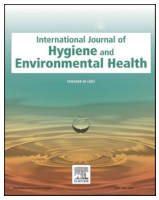
International Journal of Hygiene and Environmental Health 256 (2024) 114297

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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



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

Suman Barat [a,1], Robert B. Hood [b,1], Metrecia L. Terrell [b], Penelope P. Howards [b], Jessica B. Spencer [c], Tamar Wainstock [d], Hillary Barton [a], Melanie Pearson [a], James S. Kesner [e], Juliana W. Meadows [f], Michele Marcus [a,b], Audrey J. Gaskins [b,*]

[a] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA
[b] Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA
[c] Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Emory University School of Medicine, Atlanta, GA, USA
[d] Department of Public Health, Faculty of Health Sciences, Ben-Gurion University, Be'er Sheva, Israel
[e] Division of Applied Research and Technology, National Institute for Occupational Safety & Health, Cincinnati, OH, USA
[f] Health Effects Laboratory Division, National Institute for Occupational Safety & Health, Cincinnati, OH, USA

ARTICLE INFO

Keywords:
Biphenyl compounds
Brominated flame retardants
Polybrominated biphenyls
Menstrual cycle
Progesterone
Two-generation study

ABSTRACT

Background: 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 41 women who were born after the PBB contamination incident (1973–1974) and were prenatally exposed to PBBs, 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 ($E_1$3G), pregnanediol 3-glucuronide (Pd3G), and follicle stimulating hormone (FSH).
Results: Women in our study were, on average, 27.5 (SD:5.3) years old 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 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) μg/mg creatinine. There were no meaningful differences in average cycle length, follicular or luteal phase cycle length, bleed length, or creatinine-adjusted $E_1$3G 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.

1. Introduction

Polybrominated biphenyls (PBBs) are a class of highly stable brominated flame retardants that were once used in the manufacturing of plastics and electronics. Although the production of PBBs has ceased in the United States as of the 1970s, health concerns remain due to their persistence in the environment, their ability to accumulate in food products, and their long biological half-life (United States Environmental Protection Agency, 2017; Hood et al., 2023; Terrell et al., 2008). There is also evidence that PBB has estrogen-mimicking endocrine disrupting properties (Curtis et al., 2019), which could have long-term impacts on individuals exposed in-utero (Small et al., 2011). Previous studies have already demonstrated that those exposed to PBB, and its endocrine disrupting properties, in-utero are at higher risk for lower

* Corresponding author. 1518 Clifton Road, CNR 3017, Atlanta, GA, 30322, USA.
E-mail address: audrey.jane.gaskins@emory.edu (A.J. Gaskins).
[1] Indicates that both authors contributed equally for first authorship.

https://doi.org/10.1016/j.ijheh.2023.114297
Received 20 June 2023; Received in revised form 12 October 2023; Accepted 23 November 2023
Available online 1 December 2023

birthweight (Givens et al., 2007), low 1-min Apgar score (Terrell et al., 2015), earlier menarche (Blanck et al., 2000), and adverse pregnancy outcomes (Small et al., 2011).

The Michigan PBB registry is one of the longest-running cohort studies in the country and was established to examine the health effects of the widespread environmental contamination with PBBs. During the early 1970s, PBBs were accidently introduced into the food supply when it was shipped in place of an animal feed supplement. Research based on the Michigan PBB registry has shown associations between exposure to PBB and many reproductive health outcomes (Small et al., 2011). In 2005, a menstrual function prevalence study among women exposed to PBBs from their diet suggested that higher exposure was associated with shorter menstrual cycle length and longer bleed length among women with past year weight loss (Davis et al., 2005). A follow-up prospective study 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 (Howards et al., 2019).

Because the Michigan PBB registry is multigenerational, there is the rare opportunity to use the data collected from the registry to assess health outcomes in individuals exposed in-utero. There is evidence that in-utero exposure to PBBs is associated with health outcomes like increased odds of spontaneous abortion (Small et al., 2011), delayed puberty in males (Small et al., 2009), and earlier age at menarche (Blanck et al., 2000). 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 (sex steroids and gonadotropins). Therefore, our objective was to examine the association between in-utero exposure to PBBs and menstrual cycle function in adulthood.

2. Methods

2.1. Study population

The Michigan Department of Community Health (now the Michigan Department of Health and Human Services; MDHHS) established the Michigan Long-Term PBB Study (now the Michigan PBB registry) between 1976 and 1977 (Carter 1976; Fries 1985). The original cohort enrolled over 4800 individuals who lived on contaminated farms, ate food from contaminated farms or who worked at the chemical plant that produced PBB in Michigan (Landrigan et al., 1979) and their family members. MDHHS ceased management of the registry in 2003 and Emory University took over the management and expanded the original cohort (Chang et al., 2020). Today, the Michigan PBB registry's research is guided by a community-academic partnership between several community groups who represent those affected and Emory University. The registry includes people exposed to PBBs through occupational settings and/or consumption of contaminated food products and their children and grandchildren (Chang et al., 2020; Fries 1985). A subset of women from the Michigan PBB registry were recruited to participate in a longitudinal study on menstrual cycle function (Howards et al., 2019). In brief, women from the Michigan PBB registry who were aged 18–45 years (i.e. born before and after the contamination incident), 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). Women who participated in the study had a non-fasting venous blood draw (10 mL) and completed a health questionnaire that included medical history, current medication use, behaviors, and demographics. Participants also 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 self-administered web-based in-depth health questionnaire for females to gather more information about reproductive history. Both questionnaires collected baseline data on medical history and reproductive function and were used to determine study eligibility.

