Chlorination By-Products in Drinking Water and Menstrual Cycle Function

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We analyzed data from a prospective study of menstrual cycle function and early pregnancy loss to explore further the effects of trihalomethanes (THM) on reproductive end points. Premenopausal women (*n* = 403) collected urine samples daily during an average of 5.6 cycles for measurement of steroid metabolites that were used to define menstrual parameters such as cycle and phase length. Women were asked about consumption of various types of water as well as other habits and demographics. A THM level was estimated for each cycle based on residence and quarterly measurements made by water utilities during a 90-day period beginning 60 days before the cycle start date. We found a monotonic decrease in mean cycle length with increasing total THM (TTHM) level; at > 60 µg/L, the adjusted decrement was 1.1 days [95% confidence interval (CI), –1.8 to –0.40], compared with ≤ 40 µg/L. This finding was also reflected as a reduced follicular phase length (difference –0.94 day; 95% CI, –1.6 to –0.24). A decrement in cycle and follicular phase length of 0.18 days (95% CI, –0.29 to –0.07) per 10 µg/L unit increase in TTHM concentration was found. There was little association with luteal phase length, menses length, or cycle variability. Examining the individual THMs by quartile, we found the greatest association with chlorodibromomethane or the sum of the brominated compounds. Incorporating tap water consumption showed a similar pattern of reduced cycle length with increasing TTHM exposure. These findings suggest that THM exposure may affect ovarian function and should be confirmed in other studies.
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In previous studies of tap water consumption conducted in California by the Department of Health Services, we reported a 10–50% increased risk of spontaneous abortion with tap versus bottled water consumption (Neutra et al. 1992; Swan et al. 1992; Windham et al. 1992). To investigate these findings further, we initiated several studies that included a large prospective study of pregnancy outcome in three regions of California. Because of increased concern about the health effects of chlorination by-products, in the follow-up we examined pregnancy outcome by trihalomethane (THM) levels, the most prevalent class of such by-products measured quarterly by the water utility companies. In that study, women with high consumption of tap water (≥ 5 glasses/day) containing high levels of total trihalomethanes (TTHM) (≥ 75 µg/L) had an increased odds of spontaneous abortion on the order of 80% (Waller et al. 1998a, 2001). Some other studies have examined risks of spontaneous abortion, stillbirth, birth defects, or fetal growth retardation in relation to water chlorination by-products, with varying results. Although the epidemiological studies are limited, together they suggest a slightly increased risk of adverse reproductive and pregnancy outcomes (Bove et al. 1995; Dodds and King 2001; Dodds et al. 1999; Gallagher et al. 1998; King et al. 2000; Klotz and Pyrch 1999; Kramer et al. 1992; Savitz et al. 1995) that should be examined further (Nieuwenhuijsen et al. 2000).

One recommendation has been to study female reproductive function, including fecundity and menstruation (Reif et al. 1996). The menstrual cycle appears to influence important aspects of women's physiologic function, yet studies of determinants of menstrual function, including environmental risk factors, are lacking (Harlow and Ephross 1995). Therefore, to determine whether THMs are associated with other reproductive end points and to identify possible mechanisms, we examined data from our second follow-up study. This Women's Reproductive Health Study was a prospective study designed to examine a number of exposures in relation to early fetal loss, time to pregnancy, and menstrual function among reproductive-age women. These included tap water consumption, smoking, and solvents. This report is the first, to our knowledge, to examine menstrual cycle function in relation to THM levels and tap water consumption; another report will examine time to pregnancy in these data.

Materials and Methods

The data collection and analytic methods for the Women's Reproductive Health Study is described in detail elsewhere (Waller et al. 1998b; Windham et al. 2002) and summarized below. The study protocol was approved by the institutional review boards of both Kaiser Permanente and the California Department of Health Services, and the participants provided written consent.

Married women of reproductive age (18–39 years old) who were members of the Kaiser Permanente Medical Care Program in Northern California and attended clinic facilities in the same region as our earliest water studies were the target population. Almost 6,500 were screened by a short telephone interview to identify women who were more likely to become pregnant (a menstrual period within past 6 weeks, no surgical sterilization, not currently using birth control pills or intrauterine devices, or noncontracepting less than 3 months) and willing to collect and freeze first morning urine samples daily for 6 months or until they became pregnant. Participants were enlisted between May 1990 and June 1991. Of the 1,092 eligible women identified, 553 agreed to participate, but 89 dropped out during urine collection and 61 became ineligible (e.g., because of moving, early pregnancy, starting birth control pills), leaving 403 women who collected urine during 2–9 menstrual cycles (average 5.6). Before urine collection, participants completed a detailed baseline interview by telephone that asked about their water consumption and numerous potential confounders. Women filled out a daily diary during urine collection

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to record vaginal bleeding (number of pads or tampons/day), among other items.

