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In-utero exposure to polybrominated biphenyl (PBB) and menstrual cycle function in adulthood

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B.S., University of Georgia, 2018
Emory University
2021

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science in Public Health
in Environmental Health & Epidemiology
2021

Abstract

In-utero exposure to polybrominated biphenyl (PBB) and menstrual cycle function in adulthood

By Suman Barat

Background/Aim: There is evidence that in-utero exposure to PBBs, and similar chemicals, are associated with several adverse reproductive health outcomes including altered pubertal timing; however, less is known about the effects of in-utero exposure to PBBs on menstrual cycle function and reproductive hormone levels in adulthood.
Methods: For this menstrual cycle study, we recruited reproductive-aged women in the Michigan PBB Registry who were not pregnant, lactating, or taking hormonal medications (2004-2014). A total of 42 women who were born after the PBB contamination incident (1973-1974), who were only exposed in-utero, were included in this analysis. We estimated in-utero PBB exposure using maternal serum PBB measurements taken after exposure and extrapolated to time of pregnancy using a PBB elimination model. Women were followed for up to 6 months during which they provided daily urine samples and completed daily diaries. The urine samples were assayed for estrone 3-glucuronide (E13G), pregnanediol 3-glucuronide (Pd3G), and follicle stimulating hormone (FSH).
Results: Women in our study were, on average, 27.5 (SD:5.3) years and contributed 4.9 (SD:1.9) menstrual cycles of follow-up. Compared to women with low in-utero PBB exposure (≤1 ppb), women with medium (>1.0-3.0 ppb) and high (>3.0 ppb) exposure had significantly higher maximum 3-day mean Pd3G levels during the luteal phase. Specifically, the age- and creatinine-adjusted maximum 3-day mean luteal phase Pd3G levels (95% CI) in increasing categories of in-utero PBB exposure were 9.2 (4.6,13.9), 14.8 (11.6,18.0), and 16.1 (12.9,19.3) ug/mg. There were no significant differences in average cycle length, follicular or luteal phase cycle length, bleed length, or creatinine-adjusted E13G or FSH levels by category of in-utero PBB exposure.
Conclusion: Higher exposure to PBB in-utero was associated with increased progesterone levels across the luteal phase, however, most other menstrual cycle characteristics were largely unassociated with in-utero PBB exposure. Given our modest sample size, our results require cautious interpretation.

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Introduction.

Polybrominated biphenyls (PBBs) are a class of highly stable brominated flame retardants which persist in the environment and can be found in air, soil, seafood, meat, milk and dairy products.[1] The Michigan PBB cohort is one of the longest running cohort studies to examine the health effects of a widespread environmental contamination event of PBBs. Research based on the Michigan PBB Cohort has shown associations between exposure to PBB and many reproductive health outcomes.[2] In 2005, a menstrual function prevalence study suggested that PBB exposure was associated with changes in menstrual cycle length and bleed length.[3] A follow-up prospective study that was published in 2019, showed that PBB concentrations measured during early childhood were associated with lower concentrations of endogenous estradiol metabolites throughout the menstrual cycle in adulthood.[4]

Because the Michigan PBB cohort is multi-generational, there is the rare opportunity to use the data collected from the registry to assess health outcomes in individuals who were exposed in-utero, to investigate if there are intergenerational health effects of PBB that pass onto the next generation. There has been evidence to show that in-utero exposure to PBBs is associated with health outcomes like increased odds of spontaneous abortion[2], delayed puberty in males[5], and age at menarche[6]. However, there is limited research on the effects of in-utero exposure to PBBs or similar chemicals, specifically for menstrual cycle function and reproductive hormone levels.

Methods.

