Exploring reproductive associations of serum polybrominated diphenyl ether and hydroxylated brominated diphenyl ether concentrations among women undergoing *in vitro* fertilization

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STUDY QUESTION: Are serum concentrations of polybrominated diphenyl ethers (PBDEs) and hydroxylated brominated diphenyl ethers (OH-BDEs) associated with IVF endpoints?

SUMMARY ANSWER: Positive associations were observed for BDE153 and several OH-BDEs with IVF endpoints.

WHAT IS KNOWN ALREADY: PBDEs have been voluntarily phased out of production in the USA and EU due to their persistence and toxicity to humans and ecosystems. PBDEs have been associated with implantation failure among women undergoing IVF, yet some animal studies suggest greater toxicity from their metabolites, OH-BDEs.

STUDY DESIGN, SIZE, DURATION: We evaluated a subset of 215 women (contributing 330 IVF cycles) enrolled between 2005 and 2016 in a longitudinal cohort based at Massachusetts General Hospital Fertility Center.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The following PBDEs were quantified: 47, 99, 100, 153 and 154 and the following OH-BDEs: 3-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47 and 4-OH-BDE49. Clinical endpoints of IVF treatments were abstracted from electronic medical records. Associations of log-transformed PBDEs and OH-BDEs with IVF outcomes were assessed using multivariable generalized mixed models and cluster weighted generalized estimating equation models adjusted for lipids, age, BMI, race, year of sample collection, IVF protocol and FSH levels. Outcomes were adjusted to represent a percent change in outcome with an increase equal to the magnitude of the difference between the 75th and 25th percentiles for each specific compound (interquartile range (IQR) increase).

MAIN RESULTS AND THE ROLE OF CHANCE: Detection frequencies were highest for congeners 47 and 153 (82% $\geq$ method detection limit (MDL)) and metabolites 3 and 5-OH-BDE47 and 4-OH-BDE49 (92% $>$ MDL). PBDE and OH-BDE geometric mean concentrations declined by up to 80% between participants recruited in 2005 and those recruited in 2016. An IQR increase of BDE153 was associated with an increase in the probability of implantation (relative risk (RR) = 1.26, 95% CI: 1.16, 1.36), clinical pregnancy (RR = 1.32, 95% CI: 1.19, 1.46) and live birth (RR = 1.34; 95% CI: 1.15, 1.54). An IQR increase in 3 and 5-OH-BDE47 was associated with increased probabilities of implantation (RR = 1.52; 95% CI: 1.11, 2.09), clinical pregnancy (RR = 1.66; 95% CI: 1.17, 2.36), and live birth (RR = 1.61; 95% CI: 1.07, 2.40). When models were stratified by race (White (86%)/Other race (14%)), associations remained positive for White women, yet inverse associations

were observed for Other race women. An IQR increase in BDE47 was associated with a 46% decreased probability of clinical pregnancy (95% CI: 0.31, 0.95) for Other race women.

LIMITATIONS, REASONS FOR CAUTION: Despite the long half-lives of PBDEs and OH-BDEs, exposure misclassification is possible for women who underwent multiple treatment cycles over several months or years. It is also possible another medium, such as follicular fluid would be optimal to characterize exposure. We also tested associations for multiple congeners and metabolites with multiple outcomes.

WIDER IMPLICATIONS OF THE FINDINGS: Detections of serum concentrations of PBDEs and OH-BDEs were highest in the early years of the study and suggests that the phase-out of these compounds has contributed to a decrease in exposure. The negative associations found for PBDEs and IVF outcomes among other race women suggests the potential for racial disparity. Potential racial disparities in PBDE exposure and exploration of alternative flame retardants with reproductive health outcomes should be the focus of future investigations.

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Key words: polybrominated diphenyl ethers / hydroxylated diphenyl ethers / *in vitro* fertilization / reproductive health / race

Introduction

Approximately 15% of couples in the USA and 80 million couples worldwide are affected by infertility, defined as the inability to conceive after 1 year of unprotected intercourse (Thoma et al., 2013; Eisenberg et al., 2015; Luke, 2017). The annual number of treatment cycles using ART increased by 13% from 2013 to 2015. In 2016, 1.8% of all live births in the USA were a result of ART. Infertility treatment (including diagnosis and sequelae) in the USA is suspected to cost \$5 billion annually, which is easy to conceptualize as 1 cycle of *in vitro* fertilization (IVF) has an average cost of \$12,400. Environmental exposures including particulate matter, heavy metals, pesticides and persistent organic pollutants (POPs) have been associated with infertility (Buck Louis et al., 2013; Choe et al., 2017; Ingle et al., 2017).

Among POPs that have been associated with infertility are polybrominated diphenyl ethers (PBDEs), a prominent class of flame retardants (FRs) found in furniture, carpeting, electronics and plastics (ATSDR 2015). While there are potentially 209 different brominated diphenyl ether (BDE) congeners (having various bromine substitution patterns), only a handful of congeners are routinely detected in the environment and in human tissues and reflect the dominant congeners present in the commercial mixtures referred to as Penta-, Octa- and Deca-BDE. The most frequently detected congeners, 47, 99, 100, 153 and 154 are often found in (but not exclusively) the PentaBDE mixture (Dishaw et al., 2014). OctaBDE is primarily comprised of congeners 183, 196, 197 and 203 (153 and 154 can be found in PentaBDE and OctaBDE), while 207 and 208 can be found in both OctaBDE and DecaBDE, but 202 and 209 are exclusively found in DecaBDE (ATSDR 2015). Due to these mixtures' toxicity to humans and ecosystems, PentaBDE and OctaBDE mixtures were phased out of production in 2004 and DecaBDE mixtures were phased out in 2013 (Dodson et al., 2012). During manufacturing, PBDEs are physically added to the polymers and the absence of a covalent bond allows them to leach into surrounding environments (Weijjs et al., 2015). Their lipophilicity allows them to bioaccumulate in the environment and adipose tissues with half-lives ranging from weeks to years depending on the specific congener, which has led to widespread detection in serum among women, men, and children in the USA (Sjödin et al., 2008). PBDEs have been highly detected in dust samples from homes, offices and automobiles and it is suspected that ~82% of exposure in the USA can be attributed to house dust, while exposure in Europe is primarily from food (Domingo, 2012; Johnson et al., 2013; Watkins et al., 2013).

