Polybrominated Biphenyl Exposure and Menstrual Cycle Function

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Background: Brominated flame retardants, including polybrominated biphenyls (PBB), are persistent compounds reported to affect sex hormones in animals; less is known about potential effects in humans. An industrial accident in 1973–1974 exposed Michigan residents to PBB through contaminated food. We examined whether this exposure to PBB had long-term effects on menstrual cycle function.

Methods: In 2004–2006, we recruited reproductive-aged women in the Michigan PBB Registry who were not pregnant, lactating, or taking hormonal medications. Participants kept daily diaries and provided daily urine samples for up to 6 months. We assayed the urine samples for estrone 3-glucuronide ($E_1$3G), pregnanediol 3-glucuronide (Pd3G), and follicle stimulating hormone (FSH). We fit linear mixed models among women aged 35–42 years to describe the relation between serum PBB levels and log-transformed, creatinine-adjusted daily endocrine levels among women who were premenarchal during the exposure incident in 1973–1974 (n = 70).

Results: We observed that high (>3.0 parts per billion [ppb]) and medium (>1.0–3.0 ppb) PBB exposure were associated with lower $E_1$3G levels across the menstrual cycle and lower FSH levels during the follicular phase, compared with low PBB exposure (≤1.0 ppb). High PBB exposure was also associated with lower Pd3G levels across the cycle compared with low PBB exposure, whereas Pd3G levels were similar in women with medium and low PBB exposure.

Conclusion: Our results are consistent with a hypothesized effect of exposure to an exogenous estrogen agonist but the modest sample size of the study requires cautious interpretation.

Keywords: Biphenyl compounds; Follicular phase; Luteal phase; Menstrual cycle; Organic chemicals; Polybrominated biphenyls

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Persistent organic pollutants endure in the environment, bioaccumulate, and have long half-lives. Of concern, they may interfere with endogenous hormone function. In the 1970s, brominated flame retardants, a class of persistent organic pollutants, were widely used in household products, including foam, fabric, and electrical devices.[1,2] Concern about potential health effects of some brominated flame retardants has led the United States (US) and Europe to restrict their production. Specifically, the US banned polybrominated biphenyls (PBBs) in 1976,[3] and US companies ceased production of polybrominated diphenyl ethers (PBDEs) by 2013.[4] However, persistence of these chemicals in the environment continues to result in human exposure. In the US, 97% of a representative sample of civilians aged 12 years and older had levels of the most common PBDE congener (PBDE-47) that exceeded 4.2 ng/g lipid and 83% had levels of the most common PBB congener (PBB-153) that exceeded 0.8 ng/g lipid in 2003–2004.[5] In addition to ongoing exposure to brominated flame retardants no longer produced, others with similar properties continue to be used.[2]

A 2010 statement, highlighted concern within the scientific community regarding the potential effects of brominated flame retardants on the health of wildlife and humans.[6] Despite evidence that these compounds may affect sex hormones in animals, there has been limited research among humans.[7] Three studies have evaluated the association between exposure to brominated flame retardants and menstrual cycle

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function in humans, with inconsistent results.[8–10] Polychlorinated biphenyls (PCBs) research may also be relevant because PCBs are structurally similar to PBBs. Of studies that measured menstrual cycle length prospectively, one suggested that higher estrogenic PCB exposure was associated with longer cycles,[11] but two others suggested that higher total PCB exposure was associated with shorter cycles.[12,13] Most studies examining PCB exposure and self-reported average menstrual cycle length did not observe meaningful differences in cycle length (usually <1 day).[14–18]

Only two studies examined PCB levels and measured hormone levels. In one, the daughters of women exposed to PCB-contaminated cooking oil (n = 33) were more likely to have higher serum estradiol and follicle stimulating hormone (FSH) levels compared with daughters of unexposed women.[13] In contrast, a study of Laotian-born women in San Francisco (n = 49) did not observe meaningful associations between PCB exposure level and creatinine-adjusted urinary estrogen and progesterone metabolite levels after adjustment for potential confounders.[12]

Most prior studies have been limited to using self-reported average menstrual cycle characteristics rather than prospectively collected diary data, and those that have measured hormone levels have been small. We measured hormone levels in a prospective study of menstrual cycle function that, although small, was larger than similar previous studies. The study population included women exposed to PBB through contaminated food as children. During 1973–1974, livestock feed was accidentally contaminated with a PBB flame retardant in Michigan. An estimated 97% of Michigan residents had PBB levels greater than 1.0 ppb 5 years after the incident based on a weighted probability sample of residents in the state, with higher PBB levels detected in areas of the state with more farms with contaminated beef and dairy products.[19] In 1976–1978, the Michigan Department of Health enrolled approximate 5000 individuals who had consumed contaminated farm products into the Michigan PBB Registry. Participants in the registry have been followed for over 40 years, including the participants in our menstrual cycle function study, who collected daily urine samples for hormone measurement. In this study, we examine whether high exposure to PBB during childhood is associated with alterations in menstrual cycle function compared with low PBB exposure.

