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Urinary concentrations of bisphenol A, parabens and phthalate metabolite mixtures in relation to reproductive success among women undergoing in vitro fertilization

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ABSTRACT

Background: We have previously investigated whether urinary concentrations of bisphenol A (BPA), parabens, and phthalate metabolites were individually associated with reproductive outcomes among women undergoing in vitro fertilization (IVF) treatment. However, humans are typically exposed to many man-made chemicals simultaneously. Thus, investigating one chemical at a time may not represent the effect of mixtures.

Objective: To investigate whether urinary concentrations of BPA, parabens, and phthalate metabolite mixtures are associated with reproductive outcomes among women undergoing IVF.

Methods: This prospective cohort study included 420 women contributing 648 IVF cycles who provided up to two urine samples per cycle prior to oocyte retrieval ($N = 1145$) between 2006 and 2017 at the Massachusetts General Hospital Fertility Center, and had available urine biomarker data. Urinary concentrations of BPA, parabens, and phthalate metabolites were quantified using isotope-dilution tandem mass spectrometry. Intermediate and clinical end-points of IVF treatments were abstracted from electronic medical records. Principal component analysis (PCA) and Bayesian kernel machine regression (BKMR) were used to identify main patterns of BPA, parabens, and phthalate metabolites concentrations. We used generalized linear mixed models to evaluate the association between PCA-derived factor scores, in quartiles, and IVF outcomes, using random intercepts to account for multiple IVF cycles and adjusting for known confounders. Because of temporal trends in exposure, we conducted a sensitivity analysis restricted to women who underwent IVF cycles in the earlier years of study (2006–2012).

Results: Urinary concentrations of BPA, parabens, and most phthalate metabolites were significantly lower during the second half of the study period (2013–2017) than during the first half (2006–2012). None of the three factors derived from the PCA [di(2-ethylhexyl) phthalate (DEHP), non-DEHP, and paraben] was associated with IVF outcomes in the main analyses. Similarly, BKRM analyses did not identify any associations of individual urinary concentrations of BPA, paraben and phthalate metabolites with IVF outcomes while accounting for correlation between exposures. However, in sensitivity analyses restricted to women who underwent IVF cycles from 2006 to 2012, where concentrations of most phthalates and phenols were higher, there were decreases in implantation, clinical pregnancy, and live birth across quartiles of the DEHP factor. Specifically, women in the highest quartile of the DEHP factor had, on average, lower probabilities of implantation (−22% p, trend = 0.08), clinical pregnancy (−24% p, trend = 0.14), and live birth (−38% p, trend = 0.06) compared to women in the lowest quartile. Among this group of women, BKMR results did not identify any single contributor

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driving the decreased probabilities of live birth within the DEHP factor.
Conclusions: We confirmed that women undergoing IVF are concurrently exposed to multiple endocrine disrupting chemicals (EDCs). While we found no overall significant associations, we observed diminished pregnancy success with specific clusters of chemicals among women who underwent IVF cycles in earlier years of study, when urinary concentrations of these EDCs were higher.

1. Introduction

Endocrine disrupting chemicals (EDCs) interfere with the endocrine system, resulting in adverse health effects (Diamanti-Kandarakis et al., 2009; Gore et al., 2015; WHO, 2012). It has been estimated that the annual disease costs of EDC exposure exceeds $340 billion in the United States [2% of the gross domestic product (GDP)] (Attina et al., 2016) and $217 billion in the European Union (1% of the GDP) (Trasande et al., 2015). Given the high prevalence of infertility worldwide (Inhorn and Patrizio, 2015; Mascarenhas et al., 2012), estimated to affect about 10–15% of all couples, and the ubiquitous exposure to EDCs among women of reproductive age (CDC, 2018), special attention has been given to the potential effect of exposure to EDCs on female fecundity (Aker et al., 2016; Bloom et al., 2011; Chavarro et al., 2016; Minguez-Alarcon et al., 2016b; Minguez-Alarcon and Gaskins, 2017; Nishihama et al., 2016; Philips et al., 2018; Pollack et al., 2018).

