

Brominated flame retardants and organochlorine pesticides and incidence of uterine leiomyomata

A prospective ultrasound study

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Background: Uterine leiomyomata (UL) are hormone-responsive benign neoplasms. Brominated flame retardants and organochlorine pesticides (OCPs) can disrupt hormones involved in UL etiology.

Methods: The Study of Environmental, Lifestyle, and Fibroids is a Detroit-area prospective cohort of 1693 Black women 23–35 years of age. At baseline and approximately every 20 months for 5 years, women completed questionnaires and underwent transvaginal ultrasounds. Using a case-cohort study design, we selected 729 UL-free participants at baseline and analyzed baseline plasma samples for polybrominated diphenyl ethers (PBDEs), a polybrominated biphenyl ether (PBB-153), and OCPs. We used Cox proportional hazard models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: Compared with total PBDE plasma concentrations <50th percentile, adjusted HRs for the 50th–74th, 75th–89th, and ≥90th percentiles were 1.00 (95% CI = 0.68, 1.47), 1.04 (95% CI = 0.63, 1.68), and 0.85 (95% CI = 0.48, 1.50), respectively. HRs for PBB-153 plasma concentrations were generally similar to total PBDE plasma concentrations. Compared with total OCP plasma concentrations <50th percentile, HRs for the 50th–74th, 75th–89th, and ≥90th percentiles were 0.86 (95% CI = 0.57, 1.29), 0.73 (95% CI = 0.43, 1.22), and 0.58 (95% CI = 0.32, 1.04), respectively. HRs for individual PBDEs and OCPs were similar to their respective totals.

Conclusion: We found little support for an association between brominated flame retardant plasma concentrations and UL incidence, and some evidence of lower UL incidence with the highest OCP plasma concentrations.

Key Words: Black women; Chemicals; Dichlorodiphenyltrichloroethane; Fibroids; Flame retardants; Pesticides

Introduction

Uterine leiomyomata (UL), or fibroids, are hormone-dependent benign neoplasms of the myometrium. UL are clinically-diagnosed in 25%–30% of reproductive-aged women^{1–3} and can cause severe gynecologic morbidity such as heavy

menstrual bleeding, pelvic pain, and infertility.^{4,5} US Black women have two to three times the clinical incidence of White women and present with greater symptom severity.^{6–8} Despite their declining concentrations over time,^{9,10} brominated flame retardants and organochlorine pesticides (OCPs) are lipophilic and endocrine-disrupting, and can persist in the body long after exposure. Few epidemiologic studies have investigated the association between exposure to brominated flame retardants and OCPs with UL,^{11,12} and none have used a prospective study design or examined a population at high-risk of UL.

Polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs) were once widely used as additive flame retardants (e.g., furniture and plastics). Due to concerns about their environmental persistence and impacts on human health, the US began to discontinue the use of PBDEs in 2004, and discontinued the use of PBBs starting in the late 1970s. Current routes of exposure to PBDEs occur primarily via indoor air and dust in environments containing previously-treated materials.^{13–21} Half-lives of PBDEs in the human body range from less

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What this study adds

Our study is the first prospective ultrasound study evaluating the effects of brominated flame retardants and organochlorine pesticides on incidence of uterine leiomyomata. Black US women are a population at high risk of uterine leiomyomata and are under prioritized in the environmental health literature. Our findings suggest a lower incidence of uterine leiomyomata with higher organochlorine pesticide plasma concentrations, potentially via antiestrogenic pathways. We found no evidence of an association between brominated flame retardant plasma concentrations and incidence of uterine leiomyomata.

than a year (PBDE-28) to 4 years (PBDE-153).²² There are no known natural sources of PBBs, and the general US population are exposed from historical releases such as from previously treated products, hazardous waste sites, spills, or accidents.²³ In the United States, exposure to PBBs occurred predominantly in the 1970s when several thousand pounds of a commercial PBB mixture were inadvertently added to animal feed and distributed to Michigan farms. The primary constituent of that mixture, PBB-153, can persist in the human body for decades after exposure.^{24,25}

In the United States, the insecticide dichlorodiphenyltrichloroethane (DDT) was one of the most widely-used OCPs before it was banned in 1972. Other OCPs remained in use into the late 1980s. Despite such bans, OCPs are detectable in much of the U.S. population,⁹ the food chain^{26,27} and the ecosystem.^{28–31} Some OCPs have half-lives of up to 7 years after exposure.³² In 2001, the United States joined over 90 other nations to protect human health and the environment from the effects of OCPs. As of February 2019, the United States had yet to ratify this agreement, citing its lack of authority to implement all of its provisions.³³ OCPs, PBDEs, and PBB-153 have similar chemical structures and are all lipophilic persistent environmental chemicals. The three are also commonly reported together in national biomonitoring reports.⁹

Estrogen is believed to play a prominent role in UL development.³⁴ UL cells exhibit more estrogen receptors compared to normal myometrium.^{35,36} In vitro studies using human breast tissue show that PBDE congeners can exhibit both estrogenic (e.g., PBDE-100) and anti-estrogenic activity (e.g., PBDE-153).³⁷ Higher PBB serum concentrations are inversely associated with urinary estrogen biomarkers in premenopausal women,³⁸ which we would expect to reduce UL risk.³⁴ Higher PBB serum concentrations are also associated with earlier puberty timing,³⁹ an established risk factor for UL.³⁴ OCPs exhibit anti-estrogenic activity by inhibiting estradiol secretion in pigs⁴⁰ and can interfere with cell proliferation of human endometrial cells.⁴¹

There have been two studies examining the association of PBDEs and OCPs with UL, both case-control studies.^{11,12} Neither study measured chemical concentrations before UL incidence, nor did they systematically screen the control group for UL. Cases of UL tended to have higher PBDEs and OCP concentrations in adipose tissue compared to controls,^{11,12} but inverse or null associations were observed with serum concentrations of the same chemicals.¹¹ In 1976, the Michigan Department of Community Health began enrolling individuals exposed to PBBs and their children.^{39,42} That data has resulted in some evidence of an inverse association between PBB serum concentrations and self-reported UL.⁴³

Using data from a prospective cohort study of reproductive-aged Black women, we prospectively examined associations of plasma concentrations of PBDEs, PBB-153, and OCPs with incidence of ultrasound-detected UL.