In Phase 1, 479 women of 711 eligible women (18–45 years old) completed the interview and were all screened for the study (Fig. 1). Of these, 314 were deemed eligible, and 133 provided sufficient urine and diary data. In Phase 2, 152 women completed the questionnaire of the 172 eligible women for the study. Of these, 87 women were screened and deemed eligible, and 58 provided sufficient data. Of the women who provided sufficient data, 5 participated in both phases with 1 being born after exposure. From this pool of 191 women who provided sufficient data, only 62 women (33 from Phase 1 and 32 from Phase 2) were born after the contamination incident. All 33 of the women from Phase 1 with complete data were included for our analysis, but of the 32 women in Pahse 2, maternal PBB exposure levels were available for only 9 women. 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). Since the effect of PBB exposure in-utero may differ from exposure through diet, we focused on women prenatally exposed who also had maternal PBB levels available. The average age of the women's mothers at the time of the PBB contamination event was 19.4 years (Range: 8.2–30.6 years). 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 the Michigan PBB registry (Howards et al., 2019) that investigated PBB childhood exposure and menstrual cycle function. This study was approved by the Institutional Review Boards at Emory University and the Michigan Department of Health and Human Services.

2.2. 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 answered diary questions at approximately the same time each day and diary cards were mailed to the study site weekly. In Phase 1, participants recorded bleeding or spotting patterns, sexual intercourse (including birth control use), exercise, cigarettes smoked, consumption of alcoholic or caffeinated beverages, and other conditions like stress (using 4-point scale), fever, or any 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: inclusion of a question regarding menstrual cramping and the removal of questions regarding caffeinated beverages, fever/illness, sexual intercourse, and birth control use. Because of the differences in the diary cards, only the common questions between the two phases were utilized in the current analysis. Urine samples were collected in the morning in pre-numbered vials and stored immediately in the participants' personal freezer until sent out for laboratory analysis. Participants were also asked to write a note on the diary card 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 ($E_1$3G), and pregnanediol 3-glucuronide (Pd3G). The protocols for the urine sample analyses was slightly different in Phase 1 and 2. In Phase 1, all urine samples in the 17-day window around expected ovulation were analyzed for $E_1$3G and Pd3G. We defined day of ovulation as the day of luteal transition, which was determined by an algorithm using the ratio of E13G to Pd3G. We also measured $E_1$3G, Pd3G, and FSH in urine samples during the 5 days before menses onset through the first 5 days of the new cycle for consecutive cycles to evaluate the luteal-follicular transition. In Phase 2, all urine samples from 3 menstrual cycles were analyzed for $E_1$3G and Pd3G; plus, FSH was measured in a 14-day window (5 days before menses onset through the first 9 days of the new cycle) during the luteal-follicular transition.