Study Population. The Michigan Polybrominated Biphenyl (PBB) registry is a study of long-term health outcomes of a Michigan based population that was exposed to persistent organic pollutants in the 1970s through a food contamination event. A subset of women in this cohort

were recruited to participate in a longitudinal study on menstrual cycle function. In brief, women from the original PBB Registry aged 18-45 who were premenopausal, not pregnant or lactating, not currently taking hormonal medications, and never diagnosed or treated for cancer were eligible. Women were recruited in two phases: between 2004 and 2006 (Phase 1) and between 2013 and 2014 (Phase 2). To increase the pool of eligible women, the age range for Phase 2 was increased to include women from ages 18-54 years. Women who participated in the study had a blood draw, completed a health questionnaire with details of medical history, current medication use, behaviors and demographics, and provided daily urine collections and daily diaries for menstrual cycle function monitoring. Phase 1 women completed a computer assisted telephone interview and Phase 2 women completed a female in depth questionnaire to determine initial eligibility and collect baseline data on medical history and reproductive function.

In Phase 1, 479 women were contacted, 314 were deemed eligible, and 133 provided sufficient urine and diary data. Similarly, in Phase 2, 297 women were contacted, 87 women were deemed eligible, and 58 provided sufficient data. From this initial pool of 191 women from Phase 1 and 2, only 65 women (33 from Phase 1 and 32 from Phase 2) were born after the contamination incident and were eligible for this analysis. Since the effect of PBB exposure in utero may differ from exposure through diet, we only included women exposed in utero, with maternal PBB levels available. The women who were excluded were potentially exposed directly through consuming contaminated farm products in childhood. The majority of these women were included in a previous analysis from this cohort[4] that investigated PBB exposure in childhood and menstrual cycle function. All of the women from Phase 1 with complete data were included for our analysis, but of the 32 women in Phase 2 we were only able to determine maternal PBB

exposure levels for 10. Thus, the final sample size for our analysis was 41 women (32 women from Phase 1, 8 women from Phase 2, and 1 woman who contributed cycles during both phases). 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 completed daily diaries for up to 6 months and collected first morning urine samples for up to four menstrual cycles. Participants were given a booklet of diary cards, one for each week of participation, and were instructed to answer diary questions at the same time each day and mail the cards using prepaid postage. In Phase 1, participants recorded bleeding or spotting patterns, whether they had sexual intercourse or used birth control, whether they exercised, cigarettes smoked, use of alcoholic or caffeinated beverages, and symptoms like stress or fever/other illnesses. A comments section was included for any additional information on medications or vitamins taken, if a pregnancy test was taken, or if any other explanation was required. In Phase 2, a similar diary booklet was used with slight alterations to the questions, the main difference being the removal of questions regarding caffeinated beverages, fever/illness, sexual intercourse and birth control use, and inclusion of a question regarding menstrual cramping. Because of the differences in the diary cards, only the common questions between the two phases were utilized in our analysis. Urine samples were collected in the morning in pre-numbered vials and stored immediately in the participants personal freezer until sent out for lab analysis. Participants were also asked to note down if anything went wrong with the sample, e.g., if the sample was collected late, not frozen immediately, or if the vials were used out of numerical order.

Urine samples were assayed for primary estradiol and progesterone metabolites, estrone 3-glucuronide (E13G), and pregnanediol 3-glucuronide (Pd3G). The protocol for the urine sample analysis was slightly different in phase 1 and 2. In Phase 1, all urine samples in the 17-day window around expected ovulation (preceding the last 4 days of the cycle) were analyzed for E13G and Pd3G. In women with sufficient data during the luteal-follicular transition, E13G, Pd3G, and FSH were also measured in this 10-day window, which included menses onset. In Phase 2, all urine samples were analyzed for E13G and Pd3G and FSH was measured in the same 10-day window during the luteal-follicular transition, which included menses onset. Urine samples collected in the 10-day mid-cycle window were also analyzed for LH. Urinary E13G and Pd3G were measured in triplicate using competitive double-antibody time-resolved fluoroimmunoassays. Urinary LH and FSH were assayed in duplicate using immunofluorometric assays (PerkinElmer, Waltham, MA, USA; Cat. Nos. A031–101 and A017–201, respectively) modified and validated for analyzing urine samples.[7] 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 to PBBs in utero based on estimated maternal PBB levels at the time of pregnancy. In brief, participants in the menstrual cycle function study were connected to their mothers in the PBB registry through a maternal PBB ID. At the mother's enrollment into the PBB registry, she provided a blood sample which was analyzed for PBB-153 exposure using gas chromatography with electron capture detection. At that time, the limit of detection for PBB was 1.0 part per billion (ppb) and the coefficients of variation for PBB quantification ranged from 7-14%. A validated mixed effects decay model was then used to