Methods

Study design and population

The Study of Environment, Lifestyle, and Fibroids (SELF) is a prospective cohort study of 1693 reproductive-aged Black women residing in the Detroit, Michigan area during 2010–2012, as described in detail elsewhere.⁴⁴ Eligible participants were 23–34 years of age and self-identified as African American, Black, or partly African American. Exclusion criteria included prior clinical diagnosis of UL, cancer, or treatment for autoimmune disease. Participants who were pregnant at recruitment had enrollment delayed until at least 3 months postpartum. At baseline and approximately every 20 months for 5 years (median dates: January 27, 2012, August 29, 2013, March 26, 2015, and December 3, 2016), women completed questionnaires and underwent transvaginal ultrasound for UL detection.

For study of selected chemicals and incidence of UL, we used a case-cohort study design.⁴⁵ We analyzed data from 572 participants who were randomly selected from the 1308 UL-free participants at baseline for measurement of brominated flame retardant and OCP plasma concentrations (Figure 1), and supplemented the analytic population with all incident cases of UL that accrued outside of the subcohort (N = 157). The final analytic sample size was 729 women. Without jeopardizing statistical precision, the case-cohort approach allows for prospective exposure assessment and reduces costs from expensive biomarker assays. A comparison of key demographics comparing the full SELF cohort and the subcohort were similar (Supplemental Table 1; <http://links.lww.com/EE/A115> in Bethea et al, 2019).⁴⁶

The Institutional Review Boards of the Henry Ford Health System, National Institute of Environmental Health Sciences, and Boston University Medical Campus approved the study. The involvement of the Centers for Disease Control and Prevention (CDC) did not constitute engagement in human subjects' research. All participants provided informed consent.

Measurement of brominated flame retardants and organochlorine pesticides in plasma

At baseline, participants provided non-fasting blood samples. Samples were shipped to the National Institute of Environmental Health Sciences repository for storage at -80°C , and then shipped on dry ice in three batches to the CDC for analysis. High-resolution gas chromatography/isotope dilution high-resolution mass spectrometry⁴⁷ was used to measure baseline plasma concentrations of eleven PBDE congeners: 2,2',4-tribromodiphenyl ether (PBDE-17), 2,4,4' tribromodiphenyl ether (PBDE-28), 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47), 2,3',4',4-tetrabromodiphenyl ether (PBDE-66), 2,2',3,4,4'-pentabromodiphenyl ether (PBDE-85), 2,2',4,4',5-pentabromodiphenyl ether (PBDE-99), 2,2',4,4',6-pentabromodiphenyl ether (PBDE-100), 2,2',4,4',5,5'-hexabromodiphenyl ether (PBDE-153), 2,2',4,4',5,6'-hexabromodiphenyl ether (PBDE-154), 2,2',3,4,4',5',6-heptabromodiphenyl ether (PBDE-183), and decabromodiphenylether (PBDE-209). We also measured baseline plasma concentrations of 2,2',4,4',5,5'-hexabromobiphenyl (PBB-153) and nine OCPs: 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (*p,p'*-DDE), 2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethane (*o,p'*-DDT), 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (*p,p'*-DDT), hexachlorobenzene (HCB), β -hexachlorocyclohexane (β -HCH), γ -hexachlorocyclohexane (lindane; γ -HCH), oxychlorane, trans-nonachlor, and mirex.

The choice of brominated flame retardants and OCPs was informed by their prevalence of exposure in humans,^{9,48} and evidence of their effects on reproductive hormones and processes that could influence UL risk.^{11,12,49–52} We measured total plasma lipid concentrations using an enzymatic summation method⁵³ and reported all chemical concentrations on a lipid-adjusted basis (ng/g lipid). Laboratory personnel distributed quality control samples and reagent blanks, and coefficient of variation estimates for QC samples ranged from 3.3% to 13.9%.⁵⁴

We have previously published on the distributions of baseline plasma brominated flame retardant and OCP concentrations in our data.^{55,56} Briefly, when compared to data from the National Health and Nutrition Examination Survey,^{9,10} distributions of brominated flame retardants and OCPs in our data were generally comparable with, or slightly lower than, estimates from US Black women of a similar age range. With the exception of PBDE-153, PBDE congeners were moderately or highly correlated (Spearman correlation coefficients ranged from 0.53 to 0.95). PBDE-209 and PBB-153 weakly correlated with all other PBDE congeners (≤ 0.25). Among the OCPs, correlations were generally moderate to high (0.51–0.86).

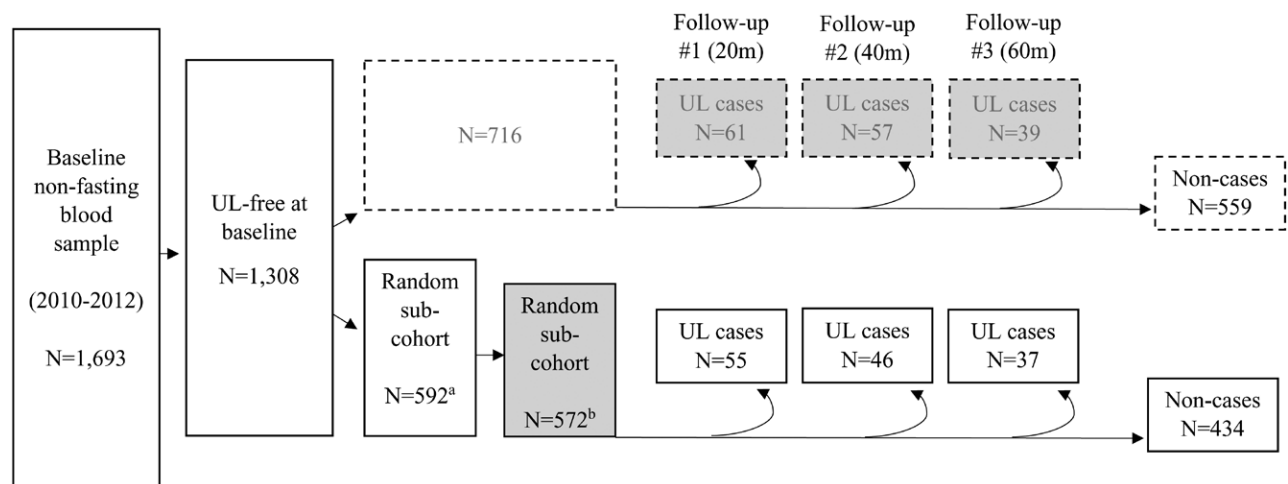


Figure 1. Case-cohort study design for the analysis of endocrine-disrupting chemical plasma concentrations and incidence of uterine leiomyomata (UL) in SELF. Dashed boxes reflect individuals outside of the random subcohort. Shaded boxes reflect the 729 individuals analyzed for baseline brominated flame retardant plasma concentrations and used in analyses. ^aPlasma for 20 of the 592 women were not available for analysis. ^bTwenty-three of the 572 women were missing follow-up data, 8 of whom were imputed as UL cases (4 at follow-up #1, 2 at follow-up #2, and 2 at follow-up #3).