METHODS

Study Population

From 2004–2006, we recruited women aged between 18 and 45 years from the Michigan PBB Registry to participate in a computer-assisted telephone interview on reproductive function. Of the 711 eligible women, 479 completed the interview. Participants in the Menstrual Cycle Function Study were recruited from interviewed women. Women who were pregnant, breastfeeding, using hormonal medications, developmentally disabled, or diagnosed with cancer and women who had not menstruated for 3 months or had a hysterectomy were ineligible (n = 165). Women who had not menstruated for 3 months were excluded to avoid including women with unrecognized pregnancies. Of the 314 women eligible for the Menstrual Cycle Function Study, 77 refused, 19 could not be contacted, 57 were lost to follow up or withdrew without participating, 27 provided diary data only, one provided urine data only, and 133 provided urine and diary data. Of the participants providing urine and diary data, 33 were born after the contamination incident and therefore only had maternal PBB levels available, and 100 participants were potentially exposed directly through consuming contaminated farm products. We hypothesized that the effect of PBB exposure in utero may differ from exposure through diet and that maternal PBB levels are not equivalent to participant PBB levels. Therefore, we limited this analysis to women potentially exposed through diet.

This study was approved by the Institutional Review Boards at Emory University and the Michigan Department of Health.

Menstrual Cycle Function Study Protocol

Study participants were asked to complete daily diaries for 6 months and collect first morning urine samples for four menstrual cycles. The urine samples were stored in the participant's freezer before shipment to the laboratory. Daily, the participant recorded the vial number, whether she had trouble with the urine collection, bleeding status (none, spotting, light, moderate, or heavy), sexual intercourse (yes or no), birth control use (none, condom, pill/patch, diaphragm, sponge, foam/jelly/suppository, withdrawal, or other), number of caffeinated beverages consumed, number of alcoholic beverages consumed, number of cigarettes smoked, level of stress (4-point scale), whether she exercised enough to increase her breathing/heart rate/sweat, whether she had a fever or illness, a list of vitamins or medications taken, and whether she took a pregnancy test. Each card covered 1 week and was mailed as completed with postage prepaid.

We assayed urine samples for the primary estradiol and progesterone metabolites, estrone 3-glucuronide ($E_1$3G), and pregnanediol 3-glucuronide (Pd3G) in a mid-cycle, 17-day window. We defined the day of ovulation as the day of luteal transition, which was determined by an algorithm using the ratio of $E_1$3G to Pd3G.[20] Additional samples were analyzed if the day of luteal transition was ambiguous to clarify whether ovulation occurred outside the 17-day window and if luteal phase days 5 and 6 were outside this window. We also measured FSH, $E_1$3G, and Pd3G in samples collected during the 5 days before menses onset through the first 5 days of the new cycle for consecutive cycles. $E_1$3G and Pd3G concentrations were measured in triplicate using a competitive double-antibody time-resolved fluorimmunoassay.[21] We assayed FSH in duplicate using noncompetitive, two-site time-resolved immunofluorometric assay

(DELFIA; Perkin-Elmer Cat. No. A017-201) modified and validated for use to analyze urine samples.[22] To adjust for the concentration of the urine samples, we measured creatinine in all samples using a Vitros 250 Chemistry Analyzer (Ortho-Clinical Diagnostics, Raritan, NJ).

Exposure Assessment

We assigned exposure based on serum PBB levels measured at enrollment into the PBB registry (1976–1978) by the Michigan Department of Community Health Bureau of Laboratories. PBB-153 was the primary congener of the PBB mixture that Michigan residents were potentially exposed to. We measured serum concentrations of PBB using gas chromatography with electron capture detection as described previously.[23,24] At the time, the limit of detection was 1.0 part per billion (ppb), and the coefficients of variation for PBB quantification ranged from 7% to 14%.[24] For analyses, we defined the reference group as women with low PBB levels at or below the limit of detection (PBB ≤ 1.0 ppb). We dichotomized PBB levels above the limit of detection at their median value into medium exposure (PBB > 1.0–3.0 ppb) and high exposure (PBB > 3.0 ppb).

Outcome Assessment

We determined onset of menses using an algorithm requiring three consecutive days without bleeding or with spotting only followed by two consecutive days of bleeding of which only one could be classified as spotting.[25] This algorithm reliably distinguishes mid-cycle spotting from onset of menses for most women. An adjustment to the algorithm was made for one cycle with 2 days of mid-cycle bleeding that corresponded to the day of luteal transition based on the hormone data. In addition, we adjusted the algorithm for a woman who reported only spotting but whose hormones indicated that she was ovulating regularly.

We examined a number of cycle-level outcomes. We classified cycles with an adequate number of urine samples, but no identified day of luteal transition, as anovulatory. We defined cycle length as the day of menses onset through the day before the next menses onset. We defined bleed length as the onset of menses to the day before three consecutive days without bleeding.[26,27] We defined the follicular phase as the day of menses onset through the day of luteal transition, and the luteal phase as the day after the day of luteal transition through the day before the next menses onset.