Specifically, we have previously investigated whether urinary concentrations of bisphenol A (BPA), parabens, and phthalate metabolites were individually associated with reproductive outcomes among women undergoing in vitro fertilization (IVF) treatment (Hauser et al., 2016; Minguez-Alarcon et al., 2015, 2016a). While we reported no significant associations between urinary concentrations of BPA and parabens in relation to IVF outcomes among women attending a fertility clinic, higher urinary DEHP metabolite concentrations were associated with lower oocyte counts, and lower probabilities of clinical pregnancy and live birth.

However, exploring the effect of one chemical at a time may not represent the real world situation whereby humans are exposed to hundreds of manmade chemicals at the same time (Birnbaum, 2012; Bobb et al., 2015). To our knowledge, the potential joint effect of urinary concentrations of BPA, paraben and phthalates on reproductive outcomes among women undergoing IVF remains unexplored. Thus, the objective of this study was to investigate whether urinary concentrations reflecting mixtures of BPA, parabens and phthalates were associated with reproductive outcomes (ovarian stimulation response, embryo quality, fertilization, implantation, clinical pregnancy, live birth) among women who underwent IVF at an academic fertility center in Boston, Massachusetts.

2. Methods

2.1. Study population

Study participants were women enrolled in the Environment and Reproductive Health (EARTH) Study, an ongoing prospective cohort established to evaluate environmental and dietary determinants of fertility (Messerlian et al., 2018). Women between 18 and 45 years old were eligible to participate and approximately 60% of those contacted by the research staff enrolled. The current analysis includes 420 women who completed at least one IVF cycle between 2006 and 2017 (n = 648 cycles) at the Massachusetts General Hospital (MGH) Fertility Center, and had available urine biomarker data providing at least one urine sample per IVF cycle for the quantification of BPA, parabens, and phthalate biomarkers.

Women were followed from study entry throughout their fertility care, pregnancy, and labor and delivery. At entry, the participant's date of birth was collected, and weight and height were measured by trained study staff. Pre-pregnancy body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. At enrollment, research staff administered sociodemographic, lifestyle, and medical history questionnaires to participants. Study participants provided additional information by completing a more comprehensive questionnaire on family, medical, reproductive and occupational history, product use, smoking history, and physical activity. The study was approved by the Human Subject Committees of the Harvard T.H. Chan School of Public Health, MGH, and the Centers for Disease Control and Prevention (CDC). Participants signed an informed consent after the study procedures were explained by trained research study staff and all the questions regarding the study were answered.

2.2. Exposure assessment

Women provided one (23%) or two (77%) spot urine samples per IVF cycle, with the first one collected between Day 3 and Day 9 of the gonadotrophin phase, and the second one collected on the day of oocyte retrieval (for fresh IVF cycles) or on day of embryo transfer (for cryo-thaw IVF cycles). Urine was collected in a sterile polypropylene specimen cup. Specific gravity (SG), which was used to correct the chemicals concentrations for urine dilution, was measured at room temperature using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA) calibrated with deionized water before each measurement. The urine was divided into aliquots, frozen, and stored at −80 °C. Samples were shipped on dry ice overnight to the CDC where they were stored at or below −40 °C until analysis.