Measurement of incident uterine leiomyomata

At baseline and at each follow-up visit, participants underwent transvaginal ultrasound by experienced sonographers for the detection of UL ≥ 0.5 cm in diameter; this is the limit of reliable detection and a standard cutoff for ultrasound research on UL.^{6,57,58} Transvaginal ultrasounds have high sensitivity and specificity compared with histologic evidence.⁵⁹ Additional details regarding UL detection, classification, and quality control assessments based on archived images are described elsewhere.⁴⁴

Covariate assessment

We collected demographic, behavioral, dietary, occupational, and medical history data via self-administered questionnaires, telephone interviews, and in-person clinic visits. For our analyses, we used the data collected at baseline, unless otherwise specified. Women completed computer-assisted interviews and questionnaires, reporting information on education, annual household income, smoking history, alcohol intake in the past year, age at menarche, contraceptive use, and childbearing and lactation history. A separate self-administered questionnaire elicited data on early-life exposures, including number of months they were breastfed in infancy. Around 85% of participants had the help of their mother to answer these questions and self-reported breastfeeding practices are highly reliable.^{60,61} At baseline, women also completed a computer-based, semi-quantitative food frequency questionnaire⁶² to provide information about their average dietary intake in the previous year. Baseline body mass index (BMI, kg/m²) was calculated from height and weight measured by technicians.

Statistical analysis

We used lipid-adjusted chemical concentrations provided by the CDC for all analyses. A priori, we restricted main analyses to chemicals with detection frequencies $\geq 60\%$: eight of the 11 PBDE congeners (PBDE-28, PBDE-47, PBDE-85, PBDE-99, PBDE-100, PBDE-153, PBDE-154, and PBDE-209), PBB-153, and four OCPs (*p,p'*-DDE, HCB, oxychlorthane, and trans-nonachlor) (Table 1). We summed across the eight PBDE congeners to evaluate total PBDE exposure, and summed across the four OCPs for total OCP exposure. We set values below the LOD to the LOD divided by the square root of two.⁶³

We used Cox proportional hazards regression models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Because baseline plasma concentrations of the selected chemicals were right-skewed, we created percentile categories of <50th (reference), 50th–74th, 75th–89th, and ≥ 90 th for each individual chemical, as well as total PBDEs and total OCPs.

Table 1.

Distribution of baseline endocrine-disrupting chemical plasma concentrations in SELF (2010–2012) (N = 729)

Chemical name	Median LOD	% detected	Median ^a (25th, 75th, 90th percentiles)
Brominated flame retardants			
PBDE-17	0.2	12.9	n/a
PBDE-28	0.2	95.3	0.8 (0.5, 1.3, 2.1)
PBDE-47	0.3	100.0	16.5 (9.1, 29.7, 53.3)
PBDE-66	0.4	9.2	n/a
PBDE-85	0.2	70.2	0.4 (0.2, 0.6, 1.3)
PBDE-99	0.3	98.9	3.0 (1.6, 5.5, 11.6)
PBDE-100	0.2	99.9	3.2 (1.9, 6.0, 11.5)
PBDE-153	0.2	100.0	4.4 (2.7, 8.0, 16.3)
PBDE-154	0.2	65.5	0.3 (0.2, 0.6, 1.1)
PBDE-183	0.4	22.2	n/a
PBDE-209	1.1	73.9	1.6 (1.0, 2.4, 3.9)
PBB-153	0.2	89.0	0.5 (0.3, 0.9, 1.7)
OCPs			
<i>p,p'</i> -DDT	1.4	49.8	n/a
<i>o,p'</i> -DDT	1.1	2.5	n/a
<i>p,p'</i> -DDE	1.4	100.0	51.2 (38.6, 69.3, 92.5)
HCB	1.4	74.5	5.9 (5.0, 7.2, 8.6)
β -HCH	1.1	36.2	n/a
γ -HCH (Lindane)	1.1	1.1	n/a
Oxychlorthane	1.1	88.5	2.1 (1.6, 2.8, 3.6)
Trans-nonachlor	1.2	91.4	2.4 (1.8, 3.4, 4.5)
Mirex	1.1	4.9	n/a

^aValues not presented for congeners with detection prevalence <60%.

LOD indicates limit of detection; n/a, not applicable; *o,p'*-DDT, 2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethane; *p,p'*-DDE, 2,2-bis(4-chlorophenyl)-1,1-dichloroethane; *p,p'*-DDT, 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane; PBB-153, 2,2',4,4',5,5'-hexabromobiphenyl; PBDE-100, 2,2',4,4',6-pentabromodiphenyl ether; PBDE-153, 2,2',4,4',5,5'-hexabromodiphenyl ether; PBDE-154, 2,2',4,4',5,6'-hexabromodiphenyl ether; PBDE-17, 2,2',4-tribromodiphenyl ether; PBDE-183, 2,2',3,4,4',5,6'-heptabromodiphenyl ether; PBDE-209, decabromodiphenyl ether; PBDE-28, 2,4,4'-tribromodiphenyl ether; PBDE-47, 2,2',4,4'-tetrabromodiphenyl ether; PBDE-66, 2,3',4',4'-tetrabromodiphenyl ether; PBDE-85, 2,2',3,4,4'-pentabromodiphenyl ether; PBDE-99, 2,2',4,4',5-pentabromodiphenyl ether.

Since there are no established cut-points for high plasma concentrations of these chemicals, we used these percentile categories to distinguish individuals with the highest chemical exposures while maintaining a high enough sample size to estimate reasonably precise associations. We explored quintile exposure categories and adjustment for dietary patterns in sensitivity analyses. For chemicals where the detection prevalence was less than 60% (PBDE-17, -66, -183, *p,p'*-DDT, *o,p'*-DDT, β -HCH, and γ -HCH), we created binary exposure categories (yes vs. no), using “not detected” as the referent group. For these low detection chemicals, we report results only for chemicals with 20 or more cases in the detection group. We used time in the study (in years) as the time scale and conditioned on age at baseline in 1-year intervals. This approach allowed for refined adjustment for these two important potential confounding variables (calendar time and age). We assumed that incident cases occurred, on average, halfway through the calendar year of detection.