We also examined daily creatinine-adjusted hormone levels relative to menses onset and relative to the day of luteal transition in addition to summary hormone outcomes adapted from definitions proposed by Baird et al. (Table 1).[28] We calculated 3-day geometric mean hormone levels adjusted for creatinine during six timeframes. We calculated maximum geometric means for the follicular phase and the luteal phase only when no samples were missing during the relevant timeframe. The maximum geometric mean was calculated by identifying the maximum value in the relevant timeframe and then calculating the geometric mean of that day, the day before, and the day after. Early follicular phase levels were calculated as the geometric mean for cycle days 2–4; preovulatory levels were based on the 3 days before the day of luteal transition, mid-luteal phase levels were based on days 5–7 of the luteal phase, and late luteal phase levels were based on the last 3 days of the cycle. Geometric means were only calculated when hormone data were available for all 3 days, and the preovulatory and luteal phase variables were only calculated when the cycle had a defined day of luteal transition.

Statistical Analyses

We used a directed acyclic graph to evaluate potential confounders.[29,30] Because our exposure was assessed when the study participants were children, we hypothesized that potential confounders such as current smoking, gravidity, and body mass index (BMI) at interview were not confounders of the relationship between PBB at enrollment and menstrual cycle function (see eFigure 1; http://links.lww.com/EDE/B540 with supporting text). Therefore, we did not include them in the models. In addition, all study participants reported being

TABLE 1. Description and Timing of Hormone Outcomes

Endocrine Outcome	Description	$E_1$3G	Pd3G	FSH
Follicular phase maximum	Geometric mean of maximum value during the follicular phase and the values for the days before and after	X		
Luteal phase maximum	Geometric mean of maximum value during the luteal phase and the values for the days before and after	X	X	
Early follicular level	Geometric mean of values for cycle days 2–4	X		X
Preovulatory level	Geometric mean of values for the 3 days before the DLT	X	X	
Mid-luteal level	Geometric mean of values for luteal days 5–7	X	X	
Late luteal level	Geometric mean of values for the last 3 cycle days	X	X	X
Daily relative to DLT	Natural log of day specific value from 14 days before DLT to 14 days after DLT	X	X	
Daily relative to menses	Natural log of day specific value from 5 days before menses to cycle day 5	X	X	X

An X indicates that outcome was calculated for that hormone.
DLT indicates day of luteal transition; $E_1$3G, estrone-3-glucuronide; FSH, follicle stimulating hormone; Pd3G, pregnanediol-3-glucuronide.

white so it was not necessary to adjust for race. However, all models are adjusted for age because the outcomes are known to change with increasing age.

We fit marginal repeated measures linear models to describe the relationship between PBB exposure and the natural log of daily hormone levels adjusted for creatinine. One set of models defined cycle day relative to the day of luteal transition (plus or minus 14 days) and the other set of models defined cycle day relative to menses onset (from 5 days before menses onset through cycle day 5). We used composite models to account for the nested repeated measures design of cycle days nested within menstrual cycles and menstrual cycles nested within women. We applied an unstructured covariance structure for the menstrual cycles and an autoregressive covariance structure for cycle days.[31] To stabilize the models, we limited the number of cycles contributed per woman to five for the models centered on day of luteal transition, which dropped at most one eligible cycle for a few women. The models included all cycles that had any hormone data during the relevant timeframe. All models were adjusted for age as a continuous variable centered on the mean age of women in the study (38 years). We output predicted mean hormone levels by cycle day for PBB exposure at the study population's average age. In addition, we calculated age-adjusted differences in daily predicted mean hormone levels and 95% confidence intervals for medium PBB compared with low PBB and for high PBB compared with low PBB.

We also fit linear mixed models with random intercepts for the analyses of cycle length outcomes and the 3-day geometric mean hormone outcomes. The models included fixed effects for categorized PBB exposure and age as a continuous variable centered on the mean age of the study population. We output predicted means for the outcomes by PBB exposure level for the average age of the women in the study.

RESULTS

Of the 100 women who were exposed to PBBs through consumption of contaminated farm products, we excluded two women because they took hormones during the cycles when they provided urine samples and one, because of the integrity of her hormone data, was questionable. We further excluded four women exposed to PBBs after menarche because we hypothesized that the long-term effect of PBBs on menstrual cycle function could differ depending on the timing of exposure. Thus, limiting our analyses to women who were exposed to PBBs before menarche provided a more homogenous population. Furthermore, there were no women in the lowest PBB exposure group under the age of 35 and no women in the middle exposure group over the age of 42. Therefore, we restricted our analyses to women between the ages of 35 and 42 because of the strong effect of age on hormone levels. After exclusions, 70 women contributed to our analyses.

These 70 women contributed a mean of four fully observed cycles and one partially observed cycle. The number of cycles available for analysis varied by outcome. Of the 340 eligible cycles, approximately a quarter (n = 81) were missing urine samples that prevented us from determining the day of luteal transition. Among the remaining cycles, 93% met the day of luteal transition criteria: 17 cycles did not meet the strict criteria, usually due to a missing urine sample, but were judged likely to have had a day of luteal transition; and one cycle (of a 38-year-old woman with high PBB exposure) did not have a day of luteal transition. Cycle length is missing for partially observed cycles; luteal and follicular phase lengths are missing for cycles without a known day of luteal transition or known timing of menses onset; and cycle average hormone outcomes are missing if a urine sample was not provided in the relevant timeframe.