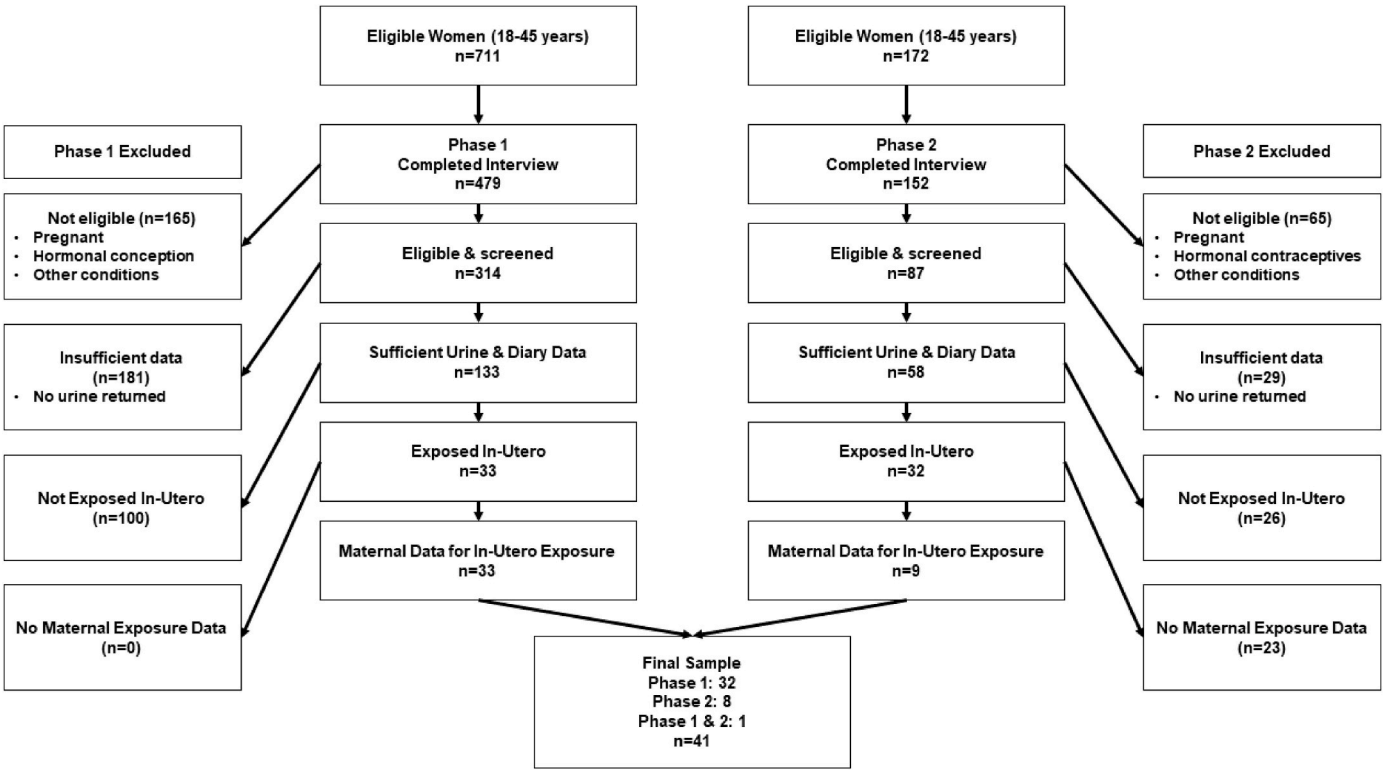


Fig. 1. Flowchart of participants in Phase I and Phase II of the Menstrual Cycle Function study in the Michigan PBB Registry.

Urinary $E_1$3G and Pd3G were measured in triplicate using competitive double-antibody time-resolved fluoroimmunoassays (Kesner et al., 1994). Urinary FSH was 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 (Kesner et al., 1994). 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).

2.3. Exposure assessment

Since PBBs can cross the placenta, we assigned exposure to PBBs in-utero based on the estimated maternal PBB levels at the time of pregnancy using a validated model of PBB elimination (Terrell et al., 2008). 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 (from 1976 to 1978), she provided a blood sample which was analyzed for serum PBB levels using gas chromatography with electron capture detection (min: non-detectable; median: 2.0 ppb; P95: 120.5 ppb; max: 251.7 ppb). The average time between the exposure event and the mothers' initial measurement was 3.2 years (Range: 3.0–4.0 years) and the average time between the mothers' initial measurement and their conception date for the participants was 1.1 years (Range: 4.0 to 13.0 years). At that time, the limit of detection (LOD) for PBB was 1.0 part per billion (ppb) and the coefficients of variation for PBB quantification ranged from 7 to 14% (Burse et al., 1980; Needham et al., 1981). A validated mixed effects elimination model was then used to estimate the mother's serum PBB level at time of pregnancy based on a general linear mixed model, which attributes unique intercept and slope estimates for each woman and has been described elsewhere (Terrell et al., 2008). Briefly, the model calculates a subject-specific rate of elimination of serum PBB levels based on age and BMI at initial measurements as time-independent covariates and time since exposure, smoking history, pregnancy and breastfeeding status as time-dependent covariates. These parameters are then used to estimate serum PBB levels outside of the range of measurements. For example, if a participant's elimination rate, or half-life, is 12 years, and their observed serum PBB level is 2 ppb in 1990, we would estimate their value in 1978 to be 4 ppb. In our study, 45% of the conception dates occurred before the mother's initial measurements while the remaining 55% of conceptions occurred after the initial measurements. The validated elimination model was confirmed by comparing results from a previously developed ordinary least squares (OLS) model (Terrell et al., 2008). For purposes of analysis, we categorized in-utero PBB exposure into low (PBB $\leq$1.0 ppb; values at or below LOD), medium (PBB $>$1.0–3.0 ppb) and high exposure (PBB $>$3.0 ppb) based on previous analyses in the Michigan PBB registry (Howards et al., 2019).