We decided on covariates a priori using the literature and directed acyclic graphs (Supplemental Figure 1; <http://links.lww.com/EE/A115>). Covariates in models were: BMI (<25, 25–29, 30–34, ≥ 35 kg/m²), education (\leq high school/general education development [GED], some college, \geq college degree), income (<\$200,000, \$20,000–50,000, >\$50,000 USD), cigarette smoking (never, former, current <10 cigarettes/day, current ≥ 10 cigarettes/day), alcohol intake in the past year (low [< 10 drinks/year], moderate [6 drinks on days when they drink and no more than once per month intake of ≥ 4 drinks per sitting], heavy [≥ 6 drinks on days when they drink or ≥ 4 drinks per sitting at least twice a month]), age at menarche (≤ 10 , 11, 12, 13, ≥ 14 years), parity (0, 1, 2, ≥ 3 births), years since last birth (<2, 2–4, 5–9, ≥ 10), lactation history (none, 1–3 months, > 3 months), having been breastfed in infancy (none, < 3 months, ≥ 3 months), and total plasma lipids (mg/dl). O'Brien and colleagues⁶⁴ showed that adjustment for covariates and total lipids resulted in the least biased effect estimates relative to approaches that involved no lipid adjustment or only covariate adjustment. Given the strong association between exposure to breastfeeding in infancy and PBB-153 plasma concentrations in adulthood,⁵⁵ we further adjusted for breastfeeding in infancy in PBB-153 analyses only.

We conducted a sensitivity analysis among non-current users of depot medroxyprogesterone acetate (DMPA) (N = 683, 96% of 729), a strong determinant of UL. Due to increases in UL incidence with increasing premenopausal age, and to examine the possibility that the association between exposure to chemicals and UL incidence might vary with age (e.g., lead to earlier onset of UL), we stratified by median baseline age (<29 vs. ≥ 29 years). We also stratified by baseline BMI (<30 vs. 30–50 kg/m²) because these chemicals are lipophilic and inversely associated with BMI. We trimmed at the upper 95th percentile due to concerns of differential UL detection among individuals with the highest BMI. Due to small case numbers, we used the following exposure categories in sensitivity analyses: <50th, 50th–74th, and ≥ 75 th. To explore whether associations were impacted by co-exposure to multiple chemical classes (PBDEs, PBB-153, and OCPs), we additionally adjusted for each chemical class in sensitivity analyses (e.g., in total PBDE models we additionally adjusted for PBB-153 exposure and vice versa). A comprehensive mixtures assessment is forthcoming and therefore beyond the scope of this article.

We used multiple imputation to impute missing covariate, exposure, and outcome data. We generated five imputation datasets, and statistically combined estimates and standard errors across the datasets.⁶⁵ The percentage missing data for any given variable ranged from 0% (e.g., age) to 5% (e.g., breastfed in infancy). For the 23 women in our subcohort with no follow-up data (3.2%), we imputed their at-risk person-time (in years) and outcome status. This imputation resulted in eight imputed cases (Figure 1). We imputed plasma chemical concentrations for <4% of participants because their specimen measurements

did not meet CDC quality control criteria (N = 10 for PBDE-17, N = 8 for PBDE-85, N = 29 for HCB, N = 1 γ -HCH [lindane], N = 4 for oxychlorane, N = 1 for trans-nonachlor, N = 3 for *o,p'*-DDT, N = 4 for *p,p'*-DDE, and N = 22 for mirex). We used SAS version 9.4 (Cary, NC) for all statistical analyses.

Results

We identified 295 UL cases over the course of 2931 person-years of follow-up (Figure 1). Five participants were censored at their age of hysterectomy, and 429 participants showed no evidence of UL during follow-up and were censored at their last ultrasound visit (approximately 7% were censored before the end of follow-up).

At baseline, the mean age was 28.6 years and mean BMI was 33.6 kg/m². Approximately 60% of participants had a BMI ≥ 30 kg/m². Approximately 21% had ≤ 12 years of education, 46% reported an annual income of <\$20,000, 19% were current cigarette smokers, 20% were heavy alcohol consumers in the past year, and 62% were parous. Plasma PBDE concentrations were positively associated with lower education, lower annual income, heavy cigarette smoking, and heavy alcohol drinking (Table 2). Plasma PBB-153 concentrations were strongly positively associated with having been breastfed for ≥ 3 months in infancy (Table 2). BMI and total plasma lipids were generally inversely associated with plasma concentrations of PBDEs, PBB-153, and OCPs (Table 2).

Compared with the <50th percentile of total plasma PBDE concentrations, adjusted HRs for the 50th–74th, 75th–89th, and ≥ 90 th percentiles were 1.00 (95% CI = 0.68, 1.47), 1.04 (95% CI = 0.63, 1.68), and 0.85 (95% CI = 0.48, 1.50), respectively (Figure 2 and Supplemental Table 1; <http://links.lww.com/EE/A115>). HRs for individual plasma PBDE concentrations (≥ 90 th vs. <50th percentile) ranged from 0.80 for PBDE-154 (95% CI = 0.44, 1.45) to 1.19 for PBDE-28 (95% CI = 0.69, 2.05) (Figure 2 and Supplemental Table 1; <http://links.lww.com/EE/A115>). The HR for plasma PBB-153 concentrations (≥ 90 th vs. <50th percentile) was 0.78 (95% CI = 0.44, 1.39) (Figure 2 and Supplemental Table 1; <http://links.lww.com/EE/A115>). Compared with total plasma OCP concentrations <50th percentile, HRs for the 50th–74th, 75th–89th, and ≥ 90 th percentile were 0.86 (95% CI = 0.57, 1.29), 0.73 (95% CI = 0.43, 1.22), and 0.58 (95% CI = 0.32, 1.04), respectively (Figure 3 and Supplemental Table 1; <http://links.lww.com/EE/A115>). HRs for individual plasma OCP concentrations (≥ 90 th vs. <50th percentile) were similar: 0.65 for *p,p'*-DDE (95% CI = 0.36, 1.17), 0.65 for HCB (95% CI = 0.37, 1.15), 0.63 for oxychlorane (95% CI = 0.34, 1.14), and 0.64 for trans-nonachlor (95% CI = 0.35, 1.18) (Figure 3 and Supplemental Table 1; <http://links.lww.com/EE/A115>). The HR comparing individuals with and without detectable levels of *p,p'*-DDT (50% detection in our cohort) was 0.65 (95% CI = 0.46, 0.90).