Women with high PBB levels (n = 23) were younger on average than women with medium exposure to PBB (n = 34) and women with low PBB levels (n = 13) (Table 2). The women with the highest exposure were also the least likely

TABLE 2. Characteristics of Study Participants by Polybrominated Biphenyl Exposure Level

Variable	≤1.0 ppb n (%)	>1.0–3.0 ppb n (%)	>3.0 ppb n %
Age (years)			
35–39	8 (62)	20 (59)	18 (78)
40–42	5 (38)	14 (41)	5 (22)
Education			
High school	2 (15)	9 (26)	6 (26)
Some college or technical school	7 (54)	14 (41)	12 (52)
College graduate	4 (31)	11 (32)	5 (22)
Income			
<$50,000/year	4 (31)	11 (34)	6 (27)
≥$50,000/year	9 (69)	21 (66)	16 (73)
Missing	0	2	1
Gravidity			
Nulligravid	3 (23)	2 (5.9)	2 (8.7)
1–2 prior pregnancies	6 (46)	14 (41)	11 (48)
3 or more prior pregnancies	4 (31)	18 (53)	10 (43)
Years from exposure to menarche			
0–4 years	5 (50)	8 (26)	8 (35)
5–9 years	4 (40)	20 (65)	15 (65)
10–14 years	1 (10)	3 (10)	0 (0.0)
Missing	3	3	0
BMI			
18.0–24.9 kg/m^2	4 (31)	20 (59)	9 (39)
25.0–29.9 kg/m^2	6 (46)	10 (29)	8 (35)
30.0–43.4 kg/m^2	3 (23)	4 (12)	6 (26)
Smoking status[a]			
Never smoker	7 (54)	25 (74)	17 (74)
Past smoker	2 (15)	3 (8.8)	5 (22)
Current smoker	4 (31)	6 (18)	1 (4.3)

Ppb indicates parts per billion.
[a]Smoking status based on information from the daily diary and the interview.

to be college graduates or current smokers. Women with the lowest exposure were the most likely to have higher education, to be nulligravid, to have a shorter time from PBB exposure to menarche, to be overweight, and to be a current smoker. Women with medium PBB exposure were the least likely to be overweight or obese and to be a former smoker.

Results from the day of luteal transition-centered daily hormone models are presented in Figure 1 and eTable 1; http://links.lww.com/EDE/B540. Predicted log creatinine-adjusted $E_1$3G (ng/mg CR) levels for a 38-year-old woman were lower across the 29-day window for women with high and medium exposure to PBB compared with those with low PBB exposure with the largest absolute differences occurring around the time of the day of luteal transition. Predicted log creatinine-adjusted Pd3G (µg/mg Cr) levels for 38-year-old women with high exposure to PBB were lower than for women with medium and low exposure to PBB across most of the 29-day window. Results from the menses-centered daily hormone models are presented in Figure 2 and eTable 2; http://links.lww.com/EDE/B540. Predicted log creatinine-adjusted $E_1$3G levels for a 38-year-old woman were lower across the 10-day window for women with medium and high exposure to PBB