Definition of menstrual characteristics. Daily urine samples were analyzed for metabolites of estrogen (estrone conjugates) and progesterone (pregnanediol-3-glucuronide) by enzyme-linked immunoassay and adjusted for creatinine as previously reported (Munro et al. 1991; Waller et al. 1998b; Windham et al. 2002). We defined menstrual cycles by bleeding patterns. Determination of ovulatory status was based on observing a sufficient relative rise in progesterone over baseline levels (Kassam et al. 1996; Waller et al. 1998b). The day of ovulation was estimated using a previously validated algorithm that generally selects the day after the peak of the estrogen to progesterone ratio (Baird et al. 1990; Waller et al. 1998b). Steroid assays were repeated in cycles that were nonovulatory or had a late day of ovulation (n = 533); in 46% of these the abnormality was confirmed, whereas in 54% we used the more normal reassay results to be conservative. Steroid levels were also examined graphically, and in a small proportion of cycles (5.6%), we recoded the day of ovulation to better correspond to the steroid patterns. All hormone assessments were conducted blinded to TTHM level.

We calculated cycle length from the first day of menses to the day before the onset of the next menses. The cycle was divided into the follicular phase, from the first day of menses through the estimated day of ovulation, and the subsequent luteal phase, where possible. We examined mean cycle and phase lengths as well as categorizing them based on the 5th and 95th percentiles of their distributions to define short and long cycles, respectively. Thus the lengths of average or normal cycles, follicular phases, and luteal phases (e.g., referent groups) were 25–35 days, 12–23 days, and 11–14 days, respectively. Bleed, or menses, length was the number of consecutive days of reported pad use, with 8 or more days considered long. Urine collection did not necessarily begin or end on menses dates, so not all end points could be calculated for each cycle; 1,624 cycles were available for cycle length analyses, 1,514 for follicular phase, 1,424 for luteal phase, and 1,714 for menses analyses. We also examined continuous measures of cycle length variability. Variability was calculated as both a range, or the difference between a woman's longest and shortest cycle lengths, and as the variance across a woman's cycle lengths, for women with at least two complete cycles (n = 375).

Table 1. Distribution of demographic variables and potential confounders by TTHM level,[a] California Women's Reproductive Health Study, 1990–1992.

Variable	0–40 n = 184 (46%)[b]	> 40–60 n = 71 (18%)	> 60–80 n = 134 (33%)	> 80 n = 12 (3%)	p-Value[c]
	Total THM level (µg/L)				
Education					
No college	41 (46)	11 (12)	34 (38)	3 (3)	
Some college	79 (52)	25 (17)	44 (29)	3 (2)	
College graduate	64 (40)	35 (22)	56 (35)	6 (4)	0.22[d]
Race					
Hispanic	25 (48)	7 (13)	18 (35)	2 (4)	
White	140 (49)	46 (16)	89 (31)	8 (3)	
Other	19 (29)	18 (27)	27 (41)	2 (3)	0.09
Income/year					
≤ \$35,000	41 (68)	9 (15)	7 (12)	3 (5)	
> \$35,000–\$55,000	76 (48)	19 (12)	59 (38)	3 (2)	
> \$55,000–\$75,000	35 (34)	25 (24)	42 (40)	2 (2)	
≥ \$75,000	28 (40)	16 (23)	22 (31)	4 (6)	< 0.001[d]
Employed					
Yes	126 (46)	43 (16)	97 (35)	9 (3)	
No	58 (46)	28 (22)	37 (29)	3 (2)	0.35
Pregnancy history					
0 Pregnancies	25 (52)	7 (15)	14 (29)	2 (4)	
≥ 1 Pregnancies, 0 losses	106 (41)	48 (19)	96 (37)	6 (2)	
≥ 1 Pregnancies, ≥ 1 losses	53 (55)	16 (16)	24 (25)	4 (4)	0.21
BMI (kg/m^2)					
< 19.1	13 (43)	5 (17)	11 (37)	1 (3)	
19.1–27.3	129 (44)	54 (19)	99 (34)	9 (3)	
> 27.3	42 (53)	12 (15)	24 (30)	2 (2)	0.93
Age (years)					
Mean ± SD	31.7 ± 4.1	31.0 ± 4.0	32.4 ± 4.1	32.4 ± 5.2	0.12
Smoking (cigarettes/day)					
Mean ± SD	1.4 ± 5.0	1.3 ± 4.2	0.4 ± 2.9	2.7 ± 6.1	0.12
Alcohol (drinks/week)					
Mean ± SD	1.3 ± 2.5	2.5 ± 5.4	1.3 ± 2.5	2.2 ± 3.4	0.04
Caffeine (mg/day)					
Mean ± SD	137.8 ± 156.8	115.3 ± 127.6	126.1 ± 180.9	233.8 ± 235.8	0.12
Unheated tap water (glasses/day)					
Mean ± SD	2.1 ± 2.7	2.4 ± 2.5	2.5 ± 3.0	2.6 ± 2.5	0.66
Heated water (glasses/day)					
Mean ± SD	1.1 ± 1.9	1.1 ± 1.6	1.0 ± 1.7	1.8 ± 2.1	0.48
Showering (minutes/week)					
Mean ± SD	66.7 ± 40.4	65.7 ± 39.5	65.2 ± 36.2	61.3 ± 25.1	0.97
Exercise score					
Mean ± SD	17.9 ± 23.9	17.2 ± 19.9	17.9 ± 23.7	26.5 ± 36.0	0.65