As previously described (Silva et al., 2007; Ye et al., 2005), online solid-phase extraction coupled with isotope dilution-high-performance liquid chromatography-tandem mass spectrometry was used to quantify the urinary concentrations of BPA, methyl-paraben, propyl-paraben, and of eight phthalate metabolites [monoethyl phthalate (MEP); mono-n-butyl phthalate (MBP); monoisobutyl phthalate (MiBP); monobenzyl phthalate (MBzP); mono(2-ethylhexyl) phthalate (MEHP); mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP); mono(2-ethyl-5-oxohexyl) phthalate (MEOHP); mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)]. The limits of detection (LOD) ranged from 0.1 to 1.2 ng/mL. Chemical concentrations were corrected for urine dilution by SG using the following formula: $Pc = P[(1.015 - 1) / (SG - 1)]$,where Pc is the SG-corrected chemical concentration (μg/L), P is the measured chemical concentration (μg/L), and 1.015 is the mean SG level in the study population (Smith et al., 2012). The geometric mean of the SG-adjusted chemical concentrations from two spot urine samples collected during each IVF cycle was used as a measure of cycle-specific urinary chemical concentration. For cycles with only one urine sample, the chemical concentration for that single sample was used as the cycle-specific urinary chemical concentration. Samples with a chemical concentration below the LOD were assigned a value equal to the LOD divided by the square root of 2 prior to adjustment by SG as described previously (Meeker et al., 2010). During the study period, the coefficient of variation for these assays ranged from 3.5% to 7.3% for BPA, 4.1% to 14.9% for methyl-paraben, 2.7% to 5.9% for propyl-paraben, 3.1% to 5.5% for MEP, 6.6% to 8.8% for MBP, 7.7% to 9.0% for MiBP, 4.5% to 16% for MBzP, 4.7% to 8.1% for MEHP, 4.5% to 7.0% for MEHHP, 4.4% to 6.2% for MEOHP, and 4.3% to 6.1% for MECPP.

2.3. Outcome assessment

Clinical information was abstracted from the patient's electronic medical record by research staff. Serum follicle stimulating hormone (FSH) was measured in a blood sample collected on the third day of the menstrual cycle using an automated electrochemiluminescence immunoassay at the MGH Core Laboratory as previously described

(LOD = 0.1 U/L) (Mok-Lin et al., 2010). Infertility diagnosis was coded according to previously described definitions of the Society for Assisted Reproductive Technology (SART) (SART, 2015). SART diagnoses includes: 1) male factor infertility which included poor semen quantity/quality; 2) female factor infertility which included endometriosis (endometrial tissue outside the uterus), diminished ovarian reserve (diminished capacity of the ovary to provide eggs), tubal or ovulatory disorders (e.g. damaged fallopian tubes or altered ovulation), other causes; and 3) unexplained infertility (idiopathic). Women underwent one of three controlled ovarian stimulation IVF treatment protocols on day 3 of induced menses after completing a cycle of oral contraceptives: (1) luteal phase GnRH-agonist protocol, (2) follicular phase GnRH-agonist/Flare protocol, or (3) GnRH-antagonist protocol. Protocols were chosen by the treating physician after taking into consideration several factors as described elsewhere (Minguez-Alarcon et al., 2016b). In the luteal phase GnRH-agonist protocol, lupron dose was reduced at, or shortly after, the start of ovarian stimulation with FSH/hMG. FSH/hMG and GnRH-agonist or GnRH-antagonist was continued to the day of trigger with Human Chorionic Gonadotropin (hCG). Follicular response was monitored with serial ultrasounds and estradiol levels, determined at the MGH Core Laboratory using the Elecsys Estradiol II reagent kit (Roche Diagnostics). Human Chorionic Gonadotropin was administered intramuscularly approximately 36 h before the scheduled oocyte retrieval procedure to induce ovulation. Details of oocyte retrieval have been previously described (Mok-Lin et al., 2010). The peak serum E_2 concentration was defined as the highest level of E_2 preceding the oocyte retrieval and obtained on the day of hCG administration. Oocyte retrieval was completed with the presence of 3 or more lead follicles ($\geq$16 mm in diameter) and when estradiol level reached at least 600 pg/mL.