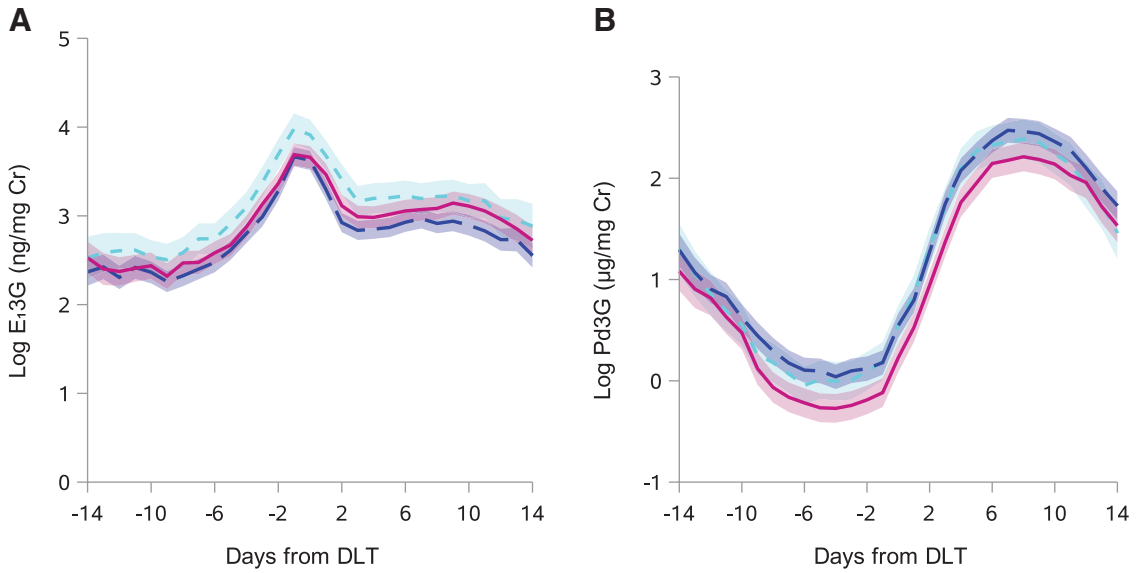

FIGURE 1. Predicted daily log creatinine-adjusted urinary hormone metabolite levels and 95% confidence intervals for a 38-year-old woman centered on the day of luteal transition (DLT) by level of polybrominated biphenyl exposure (PBB). The dotted light blue line represents ≤1.0 part per billion (ppb), the dashed dark purple line represents >1.0–3.0 ppb, and the solid red line represents >3.0 ppb. (A) Estrone 3-glucuronide ($E_1$3G) and (B) pregnanediol 3-glucuronide (Pd3G). Models are adjusted for continuous age centered on 38 years, the mean age of the study population.

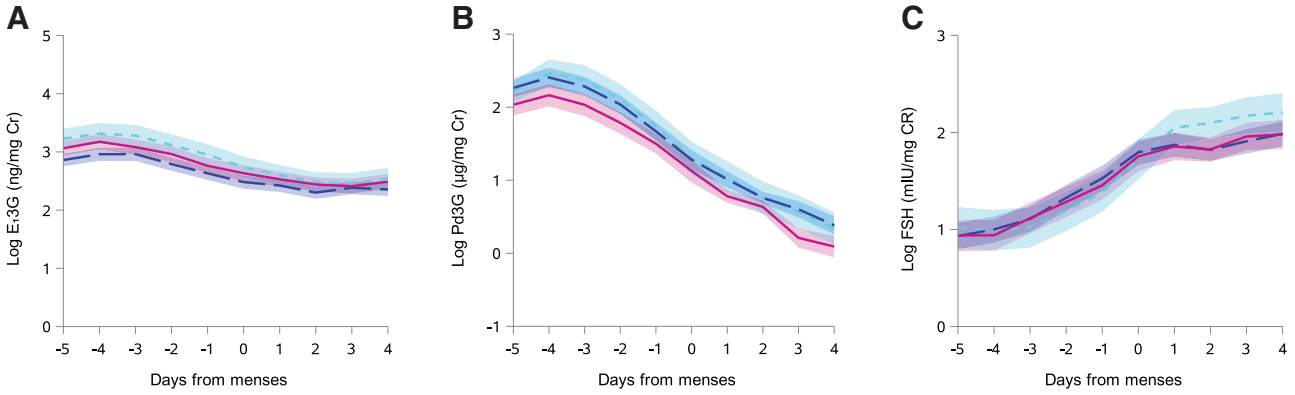

FIGURE 2. Predicted daily log creatinine-adjusted hormone metabolite and hormone levels and 95% confidence intervals for a 38-year-old woman centered on the day of menses onset by level of polybrominated biphenyl exposure (PBB). The dotted light blue line represents ≤1.0 part per billion (ppb), the dashed dark purple line represents >1.0–3.0 ppb, and the solid red line represents >3.0 ppb. (A) Estrone 3-glucuronide ($E_1$3G), (B) pregnanediol 3-glucuronide (Pd3G), and (C) follicle stimulating hormone (FSH). Models are adjusted for continuous age centered on 38 years, the mean age of the study population.

TABLE 3. Predicted Mean Cycle-Level Outcomes for a 38-Year-Old Woman by PBB Level[a]

			PBB ≤ 1.0 ppb		PBB > 1.0–3.0 ppb		PBB > 3.0 ppb	
Cycle Characteristics	**Women**	**Cycles**	**Mean**	**(95% CI)**	**Mean**	**(95% CI)**	**Mean**	**(95% CI)**
Cycle length characteristics	68	272	27	(25, 29)	28	(27, 30)	29	(27, 30)
Follicular phase length	70	241	16	(14, 18)	16	(14, 17)	16	(14, 17)
Luteal phase length	70	237	12	(11, 13)	13	(12, 14)	13	(12, 14)
Bleed length	70	319	6.0	(5.3, 6.6)	6.0	(5.6, 6.4)	5.5	(5.0, 6.0)
$E_1$3G (ng/mg Cr)								
Follicular phase max (3 day mean)	52	113	55	(46, 63)	40	(35, 46)	38	(31, 45)
Luteal phase max (3 day mean)	65	144	44	(36, 53)	31	(26, 37)	38	(32, 45)
Mean of days 2–4	69	179	15	(12, 17)	12	(10, 13)	12	(10, 14)
Mean of 3 days before DLT	69	235	43	(36, 50)	31	(26, 35)	33	(28, 38)
Mean of luteal days 5–7	69	233	27	(21, 32)	21	(17, 24)	24	(20, 29)
Mean of last 3 cycle days	68	166	25	(20, 29)	18	(15, 21)	21	(18, 25)
Pd3G (μg/mg Cr)								
Luteal phase max (3 day mean)	65	144	13	(11, 16)	13	(11, 15)	11	(9.3, 13)
Mean of 3 days before DLT	69	235	1.3	(0.9, 1.6)	1.3	(1.1, 1.5)	1.0	(0.7, 1.3)
Mean of luteal days 5–7	69	233	11	(9.3, 14)	11	(10, 13)	10	(7.9, 11)
Mean of last 3 cycle days	68	166	8.6	(6.6, 11)	8.3	(7.1, 10)	7.7	(6.2, 9.2)
FSH (mIU/mL)								
Mean of days 2–4	69	171	7.9	(5.2, 11)	8.3	(6.6, 10)	7.4	(5.3, 9.4)
Mean of last 3 cycle days	68	165	4.0	(1.7, 6.3)	4.9	(3.5, 6.3)	3.9	(2.3, 5.6)