estimate the mother's PBB level at time of pregnancy based on a general linear mixed model, which attributes unique intercept and slope estimates for each woman. Age and BMI at initial measurement were time-independent covariates, and time since exposure, smoking history, pregnancy and breastfeeding status were time-dependent covariates to determine decay rates. The validated decay model was evaluated by comparing results from a previously developed ordinary least squares (OLS) model.[8] For purposes of analysis, we categorized in utero PBB exposure into low (PBB ≤ 1.0 ppb), medium (PBB > 1.0-3.0 ppb) and high exposure (PBB > 3.0 ppb).

Outcome Assessment. Menstrual cycle function outcomes included cycle-level characteristics such as cycle length, bleed length, and follicular and luteal phase lengths which were determined by a combination of diary data and urinary hormone levels. Cycle length was defined as the number of days between the first day of one menses and the first day of the next menses. Bleed length, or menses, was defined by two consecutive days of bleeding where one of the days must be greater than spotting. The first and last day of bleeding had to be preceded and followed by at least three days of no bleeding. If this three-day rule was broken, the duration for menses was not calculated. This algorithm used to determine bleed length reliably distinguishes mid-cycle spotting from onset of menses for most women. The follicular phase length was defined as the first day of menses through the day of ovulation. The luteal phase length was the day after ovulation through the day before menses onset. Day of ovulation was based on identifying a day of luteal transition (DLT), which was determined by an algorithm examining changes in the ratio of $E_1$3G to Pd3G.[9] If no day of luteal transition was able to be identified when there were adequate urine samples, the cycle was classified as anovulatory. Of the 193 contributed

menstrual cycles, 76 were missing urine samples that prevented us from determining the day of luteal transition. Among the 117 remaining cycles, 2 cycles did not met the DLT criteria, but had adequate urine samples, and were classified as anovulatory. Both of these cycles belonged to women in the medium exposure group with above average cycle length (41 and 43 days in the cycle respectively). Cycle length was classified as missing for partially observed cycles, luteal and follicular phase lengths were determined missing for cycles without a known DLT or known timing of menses onset.

Hormone outcomes included 3-day geometric mean hormone levels, which were calculated 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 prior to 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. These hormone outcomes were adapted from definitions proposed by Baird et al. that were shown to be related to conception.[9] Although we had 41 women in our analytic sample, the sample sizes for each hormone analysis varied mostly due to women missing single days of urine collection.

Statistical Analysis. We summarized participant characteristics according to their mother's estimated PBB level when the participant was in utero. We assessed confounding using a prior knowledge in combination with directed acyclic graphs (DAGs) and descriptive statistics from our cohort. Since our exposure was in utero PBB exposure, many variables such as current smoking status, gravidity, and body mass index (BMI) at interview, were not identified as potential confounders since they were downstream of exposure and left out of the final multivariable models. In addition, since all of our study participants were White, it was not necessary to adjust for race. Due to the low number of mothers who reported smoking during pregnancy, we were unable to adjust for this variable in the models. All models, however, were adjusted for age because the cycle and hormonal outcomes are known to change with increasing age.

For models where there was one outcome per cycle (cycle-level characteristics and 3-day geometric mean hormone levels), we fit linear mixed models with a random effect for woman in order to account for the intra-individual correlations among multiple menstrual cycles per woman. The models included fixed effects for categorized in utero 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 (27.5 years). We also examined the associations between PBB exposure and the natural log of creatinine-adjusted day-specific $E_1$3G, Pd3G, and FSH levels by fitting linear mixed models with random effects for woman and cycle to account for the nesting of days within cycles and cycles within women. We adjusted for age and presented the predicted daily log-transformed $E_1$3G,

Pd3G, and FSH levels by PBB exposure level over each relevant timeframe for the average aged women in our study (27.5 years).