2.4. Outcome assessment

Menstrual cycle function outcomes included cycle-level characteristics: cycle length, menses length, and follicular and luteal phase lengths, and 12 endocrine endpoints. All of these 16 endpoints were determined by a combination of diary data and urinary hormone levels. In this study, onset of menses was defined using an algorithm requiring two consecutive days of bleeding where one of the days may be spotting. The first and last day of menses had to be preceded and followed by at least three days without bleeding, respectively. Bleed length was defined as the onset of menses to the day before the three consecutive days without bleeding. If this three-day rule was broken, the duration for menses was not calculated. This algorithm used to determine menses length reliably distinguishes mid-cycle spotting from onset of menses for most women (Jukic et al., 2008). Cycle length was defined as the number of days from the first day of one menses through the day before the onset of next menses. 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 (Baird et al., 1991). Additional samples were analyzed

if DLT was ambiguous to investigate whether ovulation occurred outside the 17-day window and if luteal phase days 5 and 6 were outside this window. If no DLT was able to be identified and 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 DLT. Among the 117 remaining cycles, 2 cycles did not meet 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 (PBB >1.0–3.0 ppb) with above average cycle length (41 and 43 days, respectively). Cycle length was classified as missing when the day of one of the bracketing menses onsets was missing, luteal and follicular phase lengths were classified as missing for cycles without a known DLT or known day of menses onset.

All of the 12 hormone outcomes were 3-day geometric mean hormone levels, calculated during six timeframes. 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 DLT, 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 for these 12 hormone outcomes 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 DLT. These hormone outcomes were adapted from definitions proposed by Baird et al. that were shown to be related to conception (Baird et al., 1991). 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.

2.5. Statistical analysis

We summarized participant characteristics according to their mother's estimated serum PBB level when the participant was in-utero. We assessed confounding using a priori knowledge in combination with directed acyclic graphs (DAGs) and descriptive statistics from the Michigan PBB registry. 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 our study participants were White, we did not 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 of the study participants because the cycle and hormonal outcomes are known to change with increasing age. 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 each of the outcomes by PBB exposure level for the average age of the women in the study (27.5 years).

3. 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 women and cycles utilized in the analysis varied by outcome, ranging from 23 women and 42 cycles for average $E_1$3G in the follicular phase to 39 women and 143 cycles for bleed length. The majority of women were younger than 35 years (90%), had a normal BMI (56%), were employed at least part-time (73%), had at least some college education (84%), were never smokers (78%), and were nulligravid (54%) (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. There were some other demographic and lifestyle characteristics that varied across categories of in-utero PBB exposure, however, none did so in a consistent direction. Between the two phases, those in phase 1 tended to be more highly exposed (>3.0 ppb: 45.5%) compared to phase 2 participants (>3.0 ppb: 22.2%) and phase 2 participants tended to be older (36–40 years: 44.5%) than phase 1 participants (36–40 years: 3.0%) (Supplemental Table 1).

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 time windows (Table 2). However, we only observed a meaningful, monotonic 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 urinary 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) μg/mg creatinine. 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 μg/mg) as compared to women with low exposure (6.3 μg/mg), while women in the highest exposure category had intermediate levels (8.3 μg/mg). After further adjustment for current BMI, which may or may not be on the causal pathway between in-utero PBB exposure and urinary

Table 1
Characteristics of participants by in-utero polybrominated biphenyl exposure level (N = 41).

	In-utero PBB Exposure Level		
	≤1.0 ppb	1.1–3.0 ppb	>3.0 ppb
	n = 10 (24.4%)	n = 15 (36.6%)	n = 16 (39.0%)
Age			
20–25 years	5 (50.0)	3 (20.0)	6 (37.5)
26–35 years	3 (30.0)	11 (73.3)	9 (56.3)
36–40 years	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)

Data are presented as N (%), unless otherwise noted.
Abbreviations: ppb, parts per billion.

Table 2
Predicted mean cycle-level outcomes for a 28-year-old woman by in-utero PBB exposure.

			In-utero PPB Exposure Level					
			≤1.0 ppb		1.1–3.0 ppb		>3.0 ppb	
	Women	Cycles	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)
Cycle Characteristics (days)								
Menstrual cycle length	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)
Menses length	39	143	5.6	(4.7, 6.4)	5.9	(5.3, 6.6)	5.7	(5.0, 6.3)
$E_1$3G (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, 38.0)	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)								
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)	14.0	(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.
Abbreviations: CI, confidence interval; Cr, creatinine; DLT, days of luteal transition; $E_1$3G, 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 (≤1.0 ppb) at an alpha level of 0.05.

progesterone levels, we still observed statistically significant associations (Supplemental Table 2).

There were no major differences in luteal phase length or menses length by categories of in-utero PBB exposure (Table 2). Cycle length and follicular phase length were slightly shorter among women with low in-utero PBB exposure levels, compared to women with medium exposure, although the 95% CIs were wide. We observed slightly higher age- and creatinine-adjusted 3-day mean follicular and luteal phase maximum $E_1$3G 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 imprecise. There were no noticeable differences in age- and creatinine-adjusted mean $E_1$3G 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.