In mammals, hydroxylated-BDEs (OH-BDEs) are formed through oxidative metabolism of PBDEs via cytochrome P450 (CYPs), specifically CYP2B6 (Feo et al., 2013). OH-BDEs are also produced naturally in marine environments and exposure can also be attributed to seafood consumption (Malmvärn et al., 2008). Like PBDEs, OH-BDEs also accumulate in the body and have been detected in human serum in adults and children (Athanasidou et al., 2008; Qiu et al., 2009; Stapleton et al., 2011). Several laboratory studies have also observed greater toxic effects from OH-BDEs compared to their parent compounds in regards to endocrine disruption, cytotoxicity and genotoxicity (Cantón et al., 2006; Hamers et al., 2008; Dingemans et al., 2010; Ji et al., 2011; Cao et al., 2018). The most common metabolites detected in humans are 3-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47 and 4-OH-BDE49 (Athanasidou et al., 2008; Qiu et al., 2009; Stapleton et al., 2011; Feo et al., 2013).

Elevated levels of PBDEs have been associated with adverse reproductive health effects in humans, including endocrine disruption, longer menstrual cycles, and time to pregnancy (TTP) (Chao et al., 2010; Harley et al., 2010; Stapleton et al., 2011; Mumford et al., 2015; Makey et al., 2016). To date, studies assessing the reproductive health effects of OH-BDEs are limited; however, several *in vitro* studies suggest they act as endocrine disruptors and elicit oxidative stress (Hamers et al., 2008; Receptor-A Ligands et al., 2008; Ji et al., 2011; Cao et al., 2018). In our present work, we expand upon previous studies in a more robust analysis investigating the association of PBDEs and pregnancy outcomes along with, to the best of our knowledge, the first study to assess these relationships with OH-BDEs using IVF as a model of intermediate developmental endpoints and pregnancy outcomes.

Materials and Methods

Study population

Study participants are a subset of women from the Environmental and Reproductive Health (EARTH) study, an established longitudinal prospective pre-conception cohort study of environmental, dietary and lifestyle impacts on reproductive health (Hauser et al., 2006). Women (18–46 years) were recruited from Massachusetts General Hospital (MGH) Fertility Center. Approximately 60% of women who were approached enrolled in the study (Mínguez-Alarcón et al., 2015). Questionnaires were administered to collect demographic information

(i.e. age, race/ethnicity, education level) at study entry. The present analysis includes 215 women (330 IVF cycles) recruited between 2005 and 2016 who contributed their own oocytes and provided a blood sample to quantify concentrations of FRs and FR metabolites in serum. The majority of women completed one to two IVF cycles during follow up. Research protocols were approved by the ethics and Research Committees of MGH, Harvard T.H. Chan School of Public Health, University of Michigan and Duke University. The study was described in detail to all participants, and informed consent was obtained from all participants.

Clinical protocols and IVF measures

During each cycle, clinical data was abstracted from the participant's electronic health record. Clinical protocols and IVF measures have been previously detailed (Mok-Lin *et al.*, 2010; Mínguez-Alarcón *et al.*, 2015). Briefly, on the third day of the woman's menstrual cycle, a blood serum sample was drawn to measure FSH and estradiol (E_2) concentrations at MGH Core Laboratory using an automated electrochemiluminescence immunoassay. Peak E_2 concentrations, defined as the highest level prior to oocyte retrieval, were obtained on the day of trigger with hCG. Infertility diagnosis was established by an MGH physician in accordance with the Society of Assisted Reproductive Technology (SART) (SART 2016). Upon infertility evaluation including infertility diagnosis and other clinical factors, one of three ovarian treatment IVF protocols was selected: (i) luteal phase gonadotrophin releasing hormone (GnRH) agonist, (ii) follicular phase GnRH agonist or 'flare' stimulation or (iii) GnRH antagonist (Vanegas *et al.*, 2017). Throughout gonadotropin stimulation and up to 2 days prior to oocyte retrieval, serum E_2 , follicle size and counts and endometrial thickness were monitored for each participant (Mínguez-Alarcón *et al.*, 2015). Once lead follicle size approached 16–18 mm and E_2 levels reached at least 500 pg/mL, oocytes were retrieved. IVF cycle was defined by oocyte retrieval. Following oocyte retrieval, an embryologist counted and classified oocytes per cycle as a germinal vesicle, metaphase I, metaphase II (M2) or degenerated. Fertilization occurred via IVF or ICSI and was confirmed 17–20 h later. Fertilization was determined by the presence of a cytoplasmic halo and two pronuclei (Veeck and Zaninovic, 2003; Carignan *et al.*, 2017). Fertilization rate was established as the number of two pronuclear embryos divided by the number of M2 oocytes. Clinical outcomes were assessed for participants who continued with embryo transfer. Successful implantation for a given transfer was confirmed when serum β -hCG levels were >6 mIU/mL, ~17 days after oocyte retrieval (Mínguez-Alarcón *et al.*, 2015). Clinical pregnancy was defined as the presence of an intrauterine pregnancy via ultrasound (≥ 6 weeks gestation) along with elevated levels of β -hCG. Live birth was defined as the birth of a neonate at or after 24 weeks gestation.