In sensitivity analyses that excluded current depot medroxyprogesterone acetate users (6% of participants), results were similar (data not shown). We observed some differences when we stratified by the median baseline age, but these differences were inconsistent and effect estimates were imprecise (Supplemental Figure 2; <http://links.lww.com/EE/A115>). Among women with a BMI 30–50 kg/m², we observed slightly stronger associations between total plasma OCP concentrations and UL incidence (HR comparing ≥ 75 th vs. <50th percentile = 0.48, 95% CI = 0.25, 0.93) (Supplemental Figure 3; <http://links.lww.com/EE/A115>). The corresponding HR among women with a BMI <30 kg/m² was 0.65 (95% CI = 0.31, 1.34).

Results were imprecise for chemicals with low detection (<60%) (Supplemental Table 2; <http://links.lww.com/EE/A115>). HRs comparing individuals with and without detectable levels were 0.85 for PBDE-17 (95% CI = 0.52, 1.39), 1.10 for PBDE-66 (95% CI = 0.65, 1.87), 1.39 for PBDE-183 (95% CI = 0.97,

Table 2.

Baseline characteristics by brominated flame retardant plasma concentrations in SELF (2010–2012) (N = 729)

	Baseline total PBDE ^a percentile					Baseline PBB-153 percentile					Baseline total OCP ^b percentile				
	<50th (<31.9 ng/g lipid)	50th–74th (31.9–56.2 ng/g lipid)	75th–89th (56.3–101.0 ng/g lipid)	≥90th (≥101.1 ng/g lipid)	<50th (<0.5 ng/g lipid)	50th–74th (0.5–0.8 ng/g lipid)	75th–89th (0.9–1.6 ng/g lipid)	≥90th (≥1.7 ng/g lipid)	<50th (<61.5 ng/g lipid)	50th–74th (61.5–82.9 ng/g lipid)	75th–89th (82.9–106.0 ng/g lipid)	≥90th (≥106.1 ng/g lipid)			
N (%)	364 (49.9)	183 (25.1)	110 (15.1)	72 (9.9)	365 (50.1)	181 (24.8)	110 (15.1)	73 (10.0)	365 (50.1)	183 (24.8)	111 (15.2)	72 (9.9)			
Mean total plasma lipids (mg/dl)	546.3	539.8	530.6	511.8	545	536	534	525	544.9	545.0	521.9	519.7			
Mean age (years)	29.1	28.3	27.8	28.0	27.5	29.4	30.1	29.5	27.5	29.5	29.9	29.5			
Mean BMI (kg/m ²)	33.8	34.3	32.7	32.4	37.0	31.3	29.1	29.2	36.3	33.6	28.7	28.2			
Education (%)															
<High school/GED	15.9	24.0	25.5	34.7	21.6	19.3	23.6	20.6	22.7	19.3	20.7	19.4			
Some college	52.5	50.8	47.3	52.8	54.8	48.6	45.5	49.3	56.2	47.0	44.1	48.6			
≥College degree	31.6	25.1	27.3	12.5	23.6	32.0	30.9	30.1	21.1	33.7	35.1	31.9			
Annual household income (%)															
<\$20,000	40.4	48.1	52.7	55.6	49.3	44.2	40.0	39.7	48.8	44.8	41.4	38.9			
\$20–50,000	41.5	37.2	30.9	27.8	37.8	37.0	40.0	32.9	37.3	38.1	38.7	34.7			
>\$50,000	18.1	14.8	16.4	16.7	12.9	18.8	20.0	27.4	14.0	17.1	19.8	26.4			
Current smoking status (%)															
Never	78.9	71.0	68.2	61.1	77.3	68.5	70.0	72.6	75.3	68.0	75.7	75.0			
Past	6.9	8.7	9.1	8.3	6.3	11.1	6.4	9.6	7.4	12.7	2.7	5.6			
<10 cigarettes/day	12.1	15.9	14.6	18.1	13.7	14.4	14.6	13.7	14.8	14.4	12.6	11.1			
≥10 cigarettes/day	2.2	4.4	8.2	12.5	2.7	6.1	9.1	4.1	2.5	5.0	9.0	8.3			
Alcohol intake (past year) ^c (%)															
None	30.2	26.8	29.1	25.0	30.1	29.3	28.2	20.6	29.9	26.5	28.8	27.8			
Moderate	52.8	50.8	50.9	45.8	50.7	51.9	49.1	56.2	51.0	54.7	48.7	48.6			
Heavy	17.0	22.4	20.0	29.2	19.2	18.8	22.7	23.3	19.2	18.8	22.5	23.6			
Age at menarche ≤10 years, %	15.4	16.9	18.2	19.4	18.6	16.0	14.6	11.0	17.5	18.2	13.5	12.5			
Breastfed ≥3 months in infancy, %	14.3	14.8	10.9	18.1	9.0	12.7	18.2	38.4	11.5	17.1	10.8	26.4			
Parity (%)															
Nulliparous	36.8	40.4	37.3	34.7	39.7	36.5	32.7	37.0	37.8	38.1	36.0	37.5			
1 birth	28.3	28.4	23.6	23.6	29.6	26.5	25.5	19.2	28.0	30.9	24.3	18.1			
2 births	17.0	14.8	20.0	20.8	15.9	19.3	15.5	21.9	16.4	18.0	20.8	20.8			
≥3 births	17.9	16.4	19.1	20.8	14.8	17.7	26.4	21.9	17.8	13.8	21.6	23.6			
Time since last birth <2 years, %	15.9	12.6	11.8	26.4	17.3	13.3	15.5	12.3	18.6	12.7	11.7	12.5			
Breastfed an infant >3 months, %	27.2	20.2	26.4	29.2	21.4	29.8	25.5	35.6	27.2	20.2	26.4	29.2			

^aTotal PBDE is the sum of PBDE congeners -28, -47, -85, -99, -100, -153, -154, and -209.

^bTotal OCP is the sum of *p,p'*-DDE, HCB, oxychlorane, and trans-nonachlor.

^cAlcohol use in past year categories: low (<10 drinks/year), moderate (<6 drinks on days when they drink and no more than once per month intake of ≥4 drinks per sitting), heavy (≥6 drinks on days when they drink or ≥4 drinks per sitting at least twice a month). GED indicates general education diploma.

1.98), 0.65 for p,p' -DDT (95% CI = 0.46, 0.90) (as mentioned above) and 0.94 for β -HCH (95% CI = 0.66, 1.33). Results using quintile exposure categories yielded similar conclusions. For example, the adjusted HR comparing the highest quintile to lowest quintile was 0.66 (95% CI = 0.39, 1.10) for total PBDEs (≥ 64.91 vs. < 18.07 ng/g lipid), 1.06 (95% CI = 0.60, 1.86) for PBB-153 (≥ 1.04 vs. < 0.30 ng/g lipid), and 0.67 (95% CI = 0.37, 1.22) for total OCPs (≥ 88.47 vs. < 42.66 ng/g lipid). Sensitivity analyses adjusted for fruit, vegetable, dairy, meat, and fish consumption yielded nearly identical results. Results additionally adjusted for co-exposure to chemical classes also did not differ. A comparison of results excluding the 23 participants with missing follow-up data and results were similar.