Embryologists determined the total number of oocytes retrieved per cycle and classified them as germinal vesicle, metaphase I, metaphase II (MII) or degenerated. Oocytes underwent either conventional IVF or intracytoplasmic sperm injection (ICSI) as clinically indicated. Embryologists determined the fertilization rate 16–20 h after insemination as the number of oocytes with two pronuclei divided by the number of mature (MII) oocytes that were either inseminated or injected. Patients undergoing cryo-thaw or donor-egg recipient cycles, underwent endometrial preparation protocols as clinically indicated. Following embryo transfer, all clinical outcomes (i.e. implantation, clinical pregnancy and live birth) were assessed identically for fresh, cryo-thaw, and donor-egg recipient cycles. Implantation was defined as a serum β-hCG level > 6 mIU/mL, typically measured 17 days (range 15–20 days) after oocyte retrieval. An elevation in β-hCG with the confirmation of an intrauterine pregnancy on an ultrasound at 6 weeks was considered a clinical pregnancy. A live birth was defined as the birth of a neonate on or after 24 weeks of gestation.

2.4. Statistical analysis

Distribution of urinary concentrations of BPA, parabens and phthalate metabolites were presented using medians ± interquartile ranges (IQRs). Differences in these distributions before and after 2012 were evaluated using Kruskal–Wallis tests. Correlations between urinary chemical concentrations were assessed using Spearman correlation coefficients. Demographic and baseline reproductive characteristics of the women were presented using median ± IQRs or counts (%). Urinary concentrations of BPA, parabens and phthalate metabolites were $\log_e$-transformed due to right skewedness, standardized to create z-scores, and were included in a principal component analysis (PCA) with varimax rotation to identify the principal components with eigenvalue greater than one (O'Rourke and Hatcher, 2013). Factor scores derived from the PCA were categorized into quartiles, with the lowest quartile considered as the reference group.

Associations of the DEHP factor scores with demographic characteristics and reproductive characteristics at study entry were evaluated using Kruskal–Wallis tests for continuous variables and chi-squared tests for categorical variables (or Fisher's exact test where appropriate). We chose to present demographic and reproductive information by the DEHP factor rather than other factor scores because DEHP-related metabolites showed associations with reproductive endpoints in an earlier analysis from the same study cohort (Hauser et al., 2016). Multivariable generalized linear mixed models were used to evaluate the associations between quartiles of the factor scores derived from the PCA and IVF outcomes, with a random intercept to account for correlation in outcomes across multiple IVF cycles per woman and adjusting for confounders. A Poisson distribution and log link function were specified for oocyte counts, a normal distribution and identity link were specified for endometrial wall thickness, and a binomial distribution and logit link function were specified for fertilization, implantation, clinical pregnancy, and live birth. To allow for better interpretation of the results, population marginal means (Searle et al., 1980) are presented adjusting for all the covariates in the model (at the mean level for continuous variables and weighted according to their relative frequencies for categorical variables). Relative changes are also presented in Supplemental tables to allow comparison of results with other environmental epidemiologic studies. Tests for linear trends across quartiles of the PCA-derived factor scores were conducted using ordinal level indicator variables for each quartile.

We also used Bayesian kernel machine regression (BKMR), a method developed for investigating chemical mixtures that flexibly models the joint effect of chemicals using a kernel function (Bobb et al., 2015; Valeri et al., 2017). The BKMR approach allows the visualization of the exposure-response association for each component of a mixture, while accounting for the correlation between the mixture components. Possible synergistic and non-linear effects can also be evaluated. Specifically, each endpoint was included in the model as a smooth function equation (represented using a kernel function) of the exposure variables, adjusted for possible confounding factors. Because the health outcome may depend on only a subset of the mixture components, variable selection was conducted to identify which of these components are responsible for the health effects of the mixture. To address collinearity of the mixture components, a hierarchical variable selection extension to BKMR was included that can incorporate prior knowledge on the structure of the mixture. Results from this analysis are presented by displaying the difference in probabilities of clinical pregnancy and live birth for a change in urinary concentration of BPA, paraben and phthalate metabolites between the 25th and 75th percentile. Measurements of each specific $\log_e$-transformed SG-adjusted BPA, paraben and phthalate metabolite concentrations were treated as continuous predictors and the other measured biomarkers of the mixture were set at their median values while adjusting for confounders.