PBDE and OH-BDE collection and measurement

Blood samples (5 mL) were collected in washed glass Wheaton vials at the study entry clinic visit. Samples were aliquoted, frozen and stored at -80°C until overnight shipment on dry ice to Dr Stapleton's lab at Duke University (Durham, NC). Serum was analyzed for five PBDE congeners: 47, 99, 100, 153 and 154 along with four OH-BDE

metabolites: 4-OH-BDE47, 5-OH-BDE47, 6-OH-BDE47 and 4-OH-BDE49. Upon arrival, samples were weighed and fortified with internal standards (monofluorinated BDE 69, 13C BDE 209 and 13C-6-OH-BDE47). Serum was diluted with water and formic acid before undergoing solid phase extraction (Oasis HLB, Waters Corp.). A 50:50 solution of dichloromethane (DCM) and ethyl acetate was used to remove both PBDES and OH-BDEs from the SPE column. Samples were then dried and rejuvenated with hexane (1 mL) followed by extract cleaning via a 1.0-g silica column. PBDEs were removed with 10 mL of hexane while 10 mL of DCM hexane solution was used for the metabolites. Gas chromatography negative chemical ionization mass spectrometry methods were used to analyze PBDES while OH-BDEs were measured using liquid chromatography tandem mass spectrometry (Stapleton *et al.*, 2011). Accuracy of the method was verified by extracting a human serum Standard Reference Material (SRM 1957) from the National Institute of Standards and Technology. Measured values were in the range of 73–97% of the certified values. Metabolites 3-OH-BDE47 and 5-OH-BDE47 are presented individually as well as combined due to co-elution problems in a few batches. Total lipids were derived from total serum cholesterol and triglycerides using the following formula: $TL\ (g/l) = [(TC \times 1.12) + (TG \times 1.33) + 1.48]$ where TL = total lipids, TG = serum triglycerides and TC = serum cholesterol (Covaci *et al.*, 2006). Missing total lipids ($n = 19$) were replaced with the median (511.5).

Statistical analysis

Demographic and reproductive characteristics for women were calculated using medians, interquartile ranges (IQRs), frequencies and percentages as appropriate. Congeners and metabolites below method detection limits (MDL) were imputed to $MDL/\sqrt{2}$ (Hornung and Reed, 1990). Geometric means (GMs), 95% confidence intervals (CIs) and selected percentiles were used to describe unadjusted (ng/g serum) and lipid-adjusted (ng/g lipid) PBDEs and OH-BDEs. Spearman correlation coefficients were used to assess the association among serum congeners and metabolites. PBDEs and OH-BDEs presented as right-skewed and were transformed by the natural logarithm for further analysis. Associations of congeners and metabolites with reproductive outcomes were confirmed to meet linearity assumptions using bivariate analyses and were treated as continuous variables. Possible demographic confounders included total serum lipids, age, body mass index (kg/m^2) (BMI), race (White/Other race), education (high school/some college, college graduate, graduate degree) and year of exposure sample collection. Reproductive characteristics considered were prior pregnancy, Day 3 FSH levels (IU/L), initial infertility diagnosis (female factor, male factor or unexplained), previous intrauterine insemination (IUI) (yes/no), previous IVF (yes/no), treatment protocol (antagonist, flare or luteal phase agonist), E_2 trigger levels (pmol/L), endometrial thickness (mm) and ICSI (yes/no). Covariates were selected if they were associated with exposure in previous cohorts, our cohort or a predictor of IVF outcomes (Harley *et al.*, 2010; Castorina *et al.*, 2011; Stapleton *et al.*, 2011; Johnson *et al.*, 2012). Final models using unadjusted PBDEs and OH-BDEs were adjusted for total serum lipid, age, BMI, race, year of exposure sample collection, Day 3 FSH levels and IVF protocol. A missing value ($n = 1$) for Day 3 FSH level was replaced with the median (6.8 IU/L).

Table 1 Demographic and reproductive characteristics for 215 women (330 *in vitro* fertilization cycles) from a subset of the EARTH cohort.

Characteristics	Median or n	(25th and 75th quartiles or %)
Demographic (n = 215 women)		
Age (years)	35	(32, 38)
Race/ethnicity		
Other race	30	(14)
White/Caucasian	185	(86)
BMI (kg/m ²)	23	(21, 26)
Ever smoker	60	(28)
Education		
High school/some college	14	(6)
College graduate	70	(33)
Graduate degree	131	(61)
Reproductive (n = 330 cycles)		
Prior pregnancy	78	(36)
Initial infertility diagnosis		
Female factor	63	(29)
Male factor	77	(36)
Unexplained	75	(35)
Previous IUI	83	(39)
Previous IVF	46	(21)
Day 3 FSH levels ^a , IU/L	6.8	(6.0, 8.3)
Treatment protocol		
Antagonist	42	(13)
Flare	60	(18)
Luteal phase agonist	228	(69)
E ₂ trigger levels ^b , pmol/L	1998	(1540, 2658)
Endometrial thickness ^c (mm)	10	(8.5, 11.2)
ICSI cycles	164	(52)
Total oocyte yield	11	(7, 14)
Number of M2 oocytes	9	(6, 12)
Fertilization rate	0.75	(0.60, 0.87)
Implantation ^d	181	(55)
Clinical pregnancy ^d	175	(47)
Live birth ^d	124	(38)