Discussion

In this prospective cohort study of reproductive-aged US Black women, we found no support for a positive association between plasma brominated flame retardant concentrations

and UL incidence. We observed some evidence of an inverse association between baseline OCP plasma concentrations and UL incidence.

The range of effect estimates observed in our study was generally similar to those from a case-control study examining the association between PBDEs and OCPs with UL.¹¹ In that study, ORs for UL per one SD increase in serum PBDE concentrations ranged from 0.92 (PBDE-153) to 1.23 (PBDE-209). ORs per one-SD increase in serum OCP concentrations ranged from 0.83 (HCB) to 1.37 (p,p' -DDE). In our study, HRs comparing women in the ≥ 90 th percentile with those in the < 50 th percentile of individual PBDEs ranged from 0.80 (PBDE-154) to 1.19 (PBDE-28), and among OCPs from 0.63 (oxychlordane) to 0.65 (p,p' -DDE and HCB). For chemicals with low levels of detection, HRs comparing women with detectable levels to women without detectable levels ranged from 0.65 (p,p' -DDT) to 1.39 (PBDE-183).

Recent results from the Michigan PBB registry report on the association between total PBB serum concentrations (PBB-77

Total PBDEs^a

- < 50 th percentile (< 31.9 ng/g lipid), UL (n=153)
- 50-74th percentile (31.9-56.2 ng/g lipid), UL (n=75)
- 75-89th percentile (56.3-101.0 ng/g lipid), UL (n=43)
- e 90th percentile (e 101.1 ng/g lipid), UL (n=24)

PBDE-28

- < 50 th percentile (< 0.8 ng/g lipid), UL (n=157)
- 50-74th percentile (0.8-1.2 ng/g lipid), UL (n=62)
- 75-89th percentile (1.3-2.0 ng/g lipid), UL (n=47)
- e 90th percentile (e 2.1 ng/g lipid), UL (n=29)

PBDE-47

- < 50 th percentile (< 16.5 ng/g lipid), UL (n=152)
- 50-74th percentile (16.5-30.0 ng/g lipid), UL (n=73)
- 75-89th percentile (30.1-53.2 ng/g lipid), UL (n=42)
- e 90th percentile (e 53.3 ng/g lipid), UL (n=28)

PBDE-85

- < 50 th percentile (< 0.4 ng/g lipid), UL (N=171)
- 50-74th percentile (0.4-0.6 ng/g lipid), UL (N=65)
- 75-89th percentile (0.7-1.2 ng/g lipid), UL (N=34)
- e 90th percentile (e 1.3 ng/g lipid), UL (N=25)

PBDE-99

- < 50 th percentile (< 3.0 ng/g lipid), UL (n=153)
- 50-74th percentile (3.0-5.4 ng/g lipid), UL (n=77)
- 75-89th percentile (5.5-11.5 ng/g lipid), UL (n=41)
- e 90th percentile (e 11.6 ng/g lipid), UL (n=24)

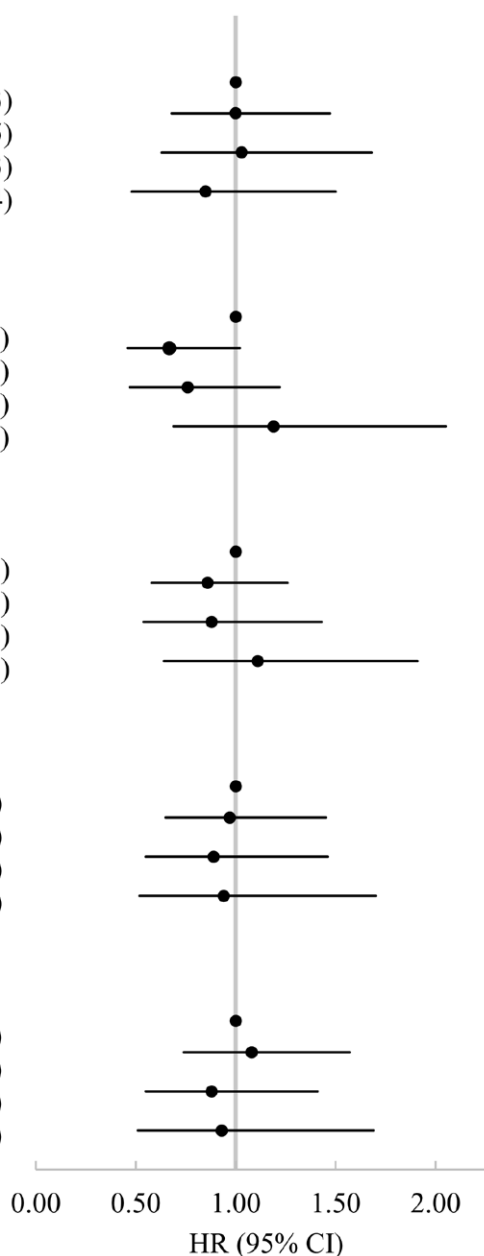
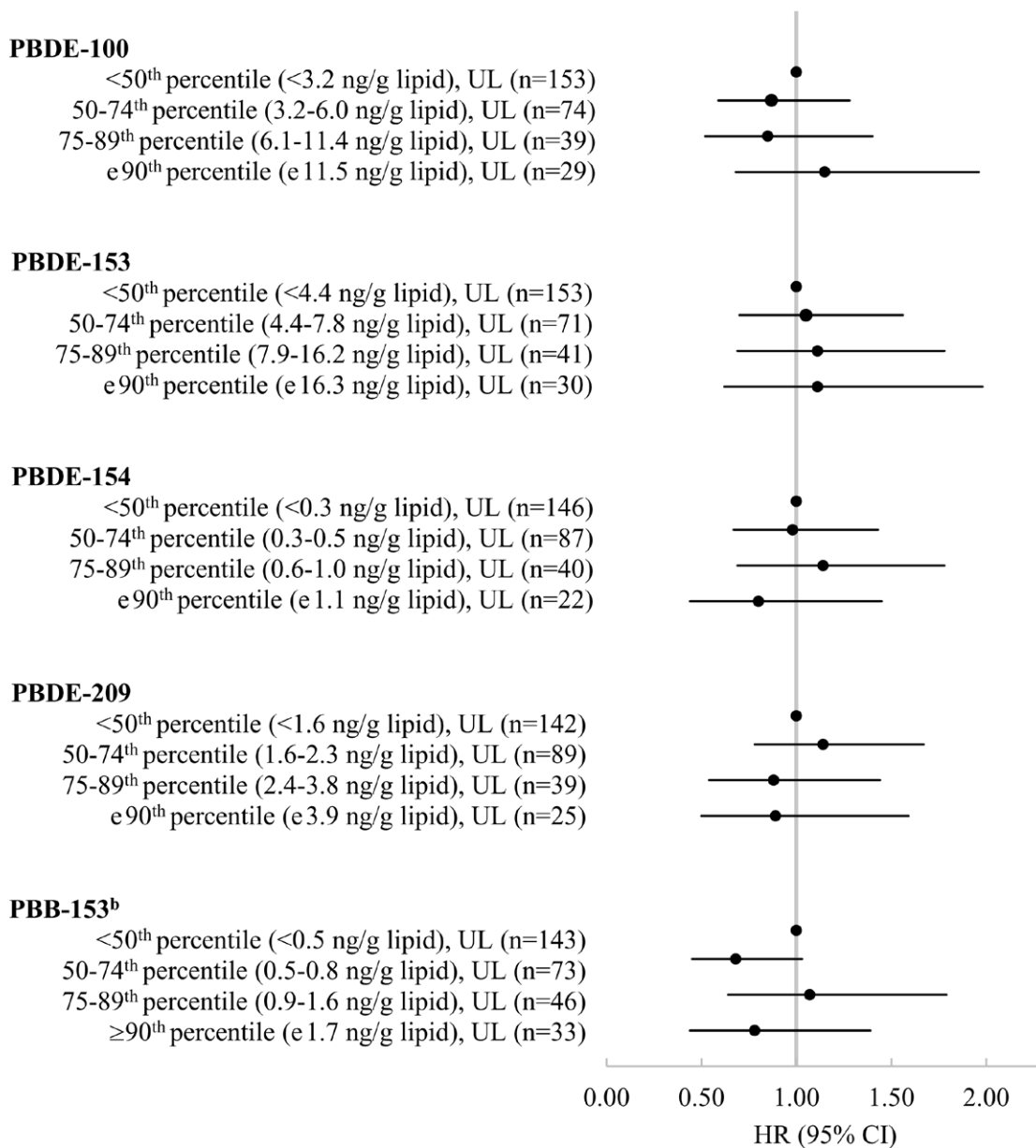