Confounding was assessed using prior knowledge on biological relevance and descriptive statistics from our study population. The variables considered as potential confounders included factors previously related to female reproductive endpoints (Rooney and Domar, 2014; Sharma et al., 2013), and factors associated with urinary chemical biomarker concentrations and reproductive outcomes in this study. Final models were adjusted for age (years), BMI (kg/m^2), year of IVF treatment cycle (year), primary SART infertility diagnosis at study entry (female, male, unexplained), having had a previous intrauterine insemination (IUI) at the MGH before joining the study (yes, no) and scores of the other two PCA-derived factors (continuous- not included in the BKMR models). Due to declining U.S. trends in exposure to these chemicals, a sensitivity analysis was conducted by restricting to women who underwent IVF cycles between 2006 and 2012 (Hauser et al., 2016). We also assessed the robustness of the findings by: 1) restricting the analysis to cycles for which women provided two urine samples; 2) restricting the analysis to one IVF cycle (first in study cycle) per woman; and 3) excluding cryo-thaw cycles. In addition, analyses were stratified by age (< 37 vs. ≥37 years), BMI (< 25 vs. ≥25 kg/m^2) and protocol type (agonist vs. other) to evaluate possible effect modification (p for interaction < 0.10). Statistical analyses were performed with SAS (version 9.4; SAS Institute Inc., Cary, NC, USA), with exception of the BKMR models, which were conducted using the R package bkmr (Bobb

et al., 2015; Bobb, 2017). Statistical tests were two-tailed and all *p*-values < 0.05 were conventionally regarded as statistically significant.

3. Results

A total of 1145 urine samples were collected from the 420 women included in this analysis. The detection frequencies for urinary concentrations of BPA, parabens (methyl-paraben and propyl-paraben), and phthalate metabolites (MBP, MiBP, MEP, MBzP, MEHP, MEHHP, MEOHP and MECPP) ranged from 73% to 99% (Table 1), and were similar to those found in U.S. females from the general population (CDC, 2018). Urinary concentrations of BPA, parabens, and phthalate metabolites were significantly lower during the second half of the study period (2013–2017) than during the first half (2006–2012), with the exception of MiBP, which increased significantly over time (Table 1). Urinary DEHP metabolite concentrations were highly correlated with each other ($r = 0.72$ to 0.98), as were urinary paraben (methyl-paraben and propyl-paraben) concentrations ($r = 0.86$) (Supplemental Table S1). Urinary concentrations of BPA and non-DEHP metabolites were weakly correlated with all other chemicals, except MBP with MiBP and MBzP ($r = 0.62$).

All urinary biomarkers concentrations were included in a PCA and three different factors were identified accounting for 43%, 16% and 16%, of the total variance in urinary phthalate metabolites, BPA, and paraben concentrations, respectively (Supplemental Table S2). Factor 1, which we refer to as the DEHP factor, was characterized by high loading scores of urinary MEHP, MEHHP, MEOHP and MECPP concentrations. Factor 2, referred to as the non-DEHP factor, had high loading scores of urinary MBP, MiBP, and MBzP concentrations. Finally, Factor 3, or the paraben factor, had loading factors for methyl-paraben, propyl-paraben and MEP loading scores. Although urinary concentrations of BPA did not exhibit high loading scores for any of the identified factors, they contributed to the DEHP factor (Supplemental Table S2).

The 420 women had a median (interquartile range [IQR]) age of 35.0 (32.0, 39.0) years and BMI of 23.1 (21.2, 26.0) kg/m^2, were predominantly white (83%), and 27% ever smoked (Table 2). Among the 420 women, 228 (54%) underwent a previous IUI at the MGH before joining the study, and female infertility was the diagnosis at enrollment for a third of the study population. Compared to women in the lowest quartile of the DEHP factor scores, women in the highest quartile were more likely to have previously undergone an IUI procedure (21% vs. 48%; *p*-value < 0.001) and to have female factor as the primary infertility diagnosis at enrollment (32% vs. 43%; p- value = 0.02); these two characteristics were positively associated with each other (p-value = 0.003) (data not shown). No other demographic and reproductive characteristics at study entry differed significantly across quartiles of DEHP factor loading scores (Table 2).