^an = 329^bn = 317^cn = 316^dn = successesOther race: Black/Asian/Other; BMI: body mass index; IUI: intrauterine insemination; E₂: estradiol; M2: metaphase II

We evaluated the associations of PBDEs and OH-BDES with intermediate IVF outcomes (total oocyte yield, M2 oocyte yield, endometrial wall thickness and fertilization rate) using multivariable generalized mixed models with random intercepts. A Poisson distribution with log link function were designated for oocyte counts while a normal distribution with identity link function was designated for endometrial thickness. A binomial distribution and logit function were designated for fertilization rate. Associations with PBDEs and OH-BDES with clinical outcomes (implantation, clinical pregnancy and live birth)

were evaluated using cluster weighted generalized estimating equation (CWGEE) models where the weight was equivalent to the inverse of the cluster size (cycle number) (Williamson et al., 2003; Huang and Leroux, 2011). CWGEEs have some advantages when accounting for multiple cycles per women (i.e. non-ignorable cluster size), since they may provide more precise estimates when compared to other common statistical approaches for dichotomous IVF outcomes (Yland et al., 2019). PBDEs and OH-BDES were modeled individually as well as summed. Metabolites 3-OH-BDE47 and 5-OH-BDE47 were

Table II Distribution of unadjusted and lipid-adjusted BDEs and OH-BDEs among 215 women from the EARTH cohort.

Percentiles									
BDEs	N > MDL	(%)	GM	(95% CI)	25th	50th	75th	95th	Max
Unadjusted (ng/g serum)									
BDE47	175	82.5	12.5	(11.1, 14.2)	6.3	11.1	24.1	70.7	191.2
BDE99	122	59.2	2.5	(2.2, 2.8)	<MDL	2.0	4.5	12.3	67.4
BDE100	149	71.0	2.3	(2.1, 2.6)	<MDL	1.9	3.9	14.3	42.8
BDE153	201	94.0	12.0	(10.5, 13.8)	6.2	10.3	22.7	80.1	256.5
BDE154	108	53.1	3.1	(2.8, 3.4)	<MDL	3.3	4.8	10.7	26.7
BDE sum			38.7	(34.9, 42.9)	21.4	34.8	68.0	160.9	287.0
Lipid-adjusted (ng/g lipid)									
BDE47			28.1	(25.3, 31.3)	13.4	24.6	57.8	145.9	326.5
BDE99			5.5	(4.9, 6.1)	2.5	5.0	10.6	31.4	162.7
BDE100			5.1	(4.6, 5.7)	2.3	4.2	10.8	29.9	73.0
BDE153			26.3	(23.3, 29.7)	13.0	24.5	47.7	172.3	690.3
BDE154			7.3	(6.7, 7.9)	4.8	7.0	12.2	24.7	62.3
BDE sum			86.3	(78.6, 94.7)	44.2	78.9	157.8	365.4	758.7
OH-BDEs									
Unadjusted (ng/g serum)									
3-OH-BDE47 ^a	76	93.8	0.14	(0.11, 0.19)	0.07	0.12	0.36	0.92	2.0
3-OH-BDE47 & 5-OH-BDE47 ^b	132	98.5	0.59	(0.49, 0.72)	0.29	0.60	1.3	3.3	9.9
5-OH-BDE47 ^a	1	1.0	0.01	(0.01, 0.01)	<MDL	<MDL	<MDL	<MDL	0.81
6-OH-BDE47	134	62.3	0.09	(0.07, 0.10)	<MDL	0.08	0.21	0.96	2.3
4-OH-BDE49	198	92.0	0.09	(0.08, 0.11)	0.04	0.09	0.18	0.62	1.7
OH-BDE sum			0.65	(0.56, 0.74)	0.30	0.62	1.3	4.0	10.7
Lipid-adjusted (ng/g lipid)									
3-OH-BDE47 ^a			0.32	(0.27, 0.41)	0.13	0.31	0.94	1.8	3.7
3-OH-BDE47 & 5-OH-BDE47 ^b			1.3	(1.1, 1.5)	0.64	1.4	2.6	8.1	19.1
5-OH-BDE47 ^a			0.02	(0.01, 0.02)	<MDL	<MDL	<MDL	<MDL	1.7
6-OH-BDE47			0.19	(0.17, 0.22)	0.08	0.18	0.45	1.6	4.2
4-OH-BDE49			0.20	(0.18, 0.23)	0.09	0.21	0.44	1.2	4.1
OH-BDE sum			0.33	(0.29, 0.37)	0.16	0.35	0.74	2.1	5.6

MDL: method detection limit; GM: geometric mean; CI: confidence interval; ^an = 81; ^bn = 134

initially modeled individually; however, due to co-eluting for some batches we decided to present their findings as a collective sum (3-OH-BDE47, 5-OH-BDE47, 3-OH-BDE47 and 5-OH-BDE47). Original effect estimates are presented in tables, and to increase interpretability, outcomes in figures and text were adjusted to represent a percent change in outcome with an increase equal to the magnitude of the difference between the 75th and 25th percentiles (IQR increase) in PBDE or OH-BDE which was calculated using the formula: $((75\text{th percentile}/25\text{th percentile})^{\beta} - 1) \times 100$ (Watkins *et al.*, 2015).