4. Discussion

In our prospective study of 41 women exposed in-utero to PBB through a food contamination event in Michigan in the 1970s, we found suggestive evidence that higher in-utero exposure to PBB was associated with slightly higher maximum progesterone levels during the luteal phase of the menstrual cycle. Other menstrual cycle characteristics, however, were not statistically significantly associated with in-utero PBB exposure. Observing elevated progesterone levels is interesting given its potential association with increased risk of breast cancer (Coelingh Bennink et al., 2023). In the Michigan PBB registry, we have observed evidence for an association between direct PBB exposure during childhood and adulthood and higher risk of breast cancer (Henderson et al., 1995; Terrell et al., 2016). Although speculative, our results could be one of many potential mechanisms through which PBB exposure leads to elevated breast cancer risk. In addition, we observed some differences in adult demographic characteristics by in utero PBB exposure. For example, we observed those with medium and high in utero exposure to PBB had a slighter higher adult BMI compared to those with low in-utero exposure. These findings are particularly interesting given the potential association between obesity and progesterone (Faulkner et al., 2019).

To our knowledge, only one other study has investigated PBB exposure and menstrual cycle function (Howards et al., 2019). This previous study came from the same Michigan PBB registry of women eligible for this study, but instead focused on women who had been exposed to PBB through diet during childhood. Howards et al., found that women with high (>3.0 ppb) PBB exposure during childhood had lower $E_1$3G and Pd3G levels across the menstrual cycle and lower FSH levels during the follicular phase as compared to women with low childhood PBB exposure (≤1.0 ppb) (Howards et al., 2019). Those findings for Pd3G levels, in particular, are quite different from 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.[17] It's also worth noting that in addition to the 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 Phase 1 and 2 did not overlap.

There is also a limited, but relevant, literature on the impact of in-utero exposure to similar persistent, endocrine disrupting chemicals such as polychlorinated biphenyls (PCBs) and per- and polyfluoroalkyl substances (PFAS) on menstrual cycle function. For example, a comparable study from Taiwan, which evaluated menstrual cycle function in adolescent daughters of women exposed to PCB-contaminated cooking oil found that higher in-utero PCB exposure was associated with increased estradiol and FSH levels and shortened bleeding periods (Yang et al., 2005). In contrast, when the exposed mothers were examined, the authors found very few differences in menstrual cycle function associated with PCB levels, with the exception of longer bleeding periods (Yang et al., 2011). 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. However, similar to our dataset, these studies were small and the differences could be due to small sample size and the wide natural variability of hormones. 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 (Kristensen et al., 2013), increased testosterone concentrations (Maisonet et al., 2015), and reduced dehydroepiandrosterone (DHEA) concentrations (Jensen et al., 2020) 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, the same class of chemicals as PBB, was related to early onset of puberty and increased incidence of multi-oocyte follicles and that this was likely due to the downregulation of pathways that are fundamental for ovarian function like hypoxia inducible factor 1 subunit alpha (HIF1A), CAMP responsive element binding protein 1 (CREB1), epidermal growth factor (EGF), β-estradiol, and peroxisome proliferator-activated receptor (PPAR) pathways (Allais et al., 2020). While their 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 (Jin et al., 2019). 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 (Li et al., 2020) and the other on prenatal PCB & DDT exposure in rats (Jonsson et al., 1975), 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 hypothalamic-pituitary-ovarian 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 h PCB153 exposure and a concentration dependent increase in progesterone secretion after 72 h of exposure(Augustowska et al., 2001), 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, provide daily urine samples, and show up for an in person visit for a blood draw, we had a limited number of participants that were eligible and willing to participate in the study. Therefore, our results must 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) or unmeasured confounding. 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. In addition, we utilized strict eligibility criteria because several factors (e.g., hormonal contraception, endometriosis, cancer diagnosis and treatment, etc.) can influence the menstrual cycle. Therefore, our results may not be generalizable to individuals with these conditions or treatments and would miss any potential associations between PBB and menstrual cycle dysfunction mediated by these conditions. Conversely, because of our low power, it is possible that we failed to detect small but clinically meaningful differences in menstrual cycle characteristics by levels of in-utero PBB exposure. An additional weakness was that maternal PBB concentrations were not directly measured during pregnancy but rather estimated using a PBB elimination model (Terrell et al., 2008), 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. Our sample size varied due to missing data, most often due to missing urine samples, which further limited the precision of some analyses. Lastly, it is possible that women included in our sample were going through perimenopausal shift which would have altered cycle length or regularity. However, given that 90% of our sample was 35 or younger (n = 37) and thus far there is no evidence to suggest that PBB increases the risk of early menopause, it is unlikely that many women, if any, were perimenopausal.

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 (0.58, 95% CI: 0.44 to 0.96, N = 10) (Sjodin et al., 2008; National Center for Health Statistics, 2018). It is possible that heterogeneity in our low exposure group may have masked differences between the lowest and highest exposure groups. 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 the daughters of affected residents of Michigan.