Figure 2. (Continued).



^a Total PBDEs = sum of PBDE congeners -28, -47, -85, -99, -100, -153, -154, and -209.

^b In PBB-153 models, we additionally adjusted for having been breastfed in infancy

All models adjust for body mass index, education, income, cigarette smoking, alcohol use, age at menarche, parity, years since last birth, lactation history, and total plasma lipids (mg/dl)

Figure 2. HR and 95% CIs for baseline plasma concentrations of brominated flame retardants and incidence of uterine leiomyomata (UL) in SELF over a 60-month follow-up (N = 729). ^aTotal PBDEs = sum of PBDE congeners -28, -47, -85, -99, -100, -153, -154, and -209. ^bIn PBB-153 models, we additionally adjusted for having been breastfed in infancy. All models adjust for BMI, education, income, cigarette smoking, alcohol use, age at menarche, parity, years since last birth, lactation history, and total plasma lipids (mg/dl).

+ PBB-101 + PBB-153 + PBB-180) and UL prevalence (odds ratio per one-unit increase in total PBB = 0.91, 95% CI = 0.56, 1.43).⁴³ Participants were between the ages of 18–59, and were predominately Non-Hispanic White (95%), and had their blood collected and reproductive history assessed at the same time. Our observation of inconsistent incidence of UL with increasing plasma PBB-153 concentrations is consistent with these findings. While we did not assess other PBB congeners, PBB-153 is the primary constituent of PBB mixtures and can persist in the human body for many decades after exposure.^{24,25}

Qin and colleagues¹² collected abdominal adipose tissue samples from individuals participating in elective surgeries at hospitals and clinics in Hong Kong. Participants were between the ages of 28–54, and consisted of 24 UL cases and 20 liposuction controls. Researchers retrieved samples from UL cases after the removal of UL tumors. Controls self-reported no history of UL on questionnaires. Comparing UL cases to controls, Qin and colleagues observed evidence of higher subcutaneous fat concentrations of PBDEs (-47, -85, -99, -100, -119, and -153) and OCPs (HCB, β -HCH, γ -HCH, p,p' -DDE, p,p' -DDD, and

Total OCPs^a

<50th percentile (<61.5 ng/g lipid), UL (n=145)
 50-74th percentile (61.5-82.9 ng/g lipid), UL (n=82)
 75-89th percentile (83.0-106.0 ng/g lipid), UL (n=43)
 e90th percentile (e 106.1 ng/g lipid), UL (n=25)

***p,p'*-DDE**

<50th percentile (<51.2 ng/g lipid), UL (n=145)
 50-74th percentile (51.2-69.4 ng/g lipid), UL (n=83)
 75-89th percentile (69.5-92.4 ng/g lipid), UL (n=44)
 e90th percentile (e 92.5 ng/g lipid), UL (n=23)

Hexachlorobenzene

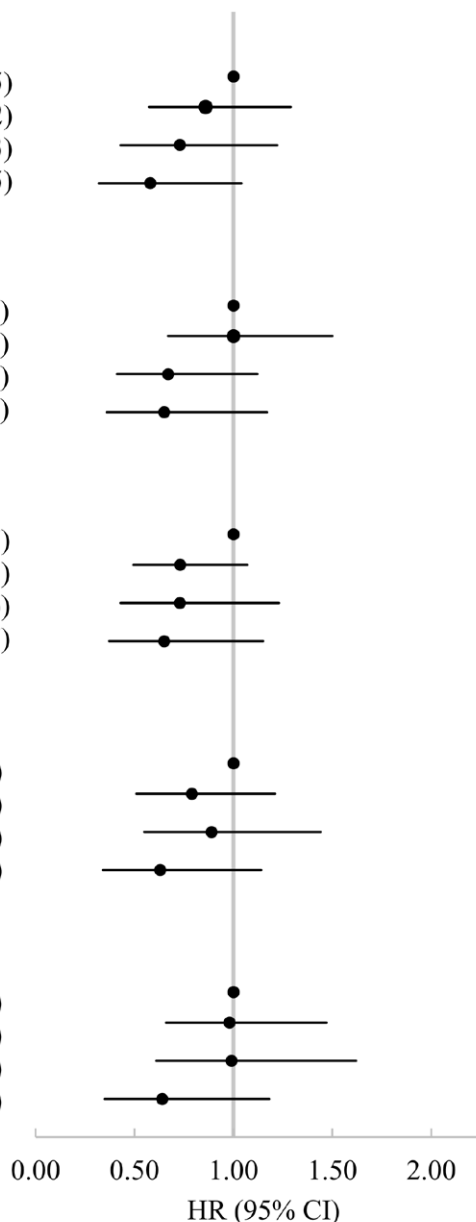
<50th percentile (<5.5 ng/g lipid), UL (n=147)
 50-74th percentile (5.5-7.0 ng/g lipid), UL (n=75)
 75-89th percentile (7.1-8.5 ng/g lipid), UL (n=46)
 e90th percentile (e 8.6 ng/g lipid), UL (n=27)