We conducted several sensitivity analyses. To further explore our observation of higher PBDE and OH-BDE concentrations among women in the first half of the study, we stratified our analysis by year (<2010, ≥2010) and reran our models. We also reran our models stratified by race (White/Caucasian or Other race) as prior studies have observed variability in PBDE exposure by race (Stapleton *et al.*, 2011). Finally, we reran our models including only first cycles to account for possible bias of overrepresenting less fertile women

who contributed more than 1 cycle in our main models. Analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC) and R version 3.3.5.

Results

Demographic and reproductive characteristics of this cohort (n = 215 women) are described in Table I and are similar to previous subsets from the EARTH cohort (Mínguez-Alarcón *et al.*, 2015; Carignan *et al.*, 2017). Our sample was comprised of predominantly White/Caucasian women (86%) with a normal BMI (median = 23) in their mid-thirties (median = 35 years). Few women (28%) reported ever smoking and over half had graduate degrees (61%). Only 36% of women reported having a prior pregnancy. The majority of initial infertility diagnoses were male factor (36%), followed by unexplained (35%), then female factor (29%). More women had undergone a previous IUI (39%)

Figure 1 Geometric means of polybrominated diphenyl ethers (PBDEs) and hydroxylated brominated diphenyl ethers (OH-BDEs) (ng/g lipid) from 215 women from the EARTH cohort by year (2005–2015) of sample collection. (Only one sample was collected in 2016 and not included.) Number of samples per year: 2005: $n = 4$, 2006: $n = 15$, 2007: $n = 16$, 2008: $n = 35$, 2009: $n = 47$, 2010: $n = 46$, 2011: $n = 48$, 2012: $n = 39$, 2013: $n = 36$, 2014: $n = 32$, 2015: $n = 8$.

Figure 2 Spearman correlation coefficients of polybrominated diphenyl ethers (PBDEs) and hydroxylated brominated diphenyl ether (OH-BDEs) (ng/g lipid) among 215 women from the EARTH cohort.

compared to IVF (21%). Median day three FSH levels were 6.8 IU/L (IQR: 6.0–8.3). Considerably more women underwent luteal phase agonist protocol (69%), compared to flare (18%), and antagonist

(13%). The median peak E_2 levels were 1998 pmol/L and endometrial thickness was 10 mm. Approximately half of cycles (52%) underwent ICSI.

Distributions of PBDEs and OH-BDEs are presented in Table II as unadjusted (ng/g serum) and lipid-adjusted (ng/g lipid). Congeners 47, 100, and 153 were frequently detected (70% > MDL). Concentration levels (GM) of BDE47 and BDE153 were approximately five times greater than BDEs 99 and 100. Metabolites 3-OH-BDE47, co-eluted samples of 3-OH-BDE47 and 5-OH-BDE47, and 4-OH-BDE49 were frequently detected (92% > MDL). The combined concentrations of 3-OH-BDE47 and 5-OH-BDE47 were the highest (GM = 1.3 ng/g lipid) of all metabolites. 3-OH-BDE47 concentrations (GM = 0.32 ng/g lipid) were slightly higher compared to 6-OH-BDE47 and 4-OH-BDE49 (GM = 0.19 ng/g lipid and GM = 0.20 ng/g lipid).

Yearly trends of lipid-adjusted PBDE and OH-BDE concentrations (GMs) throughout the study are shown in Fig. 1. Concentrations were highest for BDE47 (GM = 67 ng/g/lipid) and BDE154 (GM = 20 ng/g lipid) in 2005. BDE99 peaked in 2008 (GM = 9 ng/g lipid), while BDE100 and BDE153 did not peak until 2009 (GM = 8 ng/g lipid and GM = 54 ng/g lipid). By 2014, concentrations of congeners 99, 100 and 153 had decreased by 66, 63 and 63% (GM = 3 ng/g lipid, GM = 3 ng/g lipid and GM = 20 ng/g lipid, respectively). Between 2005 and 2015 concentrations of BDE154 decreased by 50%, while concentrations of BDE47 decreased by almost 80%. Similar trends

Figure 3 Adjusted relative risk (RR) (95% CIs) for clinical outcomes among women with an interquartile range increase in polybrominated diphenyl ether (PBDE) concentrations (ng/g serum). Models adjusted for total serum lipid, age, BMI, race (White/Other race), year of BDE sample collection, Day 3 FSH levels, IU/L, and IVF protocol (antagonist, flare, luteal phase agonist). RR: relative risk.

were observed for OH-BDES. 3-OH-BDE47 and 5-OH-BDE47 concentrations decreased by 80% over the study period, yet had a slight peak in 2010 (GM = 37 ng/g lipid). Although initial concentrations of 6-OH-BDE47 and 4-OH-BDE49 were not as high at the beginning of the study compared to other metabolites, concentrations still decreased substantially (75 and 57%, respectively).

Several demographic and clinical characteristics were associated with PBDE and OH-BDE exposure (data not shown). Correlations between BMI and BDE100 were positive ($r = 0.15$), yet negative for BDE153 ($r = -0.15$). Other race women had higher concentrations of BDE47, BDE99, 3 and 5-OH-BDE47 and 4-OH-BDE49 compared to White women. Current or past smokers had higher concentrations of BDE47 and BDE99 than non-smokers. Nulliparous women had higher concentrations of congeners 99, 100 and 153.