[a]Each woman's cycle-specific TTHM measurements are averaged, and the average value is used; cycle-specific measures are based on an exposure window: 60 days before the last menstrual period to 30 days after the last menstrual period of each cycle. [b]Numbers may not add up to column totals because of missing values. [c]p-Value is for chi-square test of independence for categorical analyses, and for continuous analyses represents a test of H_0; all TTHM categories have the same mean unless otherwise specified. [d]p-Value for Fisher's exact test.

Exposure assessment. During the baseline interview, women were asked the amount (in 8-ounce glasses) of usual daily consumption of unheated tap water (or drinks made from unheated tap water) at home, drinks made with hot tap water at home, and bottled water. The number of showers taken per week at home and their duration was also ascertained, from which we calculated minutes of showering per week.

We estimated TTHM levels in tap water in a manner similar to our previous prospective study, which was based on an average of all measurements taken by a utility (e.g., utility-wide average) (Waller et al. 1998a, 2001). The subjects' addresses during the study were geocoded and then assigned to one of the 10 appropriate water utility companies in the county. We obtained quarterly monitoring data of THM levels collected at various points (range 4–20, average 9) in the distribution system from each utility. We summed the individual THM compounds to calculate TTHMs and used that data to estimate the TTHM concentration in each woman's home tap water for each of her cycles. Because water utilities collected THM data at roughly 90-day intervals, we assigned a 90-day exposure time period for each cycle. As hormonal events that occur in previous cycles can influence subsequent cycles, we selected the time period of 60 days before to 30 days after each cycle start date. Cycle-specific TTHM levels were thus calculated by averaging all distribution system TTHM measurements taken by the subject's utility company during this 90-day window. If no water samples were taken during the window, the samples closest in time to either end of the window were averaged. If a woman had moved during the time period, the utility measures for each address were calculated, then averaged, weighting by the proportion of time spent at each address. We also averaged a woman's cycle-specific TTHM measures to obtain an estimate of her average exposure during urine collection.

We calculated cycle-specific levels for each of the four individual THM compounds (bromoform, chloroform, chlorodibromomethane, and bromodichloromethane) in a similar way. As the brominated compounds were highly correlated, we also examined the sum of the levels of the three brominated THMs.

Two ingestion metrics were calculated by multiplying the estimated cycle-specific TTHM level by the usual number of glasses of tap water consumed daily at home. We calculated the first using only unheated tap water, and for the second we used the sum of unheated and heated tap water. We converted reported glasses of water to an estimate of 0.25 L per 8-ounce glass to calculate an ingestion dose in units of micrograms per day.

We primarily examined exposure levels as categorical variables. The current U.S. Environmental Protection Agency (U.S. EPA) maximum contaminant level (MCL) for TTHM is > 80 µg/L, so initially we used that as the cutoff for the highest category. However, because of small numbers exposed at that level, we also examined and generally report > 60 µg/L as the high category (approximately the top quartile). Because there are currently no MCLs for the individual THM compounds, we categorized those by quartiles. The highest category of the ingestion metric (> 60 µg/day) represents consuming approximately three glasses of water per day containing > 80 µg/L TTHM (or equivalently, four glasses of water with > 60 µg/L TTHM). We examined showering as both minutes per week and incorporating the cycle-specific TTHM level to create combinations of low and high exposure (low showering as 0–34 min/week and high as 70 min or more; low TTHM as 0–40 µg/L and high as > 60).

Statistical analysis. We conducted analyses with the menstrual cycle as the unit of observation (Harlow and Zeger 1991). Because of expected correlation within a woman's cycles, we fit mixed models that account for repeated measures (Laird and Ware 1982; Zeger and Liang 1986) with the compound symmetry covariance structure (assumes that all repeated units, e.g., cycles, within a woman are equally correlated). We calculated mean length and differences by exposure level, as well as odds ratios (ORs) for the risk of short or long length.