[a]Predicted means are for a 38-year-old woman from models including age as a continuous variable centered on 38 years.

CI indicates confidence interval; Cr, creatinine; DLT, day of luteal transition; $E_1$3G, estrone-3-glucuronide; FSH, follicle stimulating hormone; PBB, polybrominated biphenyl; Pd3G, pregnanediol-3-glucuronide; ppb, parts per billion.

compared with women with low exposure, with the biggest absolute differences during the luteal phase, but the curves did not exhibit a dose–response pattern with PBB level. As with the day of luteal transition-centered models, predicted log creatinine-adjusted Pd3G levels for a 38-year-old woman were lower for women with the highest exposure to PBB compared with women with medium and low exposure. For FSH, the predicted log creatinine-adjusted level (mIU/mg CR) was similar across exposure levels before menses onset, but women with high and medium PBB exposure had lower FSH than the reference group after menses onset. Daily FSH levels across the 10-day window were similar for women with medium and high PBB exposure.

In Table 3, we present the predicted mean values and 95% confidence intervals for cycle-level study outcomes for a 38-year-old woman. For most outcomes, we observed only small differences across strata of PBB exposure. Average cycle length increased with increasing PBB exposure. However, women with low exposure had an average cycle length less than 28 days, and the 95% confidence intervals overlapped substantially across exposure groups. Follicular phase length and bleed length were similar across PBB exposure, but women with low PBB exposure had slightly shorter luteal phase lengths. For $E_1$3G, the 3-day geometric means were lower for women with PBB levels above the limit of detection (high and medium PBB) compared with women with low PBB exposure, but we only observed a dose–response pattern for the follicular phase maximum. For Pd3G, women with low and medium PBB exposure had similar 3-day geometric means during all timeframes. Women with high PBB exposure had a slightly lower 3-day mean for the luteal phase maximum Pd3G values, but the results were imprecise. The 3-day geometric mean values for FSH during both time periods were similar across PBB exposure levels.

DISCUSSION

We measured hormone levels in a prospective study of menstrual cycle function in Michigan women, who were exposed to PBB through contaminated food as children. We evaluated whether higher exposure to PBB in childhood was associated with alterations in menstrual cycle function compared with low PBB exposure. Exposure to PBB above 1.0 ppb was associated with lower $E_1$3G levels across the menstrual cycle and lower FSH levels during the follicular phase compared with having PBB levels at or below 1.0 ppb. These results are consistent with a physiologic hypothalamic-pituitary-ovarian axis response to exposure to an exogenous estrogen agonist. However, we did not observe a dose–response relation across levels of PBB. High exposure to PBB was also associated with lower Pd3G across the cycle compared with women with low PBB exposure, whereas women with medium PBB exposure were similar to those with low PBB exposure. Despite these differences in hormone levels, the cycle length outcomes did not differ meaningfully across PBB exposure

levels. Although cycle length increased across increasing categories of PBB exposure, the confidence intervals overlapped substantially, and all mean cycle lengths were in range with prospective studies of cycle length.[32]

To our knowledge, there are no published studies of PBB exposure and menstrual cycle function in humans for comparison, but two studies examined PCB exposure and hormone levels. A study of Laotian-born women in San Francisco (n = 49) reported no meaningful associations between PCB exposure and estradiol metabolites (estrone sulfate and estrone glucuronide) or Pd3G.[12] Many factors could contribute to the difference between these results and ours including the following: (1) differences between PBB and PCB exposure, (2) differences in relative exposure levels, and (3) difference in characteristics of study participants. A Taiwanese study compared adolescent daughters of women exposed to PCB-contaminated cooking oil to daughters of unexposed women.[13] In contrast to our study, this study suggested that estradiol and FSH were higher among the exposed group. However, the study was small (n = 38) and differed from ours with respect to route of exposure (maternal vs. participant exposure) and age of participants (adolescents vs. adults) making direct comparison difficult.