Results.

The 41 women in our study contributed a total of 193 menstrual cycles of follow-up. The mean number of cycles contributed was 4.7 (range: 1 to 8). The number of cycles utilized in the analysis varied by outcome, ranging from 19 women and 33 cycles for mean of Pd3G to 39 women and 143 cycles for bleed length. The majority of women were less than 35 years (90%), had a normal BMI (56%), were employed at least part-time (73%), had at least some college education (85%), were never smokers (78%), and were nulligravid (53%) **(Table 1).** Only three women were exposed to maternal smoking in utero. Women with medium and high exposure to PBB in utero had, on average, slightly higher BMIs in comparison to the women with low in utero PBB exposure. All other demographic and lifestyle characteristics, however, were similar across categories of in utero PBB exposure.

There were no differences in cycle characteristics including total cycle length, follicular phase length, luteal phase length, or bleed length by categories of in utero PBB exposure (**Table 2**). We observed slightly higher age- and creatinine-adjusted 3-day mean follicular and luteal phase maximum E13G concentrations among women with the highest exposure to PBB in utero as compared to women with medium and low in utero PBB exposure; however, these differences were not statistically significant. There were no noticeable differences in age- and creatinine-adjusted mean E13G concentrations during days 2-4 of the cycle, 3 days prior to DLT, during luteal days 5-7, and during the last 3 days of the cycle across categories of in utero PBB

exposure. There were also no differences in 3-day mean urinary FSH concentrations across in utero PBB exposure levels during days 2-4 and the last 3 days of the menstrual cycle.

Age- and creatinine-adjusted adjusted Pd3G concentrations were slightly lower among women in the lowest category of in utero PBB exposure as compared to women with medium or high in utero PBB exposure for all of the different time windows; however, we only observed a significant, linear trend across categories of in utero PBB exposure for 3-day mean luteal phase maximum concentrations. Specifically, the age- and creatinine-adjusted 3-day mean luteal phase maximum Pd3G levels (95% CI) in increasing categories of in-utero PBB exposure were 9.2 (4.6, 13.9), 14.8 (11.6, 18.0), and 16.1 (12.9, 19.3) ug/mg. Women with medium and high PBB exposure in utero had significantly higher age- and creatinine-adjusted maximum Pd3G levels across the menstrual cycle (14.8 and 14.2 ug/mg, respectively) as compared to women with low exposure to in utero PBB (9.7 ug/mg). Women in the medium category for in utero PBB exposure had a significantly higher age- and creatinine-adjusted mean Pd3G levels during the last 3 days of the menstrual cycle (10.4 ug/mg) as compared to women with low exposure (6.3 ug/mg), while women in the highest exposure category had intermediate levels (8.3 ug/mg).

Discussion.

In our prospective study of 41 female offspring of women directly exposed to PBB through a food contamination event in Michigan in the 1970s, we found preliminary evidence that higher in-utero exposure to PBB was associated with increased maximum progesterone levels during the luteal phase of the menstrual cycle. Most other menstrual cycle characteristics, however, were largely unassociated with in-utero PBB exposure.

To our knowledge, only one other study has investigated PBB exposure and menstrual cycle function. This previous study came from the same, larger Michigan PBB cohort of women eligible for our study but focused on the sub-set of women who had been primarily exposed to PBB through diet during childhood. The main findings from the Howards et. al paper were that women with high (>3.0 ppb) PBB exposure during childhood had lower E13G and Pd3G levels across the menstrual cycle and lower FSH levels during the follicular phase as compared to women with low PBB exposure (≤1.0 ppb).[4] The findings for Pd3G levels, in particular, are quite different to what we observed in this study. This may not be entirely unexpected as exposures experienced in utero are often hypothesized to have different biological mechanisms underlying their associations with adult reproductive function as compared to direct exposures experienced during childhood. It's also worth noting that in addition to the obvious difference in primary route of PBB exposure between the two studies, the women in our study were, on average 10 years younger than the women exposed during childhood. Therefore, it's hard to completely rule out differences in results that may be due to effect modification by age. In other words, if PBB exposure (regardless of the timing) has a differential impact on menstrual cycle function as women age, it would be challenging to differentiate this effect from effects due to differing routes of exposure since the range of ages in our two cohorts were non-overlapping.