To identify potential confounders, we examined numerous covariates, including demographics, reproductive history, and lifestyle factors, in relation to categorical TTHM levels and the ingestion metric. Smoking was calculated from the daily diary as average cigarettes/day for each cycle (Windham et al. 1999). All other variables were obtained from the baseline interview. Alcohol was calculated from frequency and amount questions into drinks per week; and caffeine was calculated from consumption of coffee, tea, and sodas as milligrams per day (Fenster et al. 1999a). Pregnancy history was categorized as no pregnancy, at least one with no losses, and at least one including one or more losses. From questions on exercise type and frequency, we calculated a measure of energy expenditure, or metabolic equivalent score. Variables associated with an end point or with exposure [i.e., age, race, education, income, employment, pregnancy history, body mass index (BMI), exercise, smoking, caffeine and alcohol consumption] were included in models examining mean cycle length or the odds of a short cycle. Each variable was removed from the model individually to examine the change in estimate (Greenland 1989). There was very little evidence of confounding of the effects of TTHM level on mean cycle length (all changes < 2%). The variable that had the greatest confounding effect on the association with short cycle was income (18% change), with slight confounding (change in estimate of 5–10%) by age, pregnancy history, BMI, and caffeine and alcohol consumption. These six variables, as well as race (because of a strong association with the ingestion metric) and smoking (associated with end point and exposure), were included in all adjusted models for ease of presentation. However, as they may not be confounders for all end points, this could decrease the precision somewhat. We calculated adjusted odds ratios (AORs) and 95% confidence intervals (CIs) for the categorical end points and adjusted differences for mean lengths, by exposure level.

Results

Participants in the study were predominantly white, parous, and educated, with a mean age of 31 years (Table 1). Only 3% of women had average TTHM levels above the MCL of 80 µg/L (or 4% of cycle-specific measures). Categorical TTHM level varied by income and, to a lesser extent, by race (Table 1). Women with higher TTHM levels smoked more and drank more caffeinated and alcoholic beverages, on average. Amount of tap water consumed and time spent showering did not vary much by TTHM level (Table 1). Sixty-three percent of women reported drinking at least some unheated tap water at home, with an average of about 2.3 glasses/day. Women who exercised, were not employed, or drank fewer caffeinated beverages were more likely to report greater unheated tap water consumption at home. Women who reported greater consumption of beverages made from heated tap water were generally older, as well as more likely to have higher consumption of caffeine (as might be expected), alcohol, and cigarettes. Women who spent more time showering tended to be younger and nonwhite.

Cycle characteristics by estimated TTHM level and water consumption. The mean cycle length was 28.8 days (SD 4.4), mean follicular phase length was 16.0 days (SD 4.4), and luteal phase length was 12.9 days (SD 1.7). Examining cycle-specific utility-wide average TTHM concentrations, we found a monotonic decrease in mean cycle length with increasing exposure category, so that the most highly exposed cycles were more than 1 day shorter after adjustment (Table 2). This decrease was reflected as shorter follicular phase length with increasing exposure, but little difference in luteal phase or menses length (Table 2). Unadjusted results were very similar to adjusted, as were the results using the average exposure over all cycles, or examining only ovulatory cycles. Cycles with TTHM levels above the MCL were also shorter by about 1 day (β = –0.99; 95% CI, –2.2 to 0.18), with an intermediate decrement of 0.7 days at the

Table 2. Menstrual cycle parameters by cycle-specific utility-wide TTHM level: mean lengths and adjusted[a] differences (95% CI).

	Estimated TTHM level (µg/L)		
Menstrual parameters	0–40	> 40–60	> 60
Cycle length			
n	716	363	545
Mean[b] ± SE	29.7 ± 0.26	29.3 ± 0.28	28.7 ± 0.28
Adjusted difference (95% CI)	Ref	–0.50 (–1.1 to 0.11)	–1.1 (–1.8 to –0.40)
Follicular phase length			
n	676	337	501
Mean[b] ± SE	16.9 ± 0.27	16.5 ± 0.29	16.0 ± 0.30
Adjusted difference (95% CI)	Ref	–0.39 (–0.98 to 0.20)	–0.94 (–1.6 to –0.24)
Luteal phase length			
n	639	318	467
Mean[b] ± SE	12.9 ± 0.09	12.8 ± 0.11	13.0 ± 0.10
Adjusted difference (95% CI)	Ref	–0.08 (–0.33 to 0.18)	0.07 (–0.20 to 0.35)
Menses length			
n	749	374	591
Mean[b] ± SE	5.3 ± 0.09	5.4 ± 0.09	5.3 ± 0.09
Adjusted difference (95% CI)	Ref	0.09 (–0.12 to 0.30)	–0.11(–0.34 to 0.12)

Ref, referent group.
[a]Adjusted for age, race, BMI, income, pregnancy history, smoking, and alcohol and caffeine consumption. [b]Unadjusted means; *n*s were lower for adjusted models due to missing data.

middle category of > 40–80 µg/L (95% CI, –1.3 to –0.10), compared with the lowest category. Examining the estimated cycle-specific TTHM concentration as a continuous variable, we found a decrement in cycle length of 0.18 days per 10 µg/L increase (95% CI, –0.29 to –0.07). The decrement in follicular phase length was nearly identical, but there was little difference in luteal phase or menses lengths. Variability in the length of a woman's cycles did not appear associated with TTHM level crudely or after adjustment. The range (about 5 days, on average) was very similar across TTHM categories, and the variance was slightly greater in the low TTHM group (data not shown).