A number of relevant studies have examined menstrual cycle length in relation to PCB or PBDE exposure and their results are inconsistent. Three studies recorded menstrual cycle length prospectively. In a study of predominantly white women in the US, cycle length did not differ meaningfully across anti-estrogenic PCB levels in adjusted models, but women in the highest tertile of estrogenic PCBs had longer cycles than women in the lowest tertile.[11] In contrast, in a study of Laotian-born women living in San Francisco, women in the highest category of PCB exposure had the shortest cycles, although cycle length did not decrease in a dose-response manner, and the confidence intervals were wide and overlapping.[12] A Taiwanese study also reported shorter menstrual cycles for daughters of women exposed to PCB-contaminated cooking oil compared with unexposed daughters, but the study was small (n = 33).[13] Self-reported average cycle length was similar among the exposed and unexposed for studies of PBDE[8,9] and of PCB[14–18] with maximum differences across exposure categories typically being less than a day. Collectively, the variability in the direction and weak association between PBDE and PCB exposure and menstrual cycle length reported in both prospective and retrospective studies are consistent with the possibility that these exposures do not affect cycle length, which would also be consistent with our findings. However, the variability in PCB results might also reflect differences in the composition of the PCB mixture if estrogenic PCBs lengthen cycles and anti-estrogenic PCBs do not.[11]

Our study provides a rare opportunity to assess the relation between brominated flame retardants and prospectively assessed menstrual cycle length, and the availability of daily hormone data is an added strength. However, our study was restricted to women aged 35–42 years, and therefore, our results may not be generalizable to younger women. Further, we excluded women who had not menstruated in the past 3 months, which limits our ability to generalize to women with irregular cycles and our ability to assess the potential for PBB exposure to interfere with ovulation.

The demanding study protocol and the requirement that women not been using hormonal contraception during this study limited the number of participants. Given the small sample size, our results should be interpreted with caution. A concern is the fact that women may naturally experience substantial variability in menstrual cycle outcomes across cycles. In small studies, it is difficult to distinguish whether observed patterns are driven by differences in exposure between women or are an artifact of the specific cycles included for each woman. We partially addressed this limitation by including up to five cycles per woman and using marginal-repeated measure linear models. However, the small number of women who were eligible to be included in the reference group remains a concern because observed differences in hormone levels between the reference group and women with detectable PBB could be driven by unstable estimates of hormone levels in the reference group.

Over 80% of our study participants had PBB levels greater than 1.0 ppb, the limit of detection at the time the assays were performed. The limit of detection for the assay used by the 2003–2004 National Health and Nutrition Examination Survey (NHANES) was 0.0025 ppb, and the geometric mean PBB level among female participants was 0.012 ppb (95% confidence interval: 0.009, 0.015),[5,33] well within our reference group. It is possible that our reference group includes women whose exposure to PBB was high enough to affect menstrual cycle function, which would move our results toward the null relative to using a reference group of women unexposed to PBB.

Another strength of the study is that PBB exposure was measured during a defined period before outcome assessment. PBB exposure before pubertal development might have long-term effects on the developing endocrine system, and this is when PBB levels were at their highest for study participants. However, it is possible that the relevant PBB exposure window is more proximal to when the outcomes were assessed. PBB has a half-life of approximately 10.8 years,[34] but the rate of elimination is affected by pregnancy, breastfeeding, smoking, and BMI.[35] Therefore, variation in PBB elimination rates could mean that the enrollment PBB level is an imperfect proxy for the PBB level more proximal to the outcome.

In our study, women with higher PBB levels had different hormone profiles from women with the lowest PBB levels, but our results should be interpreted with caution given the sample size of our study. Nevertheless, a concern remains that premenarchal exposure to PBB seems to be associated with hormone levels consistent with exposure to an exogenous estrogen decades after the PBB exposure incident. This concern

continues to be relevant, despite the fact that PBB production has decreased or ceased in many countries, because of the continued production of related brominated flame retardants.