There is also a limited, but relevant, literature on in utero exposure to similar persistent, endocrine disrupting chemicals such as polychlorinated biphenyls (PCBs) and per- and polyfluoroalkyl substances (PFAS), and menstrual cycle function. For example, a comparable study from Taiwan, which evaluated menstrual cycle function in adolescent daughters of women

exposed in utero to PCB-contaminated cooking oil found that higher in utero PCB exposure was associated with increased estradiol and FSH levels and shortened bleeding periods.[10] In contrast, when the exposed mothers were followed up, the authors found very few differences in menstrual cycle function according to PCB levels, with the exception of longer bleeding periods.[11] These two studies, which found differing results following in utero versus direct exposure to high levels of PCB, provide additional evidence that the route and timing of exposure to persistent endocrine disrupting chemicals may result in differing effects on menstrual function. There have also been multiple studies on the association between in utero exposure to PFAS and reproductive function in childhood and adolescence. These studies tended to focus on slightly different outcomes, but the results have shown that higher in utero PFAS exposure was associated with delayed menarche,[12] increased testosterone concentrations,[13] and reduced DHEA concentrations[14] in girls.

Multiple biological explanations have been proposed to explain why reproductive hormones and menstrual cycle function may be affected by in utero exposure to endocrine disrupting chemicals like PBB. For example, a study in rats found that higher in utero exposure to brominated flame retardants, a class of chemicals similar in structure and biological function to PBB, was related to early onset of puberty and increased incidence of multi-oocycte follicles and that this was likely due to the downregulation of pathways that are fundamental for ovarian function like the HIF1A, CREB1, EGF, b- estradiol, and PPAR pathways[15]. While this study did not show any significant differences in progesterone levels according to in utero exposure to brominated flame retardants, any exposure impacting ovulatory function would likely have downstream effects on progesterone production.

Regarding transgenerational effects of exposure to other endocrine disrupting chemicals, a study in pregnant rats found that maternal exposure to imazalil, a fungicide that is also an androgen receptor antagonist, was associated with increased androgen levels in the mothers but decreased androgen levels in male offspring.[17] This finding in animals further supports the notion that exposure to endocrine disrupting chemicals during pregnancy may induce hormonal changes in future generations that could be opposite to the effects observed in the initial generation. However, in contrast to our results, two studies- one focused on prenatal phthalate exposure.[18] and the other on prenatal PCB & DDT exposure in rats[19] showed a decrease in progesterone concentrations in the F1 and F2 generations with increasing exposure to these chemicals. While we observed the opposite effect, this does provide evidence that exposure to endocrine disrupting chemicals may lead to an alteration in progesterone receptors or the HPO axis. As these were animal studies, timing and dose of exposure are hard to directly compare between these studies and ours, but may be a critical consideration. For example, a study on the action of PCB congeners on proliferation and progesterone secretion in cultured in vitro porcine luteal cells showed a concentration dependent decrease in progesterone secretion after 24 and 48 hrs PCB153 exposure and a concentration dependent increase in progesterone secretion after 72 hrs of exposure[20], suggesting that duration of EDC exposure may play a pivotal role in the type of hormonal effect it has.

One of the primary limitations of our study was the small sample size. Given our strict eligibility criteria and our rigorous study protocol, which required women to complete daily diaries and provide daily urine samples, we had a limited number of participants that were eligible and willing to participate in the study. Therefore, our results should be interpreted with caution.