By categorical end points, we found some elevated risk of short cycle but little for short follicular phase at the high TTHM level (Table 3). We observed a reduced likelihood of long cycles and especially long follicular phases with increasing TTHM concentration (Table 3). Using the > 80 µg/L cutoff instead of > 60, a similar pattern for the reduced risk of long length was observed; findings were even slightly stronger for long follicular phase (AOR 0.19; 95% CI, 0.07–0.55).

Mean cycle and phase lengths did not vary much by categorized amount of unheated tap water consumed at home (e.g., ± 0.25 days for cycle length after adjustment). In contrast, increasing consumption of heated tap water was associated with decreased menstrual cycle length; cycle and follicular phase lengths were decreased by over 1 day with daily consumption of three or more drinks made from heated tap water (p = 0.05). Adjustment reduced these differences, especially including caffeine in the model; the decrement in cycle length was 0.68 days for three or more heated drinks (95% CI, –2.1 to 0.72).

Combining TTHM concentration and unheated tap water consumption into an ingestion metric, we found a somewhat U-shaped pattern with mean cycle length. The third category (> 40–60 TTHM µg/day) had the shortest mean cycle length (by 1 day), as well as somewhat reduced follicular and luteal phase lengths, but the highest category (> 60 µg/day) showed less difference (0.4 days for cycle length). Using the ingestion metric based on total home tap water consumption, we found the pattern of reduced cycle length with increasing exposure more consistent, showing an adjusted decrement in mean cycle length of slightly greater than 1 day at both of the two highest categories, so we combined them into > 40 µg/day (Table 4). There was also a decrement in follicular phase length of over 1 day (Table 4). The ORs for the categorical end points showed the pattern of decreased risk for long cycle and long follicular phase with higher exposure, similar to TTHM concentration alone.

Cycle characteristics by individual THM levels. We examined whether one of the individual THM compounds accounted for the findings with TTHM levels. All brominated compounds were associated with significantly shorter cycles, but the strongest association was with chlorodibromomethane; the adjusted decrement in mean cycle length was 1.2 days at the highest quartile (Table 5). Dose–response patterns were evident for each brominated THM. As with the TTHM level, these decrements were reflected as similar decrements in follicular phase length. Chloroform level was not associated with much decrement in cycle length (Table 5), but with a slight decrement in luteal phase length of about 0.2 days at the highest quartile (95% CI, –0.51 to 0.08). Menses length varied little by any of the four compounds, except for bromodichloromethane; menses length was slightly longer at the high quartile (β = 0.23; 95% CI, –0.01 to 0.47). Summing the highly correlated brominated compounds, we found monotonic dose–response patterns of decreasing mean cycle length and follicular phase length with increasing level (Table 5).

The odds of having a long cycle or long follicular phase were strongly reduced at the highest concentration of the summed brominated compounds (AOR 0.55; 95% CI, 0.28–1.08 and AOR 0.26; 95% CI, 0.12–0.60, respectively). This was fairly consistent for each of the individual brominated compounds as well. In addition, chloroform was associated with an increased risk of short luteal phase (AOR 2.2; 95% CI, 1.0–4.7) at the highest quartile level.

Table 3. Menstrual cycle parameters by cycle-specific utility-wide TTHM level: rates and AOR[a] (95% CI).

	Estimated TTHM level (µg/L)		
Menstrual parameters	0–40	> 40–60	> 60
Total cycles (*n*)[b]	662	345	520
Short cycle (< 24 days)			
Percent affected (*n*)	7.4 (49)	8.4 (29)	12.3 (64)
AOR (95% CI)	Ref	0.95 (0.60–1.5)	1.5 (0.95–2.5)
Long cycle (> 36 days)			
Percent affected (*n*)	8.1 (54)	5.4 (18)	5.2 (25)
AOR (95% CI)	Ref	0.71 (0.44–1.1)	0.60 (0.31–1.1)
Short follicular phase (< 12 days)			
Percent affected (*n*)	6.5 (41)	7.8 (25)	9.0 (44)
AOR (95% CI)	Ref	0.95 (0.53–1.7)	1.2 (0.70–2.1)
Long follicular phase (> 24 days)			
Percent affected (*n*)	7.6 (48)	5.1 (16)	3.1 (14)
AOR (95% CI)	Ref	0.63 (0.36–1.1)	0.37 (0.18–0.76)
Short luteal phase (< 10 days)			
Percent affected (*n*)	7.2 (39)	6.8 (18)	4.9 (19)
AOR (95% CI)	Ref	0.94 (0.53–1.7)	0.56 (0.25–1.3)
Long menses (≥ 8 days)			
Percent affected (*n*)	7.1 (48)	10.1 (34)	10.4 (55)
AOR (95% CI)	Ref	1.6 (0.94–2.7)	1.3 (0.73–2.3)