In conclusion, women who were exposed to higher levels of PBB in-utero had slightly higher urinary progesterone metabolite 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, cycle phase lengths, menses length, and urinary concentrations of estrogen and FSH, however, were largely unassociated with in-utero PBB exposure. Given our modest sample size, our results require cautious interpretation. Furthermore, because there are no standardized norms to compare our urinary hormone concentrations to, it is challenging to discern whether our findings for urinary Pd3G are being driven by abnormally low levels in women in the lowest exposure group or truly higher than average high Pd3G values in the medium and high exposure groups. While the production of PBB has decreased or ceased in most countries, our results may be still relevant due to the continued production of chemically related brominated flame retardants worldwide.

Funding

Our study was funded by grants from the National Institute of Environmental Health Sciences (R01 ES012014; R01 ES025775; R24 ES028528; P30 ES019776). Dr. Hood was funded by a NIEHS T32 Training grant (T32 ES012870).

Supplemental Table 1
Characteristics of participants by study phase (N = 41).

	All Phases N = 41	Phase I N = 33	Phase II N = 9
In-utero PBB Exposure Level			
≤1.0 ppb	10 (24.4)	8 (24.2)	2 (22.2)
1.1–3.0 ppb	15 (36.6)	10 (30.3)	5 (55.6)
>3.0 ppb	16 (39.0)	15 (45.5)	2 (22.2)
Age			
20–25 years	14 (34.1)	12 (36.4)	2 (22.2)
26–35 years	23 (56.1)	20 (60.6)	3 (33.3)
36–40 years	4 (9.8)	1 (3.0)	4 (44.5)
Education (Missing = 4)			
High school or less	6 (16.2)	5 (17.3)	1 (11.1)
Some college or technical school	9 (24.3)	7 (24.1)	2 (22.2)
College graduate or higher	22 (59.5)	17 (58.6)	6 (66.7)
Income (Missing = 3)			
< $20,000/year	11 (28.9)	9 (30.0)	2 (22.2)
$20,000-$50,000/year	12 (31.6)	10 (33,3)	2 (22.2)
>$50,000/year	15 (39.5)	11 (36.7)	5 (55.6)
Employment Status			
Unemployed, homemaker, student	11 (26.8)	10 (30.3)	1 (11.1)
Employed part-time or full-time	30 (73.2)	23 (69.7)	8 (88.9)
Gravidity			
Nulligravid	22 (53.7)	17 (51.5)	5 (55.6)
≥1 prior pregnancy	19 (46.3)	16 (48.5)	4 (44.4)
Age at menarche (Missing = 1)			
11 years	6 (15.0)	4 (12.1)	2 (25.0)
12 years	14 (35.0)	12 (36.4)	3 (37.5)
≥13 years	20 (50.0)	17 (51.5)	3 (37.5)
BMI			
18.0–24.9 kg/m^2	23 (56.1)	17 (51.5)	6 (66.7)
25.0–29.9 kg/m^2	10 (24.4)	10 (30.3)	0 (0.0)
30.0–43.4 kg/m^2	8 (19.5)	6 (18.2)	3 (33.3)
Smoking Status*			
Never	32 (78.0)	25 (75.8)	8 (88.9)
Past or Current Smoker	9 (22.0)	8 (24.2)	1 (11.1)
Maternal Smoking Status (Missing = 2)			
No	36 (92.3)	29 (90.6)	8 (100.0)
Yes	3 (7.7)	3 (9.4)	0 (0.0)

One person took part in both Phase I and Phase II.
Data are presented as N (%), unless otherwise noted.
Abbreviations: ppb, parts per billion.

Supplemental Table 2
Predicted mean urinary progesterone levels by in-utero PBB exposure levels with further adjustment for current BMI.

	Women	Cycles	In-utero PPB Exposure Level					
			≤1.0 ppb		1.1–3.0 ppb		>3.0 ppb	
			Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)
Pd3G (ug/mg Cr)								
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)
Further adjusted for BMI	30	67	9.7	(4.9, 14.5)	14.7	(11.4, 17.9)	15.9*	(12.7, 19.2)
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)
Further adjusted for BMI	35	84	6.4	(3.2, 9.5)	10.4*	(8.1, 12.7)	8.3	(6.1, 10.5)

Predicted means are for a 28-year-old woman from models including age as a continuous variable centered on 28 years.
Abbreviations: CI, confidence interval; Cr, creatinine; DLT, days of luteal transition; $E_1$3G, 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 (≤1.0 ppb) at an alpha level of 0.05.

Declaration of competing interest

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health (NIOSH). Mention of any company or product does not constitute endorsement by NIOSH. The authors have no conflicts of interest to declare.

Acknowledgements

We thank the members of the Michigan PBB Research Registry who participated in this research and further contributed to the understanding of the impacts of this large-scale chemical contamination on health. We express our gratitude to the PBB Citizens Advisory Board, the Pine River Citizen Superfund Task Force, and the Mid-Michigan District Health Department for their continued partnership in the PBB Research and their efforts to ensure that the research is relevant to the affected community and that research findings are shared with and

translated into benefits for the PBB community.