In unadjusted models, women in the highest quartile of DEHP factor score had, on average, lower total oocyte counts compared to women in the lowest quartile (10.9 vs. 11.8) (Supplemental Table S3). However, these differences did not reach significance and overall the DEHP factor scores were unrelated to the other IVF outcomes examined. Non-DEHP and paraben factor scores were also not associated with the IVF outcomes examined. These findings were confirmed in models adjusting for potential confounders (Table 3, adjusted relative differences in Supplemental Table S4). For example, women in the highest vs. lowest quartiles had probabilities of live birth of 0.32 vs. 0.37 (DEHP), 0.37 vs. 0.42 (non-DEHP), and 0.38 vs. 0.37 (paraben factors), respectively. Similarly, none of the three factors were significantly associated with any of the measured reproductive endpoints when analyses were restricted to women who provided two urine samples per cycle, one IVF cycle (first in study cycle) per woman, and also when excluding cryothaw cycles (data not shown). No effect modification by age, BMI or protocol type was observed (data not shown).

Nevertheless, in sensitivity analyses restricted to women who underwent IVF cycles between 2006 and 2012, we observed decreases in implantation, clinical pregnancy, and live birth across quartiles of the

Table 1
Distribution of SG-adjusted urinary concentrations (μg/L) of bisphenol A, parabens and phthalate metabolites among 420 women contributing 648 cycles (1145 urines) in the EARTH Study.

	Entire study period 2006–2017					2006–2012 *N* = 432			2013–2017 *N* = 216			Comparison of 2013–2017 to 2006–2012
	Detection frequency %	GM (SD)	25th	50th	75th	25th	50th	75th	25th	50th	75th	p. value
Bisphenol A (BPA)	86	1.14 (0.03)	0.71	1.10	1.75	0.87	1.31	2.09	0.49	0.76	1.23	< 0.001
Methylparaben (MPB)	99	105 (5.92)	36.6	119	302	48.4	151	344	24.5	85.7	219	< 0.001
Propylparaben (PPB)	98	17.5 (1.29)	4.61	21.4	73.2	6.71	27.7	86.1	2.76	10.1	40.8	< 0.001
Mono-n-butyl phthalate (MBP)	96	10.9 (0.38)	6.63	11.5	19.4	7.31	12.3	21.0	5.96	9.98	15.7	0.0005
Mono-isobutyl phthalate (MiBP)	97	7.31 (0.25)	4.35	7.78	12.9	3.84	6.94	12.4	5.33	9.47	14.5	0.0003
Monoethyl phthalate (MEP)	99	43.2 (2.18)	17.5	39.1	94.6	19.8	46.2	120	14.5	27.3	67.0	< 0.001
Monobenzyl phthalate (MBzP)	94	3.52 (0.15)	1.68	3.44	7.09	1.75	3.65	7.57	1.51	3.20	5.84	0.04
Mono-2-ethylhexyl phthalate (MEHP)	73	2.34 (0.11)	1.05	2.07	4.47	1.22	2.80	6.39	0.84	1.41	2.47	< 0.001
Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP)	99	11.4 (0.51)	5.41	10.1	21.2	7.48	14.3	32.7	3.95	6.03	9.09	< 0.001
Mono-2-ethyl-5-oxohexyl phthalate (MEOHP)	98	7.79 (0.34)	3.69	6.85	14.5	5.14	9.72	22.0	2.80	3.90	5.82	< 0.001
Mono-2-ethyl-5-carboxypentyl phthalate (MECPP)	100	19.5 (0.80)	9.45	16.6	34.0	13.7	24.8	53.4	6.70	9.76	15.6	< 0.001

Table 2
Demographic and reproductive characteristics [median (IQR) or n (%)] across quartiles of PCA-derived factor scores for the "DEHP factor" among 420 women contributing 648 IVF cycles in the EARTH Study.