Correlations for lipid-adjusted PBDEs and OH-BDEs (ng/g lipid) are presented in Fig. 2. All PBDEs were significantly correlated with each other. Correlations were strongest for BDE47, BDE99 and BDE100 (0.73–0.85), while correlations for BDE153 and BDE154 were slightly weaker (0.31–0.53). Moderate-to-strong correlations (0.39–0.56) were observed for all OH-BDEs. Metabolites had the strongest correlations to BDE47 (3 and 5-OH-BDE47: $r = 0.57$, 6-OH-BDE47: $r = 0.47$, and 4-OH-BDE49: $r = 0.64$). Strong correlations were also observed with 4-OH-BDE49 and BDE99 ($r = 0.59$) and BDE100 ($r = 0.47$). Associations were weakest for BDE154 and 4-OH-BDE49 ($r = 0.30$).

No associations were observed for any PBDEs or OH-BDEs with intermediate IVF outcomes (prior to implantation) in unadjusted models (Supplementary Tables SI and SII). PBDEs continued to show no association after adjustment (Supplementary Table SIII). However, in adjusted models, an IQR increase of 4-OH-BDE49 was associated with a 39 and 48% increase (95% CI: 0.5, 94 and 95% CI: 5, 109%, respectively) in total oocyte and M2 oocyte yield (Supplementary Table SIV).

Associations of PBDEs and OH-BDEs with clinical IVF outcomes are depicted in Figs 3 and 4 and represent the percent change in outcome in relation to an IQR increase in PBDE/OH-BDE concentrations. For example, an IQR increase in BDE153 was associated with increased probabilities of implantation, clinical pregnancy, and live birth (RR = 1.16; 95% CI: 1.16, 1.36, RR = 1.25; 95% CI: 1.16, 1.35, RR = 1.26; 95% CI: 1.13, 1.41). An IQR increase in 3 and 5-OH-BDE47 was associated with increased probabilities of implantation (RR = 1.28; 95% CI: 1.07, 1.53), clinical pregnancy (RR = 1.35; 95% CI: 1.11, 1.64), and live birth (RR = 1.31; 95% CI: 1.04, 1.66). An IQR increase in 6-OH-BDE47 was also associated with a 56% increase in the probabilities of both implantation and clinical pregnancy, and an 84% increase in the probability of live birth (RR = 1.56, 95% CI: 1.14, 2.14, RR = 1.56; 95% CI: 1.12, 2.18, and RR = 1.84, 95% CI: 1.26, 2.68, respectively).

When stratifying by race (White/Other race), associations with BDE153 remained positive for clinical outcomes among White women (Supplementary Fig. S1 and Supplementary Table SV). An IQR increase

Figure 4 Adjusted relative risk (RR) (95% CIs) for clinical outcomes among women with an interquartile range increase in hydroxylated brominated diphenyl ether (OH-BDE) metabolites concentrations (ng/g serum). Models adjusted for total serum lipid, age, BMI, race (White/Other race), year of OH-BDE sample collection, Day 3 FSH levels, IU/L and IVF protocol (antagonist, flare, luteal phase agonist). RR: relative risk.

in BDE47 was associated with a decreased probability of implantation (36%), clinical pregnancy (46%) and live birth (35%) for Other race (RR = 0.64; 95% CI: 0.36, 1.16, RR = 0.54; 95% CI: 0.31, 0.95, and RR = 0.65; 95% CI: 0.38, 1.13, respectively), while associations for White participants remained positive. We also observed a decrease in the probability of clinical outcomes with congeners 99, 100 and 153 for Other race women. We also observed several non-significant changes in models of OH-BDEs with clinical outcomes when stratifying by race (Supplementary Fig. S2).

Discussion

Serum concentrations of PBDEs among women in this cohort decreased over the span of the study; they were highest between 2005 and 2009 and decreased substantially from 2010 to 2015. There were no consistent associations for PBDEs and metabolites with intermediate IVF outcomes. We observed unexpected positive relationships of BDE153, 3 and 5-OH-BDE47 and 6-OH-BDE47 with clinical IVF outcomes. Positive associations remained for White women in the relationships of BDE153, and 3 and 5-OH-BDE47 with clinical outcomes. However, among Other race women, an increase in congeners 47, 99, 100 and 154 and metabolite 4-OH-BDE49

was associated with a decreased probability of clinical IVF outcomes. Although the study included a very small number of Other race women, these results suggest a possible racial disparity in PBDE exposure outcome relationships.

Serum concentrations of PBDEs in our cohort were higher compared to the general population. From a similar time period (2005–2014), concentrations in our cohort of BDE47 (GM = 28.1 ng/g lipid), BDE153 (GM = 26.3 ng/g lipid) and BDE154 (GM = 7.3 ng/g lipid) were higher than those from pooled samples of women (20–59 years) from the National Health and Nutrition Examination Survey (NHANES) (GM = 21.0 ng/g lipid, GM = 7.9 ng/g lipid, and GM = 0.4 ng/g lipid, respectively) (Centers for Disease Control and Prevention (CDC) and (NCHS), 2019). Concentrations of BDE47 among our sample were also nearly double those among pregnant women in California (CA) (GM = 14.9 ng/g lipid) and North Carolina (NC) (GM = 16.5 ng/g lipid) (Harley et al., 2010; Stapleton et al., 2011). Of the congeners in our sample, BDE47 is the most abundant in the PentaBDE mixture (25–37%) and possibly explains the higher concentrations of BDE47 compared to other congeners in our sample (ATSDR, 2015). Concentrations of BDE99 (GM = 5.5 ng/g lipid) and BDE100 (GM = 5.1 ng/g lipid) in our sample were similar to women in CA (GM = 4.4 ng/g lipid and GM = 2.8 ng/g lipid, respectively) and NC (GM = 4.7 ng/g lipid and GM = 4.2 ng/g lipid). Concentrations