References

Allais, A., Albert, O., Lefevre, P.L.C., et al., 2020. Utero and lactational exposure to flame retardants disrupts rat ovarian follicular development and. Adv. Pub. Toxicol. Sci. 175, 197–209. https://doi.org/10.1093/toxsci/kfaa044.

Augustowska, K., Wojtowicz, A., Kajta, M., et al., 2001. Polichlorinated biphenyls (PCB126 and PCB 153) action on proliferation and progesterone secretion by cultured in vitro porcine luteal cells. Exp. Clin. Endocrinol. Diabetes 109, 416–418. https://doi.org/10.1055/s-2001-18996.

Baird, D.D., Weinberg, C.R., Wilcox, A.J., et al., 1991. Using the ratio of urinary oestrogen and progesterone metabolites to estimate day of ovulation. Stat. Med. 10, 255–266. https://doi.org/10.1002/sim.4780100209.

Blanck, H.M., Marcus, M., Tolbert, P.E., et al., 2000. Age at Menarche and Tanner Stage in Girls Exposed in Utero and Postnatally to Polybrominated Biphenyl Epidemiology, vol. 11, pp. 641–647. https://doi.org/10.1097/00001648-200011000-00005.

Burse, V.W., Needham, L.L., Liddle, J.A., et al., 1980. Interlaboratory comparison for results of analyses for polybrominated biphenyls in human serum. J. Anal. Toxicol. 4, 22–26. https://doi.org/10.1093/jat/4.1.22.

Carter, L.J., 1976. Michigan's PBB incident: chemical mix-up leads to disaster. Science 192, 240–243. https://doi.org/10.1126/science.192.4236.240.

Chang, C.J., Terrell, M.L., Marcus, M., et al., 2020. Serum concentrations of polybrominated biphenyls (PBBs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in the Michigan PBB Registry 40 years after the PBB contamination incident. Environ. Int. 137, 105526 https://doi.org/10.1016/j.envint.2020.105526.

Coelingh Bennink, H.J.T., Schultz, I.J., Schmidt, M., et al., 2023. Progesterone from ovulatory menstrual cycles is an important cause of breast cancer Breast Cancer. Res. 25, 60. https://doi.org/10.1186/s13058-023-01661-0.

Curtis, S.W., Cobb, D.O., Kilaru, V., et al., 2019. Exposure to Polybrominated Biphenyl (PBB) Associates with Genome-wide DNA Methylation Differences in Peripheral Blood Epigenetics, vol. 14, pp. 52–66. https://doi.org/10.1080/15592294.2019.1565590.

Davis, S.I., Blanck, H.M., Hertzberg, V.S., et al., 2005. Menstrual function among women exposed to polybrominated biphenyls: a follow-up prevalence study. Environ. Health 4, 15. https://doi.org/10.1186/1476-069x-4-15.

Faulkner, J.L., Kennard, S., Huby, A.C., et al., 2019. Progesterone predisposes females to obesity-associated leptin-mediated endothelial dysfunction via upregulating endothelial MR. Mineralocort. Recept.) Express. Hyperten. 74, 678–686. https://doi.org/10.1161/hypertensionaha.119.12802.

Fries, G.F., 1985. The PBB episode in Michigan: an overall appraisal. Crit. Rev. Toxicol. 16, 105–156. https://doi.org/10.3109/10408448509056268.

Givens, M.L., Small, C.M., Terrell, M.L., et al., 2007. Maternal exposure to polybrominated and polychlorinated biphenyls: infant birth weight and gestational age. Chemosphere 69, 1295–1304. https://doi.org/10.1016/j.chemosphere.2007.05.031.

Henderson, A.K., Rosen, D., Miller, G.L., et al., 1995. Breast Cancer Among Women Exposed to Polybrominated Biphenyls Epidemiology, vol. 6, pp. 544–546. https://doi.org/10.1097/00001648-199509000-00014.

Hood, R.B., Terrell, M.L., Smith, A.K., et al., 2023. Elimination of PBB-153; findings from a cohort of Michigan adults. Environ. Res. 220, 115146 https://doi.org/10.1016/j.envres.2022.115146.

Howards, P.P., Terrell, M.L., Jacobson, M.H., et al., 2019. Polybrominated biphenyl exposure and menstrual cycle function. Epidemiology 30, 687–694. https://doi.org/10.1097/ede.0000000000001045.

Jensen, R.C., Glintborg, D., Gade Timmermann, C.A., et al., 2020. Prenatal exposure to perfluorodecanoic acid is associated with lower circulating concentration of adrenal steroid metabolites during mini puberty in human female infants. Odense Child Cohort Environ. Res. 182, 109101 https://doi.org/10.1016/j.envres.2019.109101.

Jin, C., Zhang, R., Fu, Z., et al., 2019. Maternal exposure to imazalil disrupts the endocrine system in F1 generation mice. Mol. Cell. Endocrinol. 486, 105–112. https://doi.org/10.1016/j.mce.2019.03.002.