Ref, referent group.
[a]Adjusted for age, race, BMI, income, pregnancy history, smoking, and alcohol and caffeine consumption. [b]Total *n* for unadjusted models of cycle length; denominators vary slightly for other parameters and are reduced slightly in adjusted models because of missing data. Percent affected is calculated using these *n*s (e.g., unadjusted).

Table 4. Menstrual cycle parameters by cycle-specific utility-wide TTHM daily consumption level:[a] mean lengths and adjusted[b] differences (95% CIs).

	Estimated TTHM consumption level (µg/day)		
Menstrual parameters	0	> 0–40	> 40
Cycle length			
n	449	717	458
Mean ± SE	29.8 ± 0.39	29.4 ± 0.28	28.5 ± 0.33
Adjusted difference (CI)	Ref	–0.23 (–1.2 to 0.77)	–1.1 (–2.2 to –0.06)
Follicular phase length			
n	402	676	436
Mean ± SE	17.1 ± 0.43	16.6 ± 0.30	15.8 ± 0.34
Adjusted difference (CI)	Ref	–0.32 (–1.4 to 0.77)	–1.1 (–2.2 to 0.03)
Luteal phase length			
n	381	636	407
Mean ± SE	12.9 ± 0.13	12.9 ± 0.10	12.9 ± 0.12
Adjusted difference (CI)	Ref	–0.005 (–0.36 to 0.34)	–0.08 (–0.46 to 0.29)
Menses length			
n	462	759	493
Mean ± SE	5.4 ± 0.12	5.2 ± 0.09	5.4 ± 0.11
Adjusted difference (CI)	Ref	–0.31 (–0.63 to 0.01)	–0.14 (–0.48 to 0.20)

Ref, referent group.
[a]Utility TTHM level (µg/L) × total home tap water consumption (glasses/day × 0.25 L/glass). [b]Adjusted for age, race, BMI, income, pregnancy history, smoking, and alcohol and caffeine consumption.

Cycle characteristics by showering. The crude mean cycle length varied little by time spent showering (Table 6). After adjustment, there was a tendency toward decreased length with any category of showering above 35 min/week, which was stronger for follicular phase than cycle length. However confidence limits were wide. Examining the dummy variable that incorporates TTHM level with showering, the results appeared driven by the high showering category; however, the number of cycles in the low showering and high TTHM (> 60 µg/L) category was very small (*n* = 82). The observed decrements had wide CIs (high showering and high TTHM: for cycle length, β = –1.2 days; 95% CI, –3.6 to 1.1, and for follicular phase length, β = –1.6; 95% CI, –4.2 to 1.1).

Discussion

We found a consistent reduction in menstrual cycle length, and a corresponding reduction in follicular but not luteal phase length, with greater estimated exposure to chlorination by-products as calculated in a number of ways. We observed a monotonic dose–response effect for the total trihalomethane concentration based on utility-wide measurements. A decrement in mean cycle length of about 1 day was seen at the level corresponding to the current U.S. EPA MCL of > 80 µg/L and similar to the level we examined in our previous studies (Waller et al. 1998a, 2001), as well as at the even lower level of > 60 µg/L. These findings were strengthened by a significant decrement in length with increasing brominated THM concentration and with TTHM as a continuous measure. Furthermore, there was a reduction in the odds of long cycles or long follicular phases at the high total and brominated THM category. An association with the brominated THMs is consistent with our previous study (Waller et al. 1998a). If causal, this could influence differences between study findings, as the proportion of TTHM represented by brominated compounds varies by water system dependent on the type of organic material present.

Attempts to quantify amount of exposure based on individual water use patterns, such as tap water consumption or showering, did not strengthen the effects greatly, but did show consistent patterns of decreased cycle length with increasing exposure for the ingestion metrics. The ingestion metric with total tap water showed a more consistent pattern than with unheated tap water alone, similar to another study (Klotz and Pyrch 1999). As THMs would volatilize out of heated water, this may implicate an additional compound(s). The associations may not have been strengthened by water use patterns because other sources of exposure are not accounted for, such as washing, cooking, and housecleaning, as well as exposures outside the home, leading to further misclassification (see below).

