morissette et al., 2016; RaphaëLle et al., 2002), though they did not adjust for diet, smoking and other predictors that may have contributed to unmeasured confounding. Meanwhile, elevated oxidative stress biomarkers have been associated with decreased semen quality both in animals and humans (Aitken et al., 2014). THMs reduced serum testosterone in rats orally treated with 1.5 mmol/kg over a 7-day period (Potter et al., 1996), and elevated blood DBCM has been associated with decreased serum total testosterone (Zeng et al., 2013).

The strengths of this study include its relatively large sample size, longitudinal study design and repeated measurements of semen quality parameters at different time points over an approximately 3-month period; and more importantly, the use of internal exposure biomarkers (i.e., blood THMs). Furthermore, our study participants were healthy men screened as potential sperm donors, who were more likely to be representative of a general population of men compared to previous studies recruiting from infertility clinics.

However, there were several notable limitations of our study. First, a single blood sample at baseline may result in exposure misclassification, which was likely to be random and thus could obscure some modest associations (Wang et al., 2019). To avoid the effects of any major routine water-use activities on baseline blood THMs, we collected blood samples from study participants in the morning (Weisel

Table 5
The percent change [%Δ (95% CI)] of repeated semen quality parameters measured during the study follow-up period (n = 5213) in relation to blood trihalomethane concentrations [a].

Trihalomethane (ng/L)	%Δ (95% CI)[b]			
	Total count	Concentration	Total motility	Progressive motility
TCM[d]				
T1 (< 12.3)	0	0	0	0
T2 (12.3–19.0)	−15.1 (−24.0, −4.9)	−8.0 (−15.7, 0.5)	1.4 (−1.6, 4.2)	1.4 (−1.6, 4.7)
T3 (> 19.0)	−15.3 (−24.3, −5.2)	−6.0 (−13.9, 2.6)	−0.9 (-3.8, 1.9)	−1.1 (−4.3, 2.1)
P for trend[c]	0.004	0.17	0.51	0.49
BDCM[d]				
T1 (< 0.71)	0	0	0	0
T2 (0.71–1.0)	−1.1 (−11.5, 10.7)	3.5 (−5.2, 12.7)	0.69 (−2.3, 3.5)	0.7 (−2.3, 4.0)
T3 (> 1.0)	1.9 (−9.2, 14.0)	6.4 (−2.5, 16.1)	1.6 (−1.4, 4.7)	1.9 (−1.1, 5.2)
P for trend[c]	0.78	0.17	0.28	0.24
DBCM[d]				
< LOD (< 0.7)	0	0	0	0
LOD-70TH (0.7–1.1)	−11.3 (−20.8, −0.9)	−2.1 (−10.1, 6.9)	0.5 (−2.3, 3.5)	0.5 (−2.7, 3.8)
> 70TH (> 1.1)	−10.3 (−19.7, 0.5)	−6.9 (−14.5, 1.6)	1.9 (−1.1, 4.7)	1.6 (−1.4, 5.0)
P for trend[c]	0.04	0.11	0.24	0.30
TBM[d]				
< LOD (< 2.0)	0	0	0	0
LOD-79TH (2.0–70.9)	−7.7 (−17.8, 3.8)	−7.5 (−15.7, 1.2)	−1.6 (−4.5, 1.4)	−1.6 (−4.7, 1.6)
> 79TH (> 70.9)	−2.7 (−13.5, 9.4)	−1.8 (−10.5, 7.7)	2.3 (−0.7, 5.4)	3.3 (−0.2, 6.7)
P for trend[c]	0.46	0.43	0.26	0.14
Br-THMs[d]				
T1 (< 3.1)	0	0	0	0
T2 (3.1–29.2)	−5.6 (−15.7, 5.4)	−6.2 (−14.1, 2.1)	−0.69 (−3.6, 2.1)	−0.9 (−3.8, 2.3)
T3 (> 29.2)	−9.2 (−18.9, 1.6)	−6.0 (−14.1, 2.6)	1.9 (−1.1, 5.0)	2.3 (−0.9, 5.7)
P for trend[c]	0.09	0.16	0.23	0.16
TTHMs[d]				
T1 (< 19.2)	0	0	0	0
T2 (19.2–51.9)	−13.1 (−22.4, −2.7)	−8.6 (−16.3, −0.2)	−0.9 (−3.8, 1.9)	−0.5 (−3.6, 2.6)
T3 (> 51.9)	−10.1 (−19.7, 0.7)	−5.8 (−13.7, 2.8)	1.6 (−1.4, 4.7)	2.3 (−0.9, 5.7)
P for trend[c]	0.06	0.17	0.29	0.15