of BDE47 among participants of this study (median = 24.6 ng/g lipid) were nearly 5-fold higher compared to women in a different cohort of women seeking fertility treatment in Boston between 1994 and 2003 (median = 5.22 ng/g lipid) and nearly 3-fold higher compared with reproductive aged women in Canada (GM = 9.0 ng/g lipid) between 2007 and 2009 (Johnson *et al.*, 2012; Oulhote *et al.*, 2016). BDE153 concentrations in our sample were also considerably higher compared to women in Canada (1.4 ng/g lipid). Higher PBDE concentrations in our cohort could be due to different sampling time periods, as recent serum concentrations may be lower due to the phase out compared to samples taken in the early 2000s. Differences in BDE153 levels may also be attributable to changes in diet over time and across geographic region.

Few studies to date have analyzed PBDE metabolites. Pregnant women in NC had slightly lower concentrations of 6-OH-BDE47 (GM = 0.11 ng/g lipid) compared to participants of this study (GM = 0.19 ng/g lipid) (Stapleton *et al.*, 2011). Concentrations of 4-OH-BDE49 among our sample were similar (GM = 0.20 ng/g lipid) to women in NC (GM = 0.17 ng/g lipid). However, among pregnant women in Indiana (IN), concentrations were higher for 5-OH-BDE47 (median = 5.7 ng/g lipid) and 6-OH-BDE47 (GM = 1.0 ng/g lipid) compared to participants of this study (median < MDL and median = 0.18 ng/g lipid, respectively). Yet 3-OH-BDE47 (median = 0.31 ng/g lipid) and 4-OH-BDE49 (median = 0.21 ng/g lipid) were higher in our sample compared to women in IN (median = 0.4 ng/g lipid and not detected, respectively).

Concentrations decreased by year of sample collection. This trend correlates to the phase-out of the PentaBDE mixture from USA markets in 2004 as BDEs 47, 99, 100 and 154 were highest in 2005 (Renner, 2004). A similar trend was also observed for a recent decade-long (2005–2014) analysis of pooled PBDE samples from NHANES where concentrations for congeners 47, 99, 100 and 154 were lower in recent years for people between the ages of 20–59 years (Sjödin *et al.*, 2019). A longitudinal study of pregnant women in CA also observed a steady decrease in congeners 47, 99, 100 and 153 between 2008 and 2014 (Parry *et al.*, 2018).

Correlations were moderate-to-strong ($r = 0.30$ – 0.85) among PBDEs, OH-BDEs and between PBDEs and OH-BDEs. Congeners 47, 99 and 100 had the strongest correlations, while congeners 153 and 154 were slightly weaker. These results were expected as congeners 47, 99 and 100 are the most prevalent congeners (by weight) in the PentaBDE mixture followed by BDEs 153 and 154 (ATSDR, 2015). Similar results were observed in serum among a sample of 137 pregnant women in NC, where BDE47 was strongly correlated to BDE99 ($r = 0.80$) and BDE100 ($r = 0.80$), yet weaker for BDE153 ($r = 0.52$) (Stapleton *et al.*, 2011). However, among a serum sample of men and women in Shanghai, China ($n = 25$), BDE47 was strongly correlated to both BDE100 ($r = 0.97$) and BDE154 ($r = 0.97$) (Xu *et al.*, 2018). They also observed a strong correlation between BDE100 and BDE154 ($r = 0.99$). Correlations for OH-BDEs were slightly weaker among our sample ($0.39 \leq r \leq 0.59$). Correlations between 6-OH-BDE47 and 4-OH-BDE49 among pregnant women in NC were slightly higher ($r = 0.62$). All metabolites in this analysis are found in the hydroxylation pathway of BDE47, which coincides with our observation of the strongest correlations between BDE47 and OH-BDEs ($0.47 \leq r \leq 0.64$) (Qiu *et al.*, 2009). Similar correlations were observed in serum among

a sample ($n = 47$) of women in Dalian, China ($0.26 \leq r^2 \leq 0.65$) (Wang *et al.*, 2016).

Our results for associations of PBDEs and OH-BDEs with intermediate IVF outcomes were overall null while results with clinical outcomes were positive, though unexpected. An increase in BDE153, 3 and 5-OH-BDE47 and 6-OH-BDE47 was associated with an increase probability of implantation, clinical pregnancy and live birth. These results are unexpected as prior studies of IVF patients found an increase in failed implantation (odds ratio (OR) = 10.0) with detectable BDE153 concentrations in follicular fluid (FF) (Johnson *et al.*, 2012). Another study found increases in BDE100 and BDE153 to be associated with a decrease in fecundability odds ratio (fOR) (fOR = 0.6 and fOR = 0.5, respectively) (Harley *et al.*, 2010). In all clinical models, metabolites had stronger and positive associations with clinical outcomes compared with the parent compound, BDE47. These results were unanticipated as previous studies suggest OH-BDEs to be more toxic than PBDEs due to their disruption of oxidative phosphorylation, which is associated with fertilization and early embryo development (Legler 2008; Legradi *et al.*, 2014; Cecchino *et al.*, 2018).