No other studies of chlorination by-products have examined menstrual cycle function, but as noted earlier, several studies have found increased risks of adverse pregnancy outcomes at higher exposure levels (Bove et al. 1995; Dodds et al. 1999; Gallagher et al. 1998; Kramer et al. 1992; Nieuwenhuijsen et al. 2000; Savitz et al. 1995; Waller et al. 1998a). An effect on the hypothalamic–pituitary system that controls hormone secretion could be an underlying mechanism leading to multiple effects on both pregnancy and ovarian function. Animal experiments have shown exogenous exposures that act on the central nervous system can affect ovulation (Stoker et al. 2001). An increase in fetal resorptions in rats exposed to bromoform and bromodichloromethane, as well as changes in hormone levels indicating disruption of the pituitary–gonadal axis (Bielmeier et al. 2001), further supports this hypothesis.

Our finding of a shortened menstrual cycle during the follicular phase indicates an alteration in menstrual cycle function and presumably ovarian function. A shorter follicular phase reflects earlier ovulation, potentially affecting oocyte maturation, endometrial thickening, and conception or contraception timing. Postovulatory aging at the time of conception has been associated with an increased risk of early pregnancy loss (Wilcox et al. 1998). Clinical studies have found lower conception rates associated with short follicular phase (Check et al. 1992; Liss et al. 2002) and an epidemiologic study found lower fecundity with short (and more variable) cycle length (Kolstad et al. 1999). A shorter cycle length has also been associated with an earlier age at menopause (Whelan et al. 1990), perhaps reflecting follicle depletion and ovarian aging, and an increased risk of breast cancer (Kelsey et al. 1993).

Our previous findings (Fenster et al. 1999a; Windham et al. 1999) with respect to

Table 5. Means and adjusted[a] differences (95% CIs) in menstrual cycle and follicular phase length by quartiles[b] of individual THM compounds.

	Quartile[b] of exposure		
Menstrual characteristics	1[c] Mean in days ± SE	2–3 Difference (95% CI)	4 Difference (95% CI)
Cycle length			
Bromoform	29.7 ± 0.26	–0.42 (–0.96 to 0.13)	–0.79 (–1.4 to –0.14)
Bromodichloromethane	29.8 ± 0.30	–0.59 (–1.2 to –0.02)	–0.74 (–1.5 to –0.02)
Chlorodibromomethane	30.0 ± 0.33	–0.69 (–1.4 to –0.02)	–1.2 (–2.0 to –0.38)
Chloroform	29.6 ± 0.30	–0.43 (–0.99 to 0.13)	–0.30 (–1.0 to 0.40)
Sum of brominated	30.0 ± 0.34	–0.72 (–1.4 to –0.04)	–1.2 (–2.0 to –0.40)
Follicular phase length			
Bromoform	16.9 ± 0.27	–0.30 (–0.83 to 0.23)	–0.78 (–1.4 to –0.14)
Bromodichloromethane	17.0 ± 0.31	–0.54 (–1.1 to 0.01)	–0.80 (–1.5 to –0.08)
Chlorodibromomethane	17.1 ± 0.34	–0.62 (–1.3 to 0.05)	–1.1 (–1.9 to –0.25)
Chloroform	16.8 ± 0.31	–0.42 (–0.96 to 0.12)	–0.13 (–0.82 to 0.56)
Sum of brominated	17.2 ± 0.35	–0.66 (–1.3 to 0.02)	–1.1 (–1.9 to –0.29)

[a]Adjusted for age, race, BMI, income, pregnancy history, caffeine and alcohol consumption, and smoking. [b]Top quartiles for each compound and the summed brominated are: ≥ 12, ≥ 16, ≥ 20, ≥ 17, and ≥ 45 µg/L, respectively. [c]Reference group; the mean provided is unadjusted with SE.

Table 6. Menstrual cycle parameters by time spent showering: mean lengths and adjusted[a] differences with 95% CIs.

	Shower time (minutes/week)			
Menstrual parameters	0–34	35–69	70–104	≥ 105
Cycle length				
n	305	499	471	307
Mean[b] ± SE	29.3 ± 0.46	29.0 ± 0.37	29.6 ± 0.38	29.1 ± 0.47
Adjusted difference (CI)	Ref	–0.70 (–1.9 to 0.47)	–0.47 (–1.7 to 0.74)	–0.68 (–2.0 to 0.62)
Follicular phase length				
n	291	459	437	287
Mean[b] ± SE	16.8 ± 0.51	16.2 ± 0.40	16.8 ± 0.41	16.3 ± 0.50
Adjusted difference (CI)	Ref	–1.1 (–2.3 to 0.22)	–0.90 (–2.2 to 0.42)	–1.2 (–2.6 to 0.26)
Luteal phase length				
n	273	428	414	270
Mean[b] ± SE	12.8 ± 0.16	12.9 ± 0.12	13.0 ± 0.13	12.9 ± 0.16
Adjusted difference (CI)	Ref	0.03 (–0.37 to 0.43)	0.11 (–0.30 to 0.52)	0.18 (–0.26 to 0.63)
Menses length				
n	330	534	483	319
Mean[b] ± SE	5.3 ± 0.15	5.5 ± 0.12	5.2 ± 0.12	5.2 ± 0.15
Adjusted difference (CI)	Ref	0.26 (–0.12 to 0.63)	–0.004 (–0.39 to 0.38)	–0.06 (–0.48 to 0.36)