Abbreviations: LOD (limits of detection); TCM (chloroform); BDCM (bromodichloromethane); DBCM (dibromochloromethane); TBM (bromoform); Br-THMs (bromotrihalomethanes); TTHMs (total THMs).

[a] Estimates were from adjusted linear mixed-effect models with random intercepts for subject identification; models were adjusted for BMI, age, education, monthly income, time since last showering/bathing, smoking status, alcohol use, season, and abstinence time.

[b] Log_{10}-transformed values that were back-transformed {100×[10^{beta}-1]} to obtain percent change.

[c] P-values were estimated by modeling blood trihalomethane tertiles as an ordinal level variable in multivariable regression models.

[d] Blood concentrations of TCM, BDCM, Br-THMs and TTHMs were divided into tertiles (33.33%, 66.66%). DBCM (< LOD, 70%) and TBM (< LOD, 79%) were constructed as a three-level categorical variable by classifying detected values below the LOD as the lowest exposure group, and dividing the detectable values equally (at the median) as the middle- and high-exposure groups.

and Jo, 1996; Zeng et al., 2013). Meanwhile, we included the time interval since last showering/bathing as a covariate in regression models, given that bathing/showering is the most influential determinants of blood THMs (Leavens et al., 2007; Nuckols et al., 2005). Second, co-pollutants, such as haloacetonitriles (HANs) and halogenated acetic acids (HAAs) that may also impair male reproductive health, were not accounted for and may be a source of unmeasured confounding (Liu et al., 2018; Zhang et al., 2017). Third, we did not examine the interactions between gene polymorphisms and THM exposure on semen quality (Infante-Rivard, 2004). For instance, the frequency of people with rs915906 TT of *CYP2E1* gene ranged from 0.58 to 0.60 in the Chinese population according to the Ensembl project (available at http://www.ensemblgenomes.org). A recent study by our group has shown that men with rs915906 TT genotype exhibited higher blood TCM and TTHM concentrations than the men with the CT/CC genotype (Yang et al., 2016). Fourth, the detection rates of individual brominated THMs were relatively low and thus our findings for these compounds may have been biased as a result of the imputation of values below the LODs (Windham et al., 2015). Therefore, additional researches are still needed to confirm our findings, explore potential underlying mechanisms (e.g., oxidative stress and genotoxicity), as well as evaluate the joint effect of THMs with other co-occurring pollutants (e.g., HAAs). Moreover, genetic analysis would be helpful to identify susceptible populations, such as those with rs915906 TT genotype of *CYP2E1* gene.

5. Conclusion

In our study population of healthy men who were exposed to TTHMs that were at or below the Chinese regulatory, we noted inverse cross-sectional associations between blood TCM concentrations and sperm count, motility and progressive motility, and between blood DBCM, Br-THMs and TTHMs and sperm count and concentration. Using a longitudinal design with repeated semen quality parameters tested at multiple time points over an appropriate 3-month period, the inverse associations between blood TCM, DBCM, Br-THMs and TTHMs and sperm count were further confirmed. Our results suggest that both individual and summary measures of exposure for Br-THMs and THMs were associated with decreased semen quality, strengthening the previous evidence that exposure to THM may impair male reproductive health.

Declaration of Competing Interest

None.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105335.

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