Jonsson Jr., H.T., Keil, J.E., Gaddy, R.G., et al., 1975. Prolonged ingestion of commercial DDT and PCB; effects on progesterone levels and reproduction in the mature female rat. Arch. Environ. Contam. Toxicol. 3, 479–490. https://doi.org/10.1007/BF02220818.

Jukic, A.M., Weinberg, C.R., Wilcox, A.J., et al., 2008. Accuracy of reporting of menstrual cycle length. Am. J. Epidemiol. 167, 25–33. https://doi.org/10.1093/aje/kwm265.

Kesner, J.S., Knecht, E.A., Krieg Jr., E.F., et al., 1994. Validations of Time-Resolved Fluoroimmunoassays for Urinary Estrone 3-glucuronide and Pregnanediol 3-glucuronide Steroids, vol. 59, pp. 205–211. https://doi.org/10.1016/0039-128x(94)90029-9.

Kristensen, S.L., Ramlau-Hansen, C.H., Ernst, E., et al., 2013. Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. Hum. Reprod. 28, 3337–3348. https://doi.org/10.1093/humrep/det382.

Landrigan, P.J., Wilcox Jr., K.R., Silva Jr., J., et al., 1979. Cohort study of Michigan residents exposed to polybrominated biphenyls: epidemiologic and immunologic findings. Ann. N. Y. Acad. Sci. 320, 284–294. https://doi.org/10.1111/j.1749-6632.1979.tb56611.x.

Li, K., Liszka, M., Zhou, C., et al., 2020. Prenatal exposure to a phthalate mixture leads to multigenerational and transgenerational effects on uterine morphology and function in mice. Reprod. Toxicol. 93, 178–190. https://doi.org/10.1016/j.reprotox.2020.02.012.

Maisonet, M., Calafat, A.M., Marcus, M., et al., 2015. Prenatal exposure to perfluoroalkyl acids and serum testosterone concentrations at 15 Years of age in female ALSPAC study participants. Environ. Health Perspect. 123, 1325–1330. https://doi.org/10.1289/ehp.1408847.

National Center for Health Statistics, 2018. National Health and Nutrition Examination Survey, 2003– 2004. Center for Disease Control and Prevention. https://wwwn.cdc.gov/nchs/nhanes/Search/DataPage.aspx?Component=Laboratory&CycleBeginYear=2003. (Accessed 28 April 2021).

Needham, L.L., Burse, V.W., Price, H.A., 1981. Temperature-programmed gas chromatographic determination of polychlorinated and polybrominated biphenyls in serum. J. Assoc. Off. Anal. Chem. 64, 1131–1137.

Sjodin, A., Wong, L.Y., Jones, R.S., et al., 2008. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. Environ. Sci. Technol. 42, 1377–1384. https://doi.org/10.1021/es702451p.

Small, C.M., Murray, D., Terrell, M.L., et al., 2011. Reproductive outcomes among women exposed to a brominated flame retardant in utero. Arch. Environ. Occup. Health 66, 201–208. https://doi.org/10.1080/19338244.2010.539640.

Small, C.M., Terrell, M.L., Cameron, L.L., et al., 2009. Utero exposure to a brominated flame retardant and male growth and development. Int. J. Child. Adolesc. Health 2.

Terrell, M.L., Hartnett, K.P., Lim, H., et al., 2015. Maternal exposure to brominated flame retardants and infant Apgar scores. Chemosphere 118, 178–186. https://doi.org/10.1016/j.chemosphere.2014.08.007.

Terrell, M.L., Manatunga, A.K., Small, C.M., et al., 2008. A decay model for assessing polybrominated biphenyl exposure among women in the Michigan Long-Term PBB Study. J. Expo. Sci. Environ. Epidemiol. 18, 410–420. https://doi.org/10.1038/sj.jes.7500633.

Terrell, M.L., Rosenblatt, K.A., Wirth, J., et al., 2016. Breast cancer among women in Michigan following exposure to brominated flame retardants. Occup. Environ. Med. 73, 564–567. https://doi.org/10.1136/oemed-2015-103458.

United States Environmental Protection Agency, 2017. Technical Fact Sheet – Polybrominated Biphenyls (PBBs). EPA. https://www.epa.gov/sites/default/files/2017-12/documents/ffrro_factsheet_pbb_11-16-17_508.pdf.

Yang, C.Y., Huang, T.S., Lin, K.C., et al., 2011. Menstrual effects among women exposed to polychlorinated biphenyls and dibenzofurans. Environ. Res. 111, 288–294. https://doi.org/10.1016/j.envres.2010.11.011.

Yang, C.Y., Yu, M.L., Guo, H.R., et al., 2005. The endocrine and reproductive function of the female Yucheng adolescents prenatally exposed to PCBs. PCDFs Chemosphere 61, 355–360. https://doi.org/10.1016/j.chemosphere.2005.02.089.