Ref, referent group.
[a]Adjusted for age, race, BMI, income, pregnancy history, smoking, and alcohol and caffeine consumption. [b]Unadjusted means (*n* corresponds to these).

exogenous exposures in this data set indicated an increased risk (2–3 times) of short cycle with greater exposure to tobacco smoke or caffeine, which translated to reductions in cycle length of 2.5 and 0.4 days, respectively. These variables were controlled for in this analysis, as were several other variables potentially associated with menstrual function. Mean cycle length is known to vary by age, but little is known about other predictors of cycle length. Some factors that we as well as others have suggested include race, body size, exercise, and stress (Fenster et al. 1999b; Harlow and Ephross 1995; Waller et al. 1998b). We found little evidence for confounding in this analysis. Nutrition may be related to menstrual cycle function, but we did not collect data on this factor, and this population of HMO members is unlikely to have severe nutritional deficiencies. More subtle effects on the endocrine system and menstrual cycling from consumption of foods containing hormonelike substances have also been suggested (Cassidy et al. 1994). However, for these foods to represent confounding factors, their consumption would have to vary with TTHM level. Other environmental exposures have been associated with changes in cycle length of a similar magnitude to this study, including 1 day shorter with higher estimated PCB exposure (Mendola et al. 1997) and 1 day longer with premenarcheal dioxin exposure (Eskenazi et al. 2002).

One concern in all studies of chlorination by-products is exposure misclassification; nearly all studies of reproductive outcomes have used existing water treatment records. Our estimation of THM level was more specific than some because it is based on actual utility measurements (vs. water source or type of treatment) within a relatively narrow time frame around the cycle start date (vs. an annual average, for example) and incorporating personal water use. Based on our previous work that compared different statistical methods for using water utility data, calculating the utility-wide average TTHM level provided a reasonable, efficient estimate (Waller et al. 2001). However, the utility measurements are made at only selected points within the distribution system and will differ from what actually comes out of an individual tap on any given day. Another source of misclassification may be the self-reported quantification of everyday habits involving exposure, as well as the lack of data collection on other sources of exposure, including those outside the home. This misclassification would be most likely to attenuate results. Conducting a type of sensitivity analysis, we found the results varied somewhat by the definition of the exposure time period; similar results were also seen for a period 90 days before the cycle start date and using the overall average of a woman's cycle-specific TTHM levels. The results were attenuated for a period starting 30 days before and extending 60 days after the cycle start date, perhaps reflecting greater misclassification, as some measurements could occur after the cycle is over. Misclassification of the end points, particularly cycle length, is less likely, and our mean lengths are well within the range of other studies (Harlow and Ephross 1995). The day of ovulation, which determines the follicular phase length, is estimated from urinary hormone patterns so may vary by a day or two from actual ovulation (Baird et al. 1990). However, this is likely to be random with respect to TTHM level.

The study sample is not representative of the general population; because of the labor-intensive nature of data collection, only about 40% of initially eligible women completed the study. This rate is similar to other studies with similar methods, despite both recruiting from occupational cohorts (Gold et al. 1995) and one for a shorter urine collection period (Whelan et al. 2002). As noted, all participants were English-speaking and tended to be well educated. In addition, the most extreme abnormalities of cycle function would not be included because of some of the eligibility requirements designed to increase the likelihood of pregnancy, and because the more normal or typical hormone patterns were included when duplicate assays were discordant. Nevertheless, the consumption, or exposure, patterns with respect to tap water and THMs were similar to our earlier studies (Swan et al. 1992, 1998; Waller et al. 1998a; Windham et al. 1992).

This study has many strengths, including its prospective design. Because women were not selected because of pregnancy or an adverse outcome, their reported consumption should have been representative of their usual patterns. The estimated TTHM level was ascertained independently using existing records and the woman's address, as well as some measures of personal water use. The menstrual parameters were based on biologic measures (e.g., the hormone levels) rather than self-reporting, collected over several cycles per participant, providing a large improvement over studies that ask for usual cycle length or subjective menstrual symptoms. Furthermore, many potential confounders were considered in these analyses.

Because this is the first study to report such findings, it will be important to confirm them. They add to the growing literature indicating effects of exposure to chlorination by-products on reproduction and expand it to include ovarian function.

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