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REFERENCES

1. NIEHS. Flame Retardants. *Factsheet*. Research Triangle Park, NC (USA), 2016.
2. Birnbaum LS, Bergman A. Brominated and chlorinated flame retardants: the San Antonio Statement. *Environ Health Perspect.* 2010;118:A514–A515.
3. EPA. Polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs). *Technical Fact Sheet*. United States, 2014.
4. ATSDR. Polybrominated diphenyl ethers. *ToxFAQs™*. Atlanta, GA (USA), 2017.
5. Sjödin A, Wong LY, Jones RS, et al. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. *Environ Sci Technol.* 2008;42:1377–1384.
6. DiGangi J, Blum A, Bergman A, et al. San Antonio Statement on brominated and chlorinated flame retardants. *Environ Health Perspect.* 2010;118:A516–A518.
7. Darnerud PO. Brominated flame retardants as possible endocrine disrupters. *Int J Androl.* 2008;31:152–160.
8. Chao HR, Shy CG, Wang SL, et al. Impact of non-occupational exposure to polybrominated diphenyl ethers on menstruation characteristics of reproductive-age females. *Environ Int.* 2010;36:728–735.
9. Chao HR, Wang SL, Lee WJ, Wang YF, Päpke O. Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from central Taiwan and their relation to infant birth outcome and maternal menstruation effects. *Environ Int.* 2007;33:239–245.
10. Davis SI, Blanck HM, Hertzberg VS, et al. Menstrual function among women exposed to polybrominated biphenyls: a follow-up prevalence study. *Environ Health.* 2005;4:15.
11. Buck Louis GM, Rios LI, McLain A, Cooney MA, Kostyniak PJ, Sundaram R. Persistent organochlorine pollutants and menstrual cycle characteristics. *Chemosphere.* 2011;85:1742–1748.
12. Windham GC, Lee D, Mitchell P, Anderson M, Petreas M, Lasley B. Exposure to organochlorine compounds and effects on ovarian function. *Epidemiology.* 2005;16:182–190.
13. Yang CY, Yu ML, Guo HR, et al. The endocrine and reproductive function of the female Yucheng adolescents prenatally exposed to PCBs/PCDFs. *Chemosphere.* 2005;61:355–360.
14. Axmon A, Rylander L, Strömberg U, Hagmar L. Altered menstrual cycles in women with a high dietary intake of persistent organochlorine compounds. *Chemosphere.* 2004;56:813–819.
15. Cooper GS, Klebanoff MA, Promislow J, Brock JW, Longnecker MP. Polychlorinated biphenyls and menstrual cycle characteristics. *Epidemiology.* 2005;16:191–200.
16. Mendola P, Buck GM, Sever LE, Zielezny M, Vena JE. Consumption of PCB-contaminated freshwater fish and shortened menstrual cycle length. *Am J Epidemiol.* 1997;146:955–960.
17. Toft G, Axmon A, Lindh CH, Giwercman A, Bonde JP. Menstrual cycle characteristics in European and Inuit women exposed to persistent organochlorine pollutants. *Hum Reprod.* 2008;23:193–200.
18. Yang CY, Huang TS, Lin KC, Kuo P, Tsai PC, Guo YL. Menstrual effects among women exposed to polychlorinated biphenyls and dibenzofurans. *Environ Res.* 2011;111:288–294.
19. Wolff MS, Anderson HA, Selikoff IJ. Human tissue burdens of halogenated aromatic chemicals in Michigan. *JAMA.* 1982;247:2112–2116.
20. Baird DD, Weinberg CR, Wilcox AJ, McConnaughey DR, Musey PI. Using the ratio of urinary oestrogen and progesterone metabolites to estimate day of ovulation. *Stat Med.* 1991;10:255–266.
21. Kesner JS, Knecht EA, Krieg EF Jr, et al. Validations of time-resolved fluoroimmunoassays for urinary estrone 3-glucuronide and pregnanediol 3-glucuronide. *Steroids.* 1994;59:205–211.
22. Kesner JS, Knecht EA, Krieg EF. Time-resolved immunofluorometric assays for urinary luteinizing-hormone and follicle-stimulating-hormone. *Analytica Chimica Acta*. 1994;285:13–22.
23. Burse VW, Needham LL, Liddle JA, Bayse DD, Price HA. Interlaboratory comparison for results of analyses for polybrominated biphenyls in human serum. *J Anal Toxicol.* 1980;4:22–26.
24. Needham LL, Burse VW, Price HA. Temperature-programmed gas chromatographic determination of polychlorinated and polybrominated biphenyls in serum. *J Assoc Off Anal Chem.* 1981;64:1131–1137.
25. Jukic AM, Weinberg CR, Wilcox AJ, McConnaughey DR, Hornsby P, Baird DD. Accuracy of reporting of menstrual cycle length. *Am J Epidemiol.* 2008;167:25–33.
26. Hornsby PP. *The effects of in utero exposure to diethylstilbestrol (DES) on the menstrual cycle [Ph D]*. University of North Carolina at Chapel Hill, 1991.
27. Reutman SR, LeMasters GK, Knecht EA, et al. Evidence of reproductive endocrine effects in women with occupational fuel and solvent exposures. *Environ Health Perspect.* 2002;110:805–811.
28. Baird DD, Weinberg CR, Zhou H, et al. Preimplantation urinary hormone profiles and the probability of conception in healthy women. *Fertil Steril.* 1999;71:40–49.
29. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology.* 1999;10:37–48.
30. Hernán MA, Hernández-Díaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol.* 2002;155:176–184.
31. Park T, Lee YJ. Covariance models for nested repeated measures data: analysis of ovarian steroid secretion data. *Stat Med.* 2002;21:143–164.
32. Harlow SD, Ephross SA. Epidemiology of menstruation and its relevance to women's health. *Epidemiol Rev.* 1995;17:265–286.
33. National Center for Health Statistics, Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey, 2003–2004. Available at: https://wwwn.cdc.gov/nchs/nhanes/Search/DataPage.aspx?Component=Laboratory&CycleBeginYear=2003. Accessed November 16, 2018.
34. Rosen DH, Flanders WD, Friede A, Humphrey HE, Sinks TH. Half-life of polybrominated biphenyl in human sera. *Environ Health Perspect.* 1995;103:272–274.
35. Terrell ML, Manatunga AK, Small CM, et al. A decay model for assessing polybrominated biphenyl exposure among women in the Michigan Long-Term PBB Study. *J Expo Sci Environ Epidemiol.* 2008;18:410–420.