Women experience natural variation in menstrual cycle characteristics across cycles so it is difficult to distinguish, in small studies like ours, whether the observed patterns are driven by differences in exposure between women or are merely due to chance (e.g. an artifact of the specific cycles we included for each woman). While we partially addressed this by including multiple cycles per woman and using marginal repeated measures linear models to account for the inherent variability in menstrual cycle function within a woman, the overall power of our study was still limited. It is also possible that, because of our low power, we failed to detect small but clinically significant differences in menstrual cycle characteristics by levels of in utero PBB exposure. Future studies, with larger sample sizes will be needed to further address this question. An additional weakness was that maternal PBB concentrations were not directly measured during pregnancy but rather estimated using a PBB decay elimination model[8], which likely led to measurement error of the exposure. Given the prospective nature of our study, however, it is highly unlikely that this error was differential with respect to menstrual cycle function and therefore would only be expected to bias the results towards the null.

Regarding generalizability, the estimated maternal PBB levels for the study participants were, on average, much higher than would be expected in the general population. For example, only ~15% of our study participants' mothers had PBB levels less than 1.0 ppb, which was the limit of detection at the time the assays were performed. For comparison, the geometric mean PBB level among female participants in the 2003–2004 National Health and Nutrition Examination Survey (NHANES) was 0.012 ppb (95% CI: 0.009 to 0.015), which is well below even the average PBB level in our lowest exposure group (N=10, 0.58 95% CI: 0.44, 0.96).[21,22] It is possible that because the "low" exposure group in our study still had significantly higher PBB

levels than the general population, this may have biased our results towards the null. Because of the high levels of in utero PBB concentrations observed in our study population, our results may not be directly generalizable to most populations beyond other affected residents of Michigan and their daughters. The results may be applicable to other populations with high PBB exposure in their diets- which would lead to higher exposure in utero exposure as well, however these populations are limited because PBB has been largely banned in the United States and multiple other countries and the incident that led to this exposure was a food contamination accident, which is not commonplace.[1]

In conclusion, women who were exposed to higher levels of PBB in-utero had increased progesterone levels across the luteal phase of the menstrual cycle compared to women with the lowest in utero exposure to PBB. Most other menstrual cycle characteristics, including cycle length, bleed length, and urinary concentrations of estradiol and FSH, however, were largely unassociated with in-utero PBB exposure. Given our modest sample size, our results require cautious interpretation. While the production of PBB has decreased or ceased in most countries, our results may be still relevant due to the continued production of related brominated flame retardants worldwile.

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Table 1. Characteristics of Study Participants by Polybrominated Biphenyl Exposure Level (N=41)

	In Utero PBB Exposure Level		
	<1.0 ppb	**1.1-3.0 ppb**	**>3.0 ppb**
Number of Women	10	15	16
Age (years)			
20-25	5 (50.0)	3 (20.0)	6 (37.5)
25-35	3 (30.0)	11 (73.3)	9 (56.3)
35-40	2 (20.0)	1 (6.7)	1 (6.3)
Education (Missing=4)			
High school or less	2 (25.0)	2 (13.3)	2 (14.3)
Some college or technical school	2 (25.0)	2 (13.3)	5 (35.7)
College graduate or higher	4 (50.0)	11 (73.3)	7 (50.0)
Income (Missing=3)			
< $20,000/year	3 (37.5)	4 (28.6)	4 (25.0)
$20,000-$50,000/year	1 (12.5)	3 (21.43)	8 (50.0)
>$50,000/year	4 (50.0)	7 (46.7)	4 (25.0)
Employment Status			
Unemployed, homemaker, student	2 (20.0)	3 (20.0)	6 (37.5)
Employed part-time or full-time	8 (80.0)	12 (80.0)	10 (62.5)
Gravidity			
Nulligravid	4 (40.0)	10 (66.7)	8 (50.0)
≥1 prior pregnancy	6 (60.0)	5 (33.3)	8 (50.0)
Age at menarche (Missing=1)			
11 years	3 (30.0)	2 (14.3)	1 (6.3)
12 years	4 (40.0)	2 (14.3)	8 (50)
≥13 years	3 (30.0)	10 (71.4)	7 (43.8)
BMI			
18.0-24.9 kg/m^2	6 (60.0)	9 (60.0)	8 (50.0)
25.0-29.9 kg/m^2	4 (40.0)	0 (0.0)	6 (37.5)
30.0-43.4 kg/m^2	0 (0.0)	6 (40.0)	2 (12.5)
Smoking Status*			
Never	7 (70.0)	13 (86.7)	12 (75.0)
Past or Current Smoker	3 (30.0)	2 (13.3)	4 (25.0)
Maternal Smoking Status (Missing=2)			
No	7 (70.0)	14 (100.0)	15 (100.0)
Yes	3 (30.0)	0 (0.0)	0 (0.0)

Abbreviations: ppb, parts per billion

*Smoking status is based on information from daily diary and interview

Table 2. Predicted Mean Cycle-Level outcomes for a 28-year-old woman by PBB level.