Oxychlorthane

<50th percentile (<2.1 ng/g lipid), UL (n=139)
 50-74th percentile (2.1-2.7 ng/g lipid), UL (n=70)
 75-89th percentile (2.8-3.5 ng/g lipid), UL (n=55)
 e90th percentile (e 3.6 ng/g lipid), UL (n=31)

Trans-nonachlor

<50th percentile (<2.4 ng/g lipid), UL (n=140)
 50-74th percentile (2.4-3.3 ng/g lipid), UL (n=80)
 75-89th percentile (3.4-4.4 ng/g lipid), UL (n=49)
 ≥90th percentile (e 4.5 ng/g lipid), UL (n=26)



^a Total OCPs = sum of *p,p'*-DDE, hexachlorobenzene, oxychlorthane, and trans-nonachlor

All models adjust for body mass index, education, income, cigarette smoking, alcohol use, age at menarche, parity, years since last birth, lactation history, and total plasma lipids (mg/dl)

Figure 3. HR and 95% CIs for baseline plasma concentrations of OCPs and incidence of uterine leiomyomas (UL) in SELF over a 60-month follow-up (N = 729).
^aTotal OCPs = sum of *p,p'*-DDE, HCB, oxychlorthane, and trans-nonachlor. All models adjust for BMI, education, income, cigarette smoking, alcohol use, age at menarche, parity, years since last birth, lactation history, and total plasma lipids (mg/dl).

p,p'-DDT). Associations persisted when they accounted for age, BMI, and seafood consumption. They did not account for child-bearing or lactation history.

An important distinction between our study (SELF) and the previous studies reporting on PBDEs, PBBs, and OCPs with UL is our prospective study design, collecting biomarker data among reproductive-aged UL-free women at baseline and then assessing UL incidence during a 5-year period. Another important difference across studies was outcome assessment: SELF systematically screened all women for UL with transvaginal ultrasound at baseline and during follow-up. The prospective screening of cases and non-cases circumvents challenges related to retrospective case-control study designs, including

the presence of prevalent asymptomatic UL in the non-case group (outcome misclassification), lack of information about UL onset, and difficulties in establishing temporality between exposure and UL.

There are several explanations for the null associations between PBDEs and UL incidence observed in our studies, and others. First, since PBDE congeners co-occur in mixtures and can exhibit both estrogenic and anti-estrogenic effects,³⁷ their counteracting effects may not have been strong enough to influence UL incidence over the course of our study. Suggestively higher UL incidence with PBDE-153 and PBDE-183 may indicate an adverse effect of some congeners; however, CIs were wide and imprecise. Second, in addition to their potential counteracting

effects, these congeners have varying degrees of persistence in the human body. For example, half-lives for the most commonly detected PBDE congeners range from 11 months (PBDE-28) to 4 years (PBDE-153).²² Therefore, plasma concentrations assessed at baseline may not reflect chemical exposures during etiologically relevant time periods of uterine development, although the etiology of UL initiation is unclear.³⁴ In comparison, PBB-153 has a half-life ranging from 11 to 29 years.^{24,25} Plasma concentrations at baseline may reflect chemical exposures in early life that may have influence the developmental trajectory of uterine tissue. Third, the levels of PBDE congeners in these populations have not been high enough to observe an adverse effect on UL incidence. Finally, PBDE concentrations may not be causally associated with UL incidence.

Our observation of a 30%–40% reduced risk of UL among women in the highest category of baseline plasma OCP concentrations agrees with studies reporting on other persistent organic pollutants. Serum dioxin concentrations from samples collected soon after exposure to a chemical explosion were associated with a 40% lower ultrasound-detected UL risk 20 years later.⁶⁶ In vitro studies show that brominated flame retardants, OCPs, and dioxins can act via similar pathways.^{41,67,68} Higher baseline plasma OCP concentrations may inhibit UL incidence through estrogenic and non-estrogenic pathways.⁶⁹ OCPs can inhibit estradiol secretion in pig ovarian follicles⁴⁰ and can inhibit ovarian tumor development and growth.⁷⁰ OCPs also inhibit cell proliferation of human endometrial cells and increase the proportion of necrotic cells.⁴¹ Our study has some limitations. First, we measured persistent chemicals in plasma and not adipose or uterine tissue. Concentrations of PBDEs and OCPs are generally higher in adipose tissues and lower in blood.^{11,71,72} The use of circulating blood concentrations adjusted for total plasma lipids can provide unbiased effect estimates.⁶⁴ Second, if the HR changed over the course of follow-up, our report of a single HR averaged over follow-up will not reflect those potential time-varying hazards.⁷³ Third, chemical exposure during developmentally sensitive periods (e.g., puberty) may have been more relevant for UL development.⁷⁴ Higher PBB concentrations before menarche are inversely associated with urinary estrogen levels in adulthood.³⁸ Blood concentrations of these chemicals are also generally lower comparing individuals of reproductive age (20–39 years) to adolescents (12–19 years),¹⁰ though it is unclear whether this represents an age effect or a time period effect. We did not have information on plasma concentrations of persistent chemicals earlier in life, nor were we able to assess the extent to which other biomarkers (e.g., estrogen) mediated observed associations.

Strengths of the present study include its prospective study design, use of state-of-the-art exposure measurement, and use of transvaginal ultrasound to identify prevalent cases at baseline and detect UL incidence approximately every 20 months. We also collected data on a wide range of potential confounders, including socio-demographics, anthropometrics, early-life factors, and reproductive history. Furthermore, we examined these associations in a population at high risk of UL.

Conclusions

We found no support for a positive association between plasma brominated flame retardant concentrations and UL incidence. We found a 30%–40% lower incidence of UL among women with the highest baseline plasma OCP concentrations, consistent with previous reports of these pollutants and UL.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

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