Although concentrations decreased for PBDEs and OH-BDEs over the study period, we did find a slight increase in BDE 47 and 153 along with 3 and 5-OH-BDE47 and 6-OH-BDE47 concentrations between 2007 and 2010. Coinciding with this time period, we also observed an increase in the likelihood of successful implantation, clinical pregnancy, and live birth rates in our cohort (Supplementary Fig. S4). Increasing IVF success rates over time could have possibly biased our results of PBDEs/OH-BDEs with clinical outcomes. Our results could also be due to residual confounding. Although we adjusted for year of sample collection in our original models and performed a stratified analysis based on year (data not shown), it is possible these quantifications of year did not appropriately account for the associations for women who underwent multiple cycles within the same year compared to their PBDE and OH-BDE concentrations measured at study entry. Seafood consumption possibly mitigated our results with OH-BDEs and IVF endpoints as diets high in seafood (i.e. omega fatty acids) are associated with positive IVF outcomes (Lass and Belluzzi, 2018). A study of Japanese women found a 20-fold increase in concentrations of 6-OH-BDE47 compared to BDE47, likely attributed to seafood consumption (Haraguchi *et al.*, 2016).

When clinical outcomes were stratified by race, on average among Other race women the probabilities of implantation, clinical pregnancy and live birth all decreased with increased concentrations of all PBDEs and OH-BDEs, except for 6-OH-BDE47. However, only the association between BDE47 and a decreased probability of clinical pregnancy reached statistical significance, which could be due to the small number of Other race women (and cycles) in our sample. Several studies have established racial and ethnic disparities in exposure to endocrine disrupting chemicals, including PBDEs among women (James-Todd *et al.*, 2016). We observed higher concentrations of PBDEs among Other race women (Supplementary Fig. S3). Similar results were observed from NHANES, which found higher levels of BDE47 and BDE99 among Mexican Americans and Blacks compared to Whites (Sjödin *et al.*, 2008). A larger analysis of NHANES data observed higher concentrations of BDE47 and BDE99 among non-Hispanic Blacks compared to all other race and ethnicity groups (Sjödin *et al.*, 2019). Higher concentrations of six PBDE congeners have been

observed among USA Black adolescent girls compared to Whites (Windham et al., 2010).

Our study is not without limitations. Despite the long half-lives of PBDEs ranging in the order of years, it is possible for exposure misclassification for women who underwent multiple cycles (over months or even years) with only a single exposure measurement (Makey et al., 2014). However, results from models only including first cycle outcomes were similar to our multiple-cycle models (Supplementary Tables SVI and SVII). Like many other studies, we measured PBDE and OH-BDEs in serum, while the measurement of chemicals in FF could potentially be an optimal medium for the specific microenvironments for reproductive studies (Lefevre et al., 2016; Huang et al., 2019). A previous IVF study comparing PBDEs in FF and serum detected weak but significant correlations (Kendall's tau-beta (T_b) = 0.15–0.38) for congeners 47, 100 and 154 (Johnson et al., 2012). We also tested associations for multiple congeners and metabolites with multiple outcomes. Lastly, a limitation of the study is that the results were very imprecise for the stratified analyses. This could be due to the small sample size among women of Other race ($n = 30$), but confidence intervals were also wide for models conducted within White women. The homogeneity of our population, similar to other IVF cohorts, may have resulted in a lack of precision of the probabilities of clinical outcomes seen in the Other race stratified models (Zota et al., 2010). However, we also observed wider confidence intervals for White models.

To the best of our knowledge, the present study is the largest prospective preconception cohort assessing the association of PBDEs and OH-BDEs on reproductive health. This is also the first study to assess the relationship between PBDEs and OH-BDEs with intermediate IVF outcomes (prior to implantation). An IVF cohort also allows for the study of many endpoints critical to a successful pregnancy but not observable in a TTP study in the general population. Our prospective study design eliminates the possibility of reverse causation. Finally, our use of a CWGEE model for clinical outcomes account for the multiple cycles per woman and provide more precise effect estimates compared to other statistical approaches (Yland et al., 2019).

Conclusion

Among our cohort, PBDEs and OH-BDEs were frequently detected in serum with concentrations highest in the early years of the study period, which coincides with the phase out of the PentaBDE mixture in 2004 (Renner, 2004). We did not observe any consistent trends of PBDEs or OH-BDEs with intermediate IVF outcomes but identified some unexpected positive relationships of clinical IVF outcomes with BDE153, 3 and 5-OH-BDE and 6-OH-BDE. Our stratified models supported prior studies of racial disparities with higher concentration of PBDEs among Other race populations. Future studies should focus on the rise in use of alternative FRs as concentrations of PBDEs continue to drop as well as employ designs to adequately explore racial or ethnic disparities with PBDE exposure and their associations with reproductive health.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

M.E.I.: data analysis, interpretation, drafting work, final approval, accountability. L.M.-A.: data interpretation, draft review, final approval, accountability. C.C.C.: data interpretation, draft review, final approval, accountability. H.M.S.: conceptualization, data interpretation, draft review, final approval, accountability. P.L.W.: design and implementation of analysis, data interpretation, draft review, final approval, accountability. J.D.F.: data acquisition, final approval, accountability. M.B.M.: data interpretation, draft review, final approval, accountability. R.H.: conceptualization, data interpretation, draft review, final approval, accountability. J.D.M.: conceptualization, data interpretation, draft review, final approval, accountability.

Conflict of interest

The authors declare no conflict of interest.

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