			PBB ≤1.0 ppb		PBB 1.1-3.0 ppb		PBB > 3.0 ppb	
	Women	**Cycles**	**Mean**	**(95% CI)**	**Mean**	**(95% CI)**	**Mean**	**(95% CI)**
Cycle Characteristics								
Cycle length characteristics	33	113	28.4	(22.3, 34.5)	33.6	(29.1, 38.0)	30.6	(26.2, 35.1)
Follicular phase length	38	102	16.4	(10.4, 22.4)	21.5	(17.1, 25.8)	18.8	(14.4, 23.1)
Luteal phase length	37	103	12.0	(10.3, 13.7)	11.4	(10.1, 12.6)	13.6	(12.4, 14.9)
Bleed length	39	143	5.6	(4.7, 6.4)	5.9	(5.3, 6.6)	5.7	(5.0, 6.3)
E13G (ng/mg Cr)								
Follicular phase max (3 day mean)	23	42	34.9	(18.2, 51.6)	35.6	(25.3, 46.3)	39.3	(28.1, 50.4)
Luteal Phase Max (3 day mean)	30	67	27.7	(17.5, 38.3)	28.2	(21.2, 35.3)	30.6	(23.5, 37.7)
Mean of days 2-4	34	89	8.9	(5.4, 12.4)	11.2	(8.7, 13.7)	8.6	(6.1, 11.1)
Mean of 3 days before DLT	38	101	26.4	(18.4, 34.4)	30.8	(24.9, 36.6)	25.0	(19.1, 30.8)
Mean of luteal days 5-7	37	104	17.0	(11.9, 22.1)	19.5	(15.5, 23.5)	18.1	(14.1, 22.1)
Mean of last 3 cycle days	35	85	15.7	(9.1, 22.4)	21.6	(16.7, 26.5)	17.3	(12.6, 22.0)
Pd3G (ug/mg Cr)								
Mean of cycle max	19	33	9.8	(6.1, 13.5)	14.9*	(12.9, 16.8)	14.7*	(12.1, 17.3)
Luteal phase max (3 day mean)	30	67	9.2	(4.6, 13.9)	14.8	(11.6, 18.0)	16.1*	(12.9, 19.3)
Mean of 3 days before DLT	38	101	1.2	(0.7, 1.7)	1.7	(1.4, 2.1)	1.6	(1.2, 1.9)
Mean of luteal days 5-7	37	104	10.0	(6.5, 13.5)	12.9	(10.1, 15.7)	14.0b	(11.2, 16.8)
Mean of last 3 cycle days	35	84	6.3	(3.3, 9.4)	10.4*	(8.2, 12.6)	8.3	(6.2, 10.5)
FSH (mIU/mL)								
Mean of days 2-4	34	80	5.2	(3.8, 6.6)	5.5	(4.5, 6.6)	5.4	(4.4, 6.4)
Mean of last 3 cycle days	34	80	2.7	(1.8, 3.7)	2.5	(1.8, 3.1)	2.6	(2.0, 3.3)

Predicted means are for a 28-year-old woman from models including age as a continuous variable centered on 28 years.
CI indicates confidence interval, Cr, creatinine, DLT, days of luteal transition; E13G, estrone-3-glucuronide; FSH, follicle stimulating hormone; PBB, polybrominated biphenyl; Pd3G, pregnanediol-3-glucuronide; ppb, parts per billion.
***Indicates the mean was significantly different from the reference group (<1.0ppb) at an alpha level of 0.05**