		DEHP factor				
	Total cohort	Q1	Q2	Q3	Q4	p-Value
	420 women 648 cycles	109 women 162 cycles	100 women 162 cycles	104 women 162 cycles	107 women 162 cycles	
Demographic characteristics						
Age, years	35.0 (32.0, 39.0)	35.0 (32.0, 39.0)	35.0 (32.0, 38.0)	35.0 (32.5, 39.0)	36.0 (33.0, 39.0)	0.47
Race, n (%)						0.16
White/Caucasian	350 (83)	95 (87)	77 (77)	85 (82)	93 (87)	
Black/Asian/Other	70 (17)	14 (13)	23 (23)	19 (18)	14 (13)	
Body Mass Index, kg/m^2	23.1 (21.2, 26.0)	23.0 (21.2, 25.8)	23.2 (21.2, 26.1)	23.1 (21.1, 25.7)	23.3 (21.2, 26.3)	0.84
Ever smoker, n (%)	112 (27)	25 (23)	33 (33)	27 (26)	27 (25)	0.40
Education, n (%)[a]						0.69
High school/some college	29 (7)	8 (7)	7 (7)	7 (7)	7 (6)	
College graduate	125 (30)	25 (23)	32 (32)	31 (30)	37 (35)	
Graduate degree	266 (63)	76 (70)	61 (61)	66 (63)	63 (59)	
Reproductive/cycle characteristics						
Day 3 FSH Levels, IU/L	6.9 (6.1, 8.3)	6.8 (6.0, 8.3)	6.8 (5.8, 7.8)	7.4 (6.0, 8.5)	7.1 (6.3, 8.4)	0.10
Initial infertility diagnosis, n (%)						0.02
Male factor	199 (31)	42 (26)	52 (32)	54 (33)	51 (31)	
Female factor	224 (34)	52 (32)	45 (28)	58 (36)	69 (43)	
Unexplained	225 (35)	68 (42)	65 (40)	50 (31)	42 (26)	
Previous IUI, n (%)	228 (35)	34 (21)	47 (29)	69 (43)	78 (48)	< 0.0001
Previous IVF, n (%)	144 (22)	28 (17)	32 (20)	45 (28)	39 (24)	0.11
Treatment protocol, n (%)						0.26
Antagonist	85 (13)	22 (14)	20 (12)	25 (15)	18 (11)	
Flare	101 (16)	25 (15)	15 (9)	27 (17)	34 (21)	
Luteal phase agonist	378 (58)	94 (58)	107 (66)	86 (53)	91 (56)	
Cryo cycle	64 (10)	18 (11)	15 (9)	19 (12)	12 (7)	
Donor recipient	20 (3)	3 (2)	5 (4)	5 (3)	7 (4)	
ICSI cycles, n (%)	303 (57)	72 (56)	80 (58)	71 (54)	80 (59)	0.86

[a] Some women (n = 35, 8%) had missing education and were included in the graduate degree category.

Table 3
Adjusted[a] early developmental and pregnancy outcomes (adjusted mean, 95% CI) by quartiles of PCA-derived factor scores from SG-adjusted urinary phenols and phthalate metabolite concentrations among 420 women undergoing 648 IVF cycles in the EARTH Study.

	Total oocyte yield (n)	MII oocyte yield (n)	Endometrial wall thickness (mm)	Fertilization (rate)	Implantation (probability)	Clinical pregnancy (probability)	Live birth (probability)
DEHP factor							
Q1	11.1 (10.2, 12.0)	9.3 (8.5, 10.1)	10.1 (9.7, 10.5)	0.71 (0.67, 0.75)	0.56 (0.47, 0.64)	0.48 (0.39, 0.56)	0.37 (0.29, 0.46)
Q2	11.7 (10.9, 12.6)	9.7 (9.0, 10.5)	10.6 (10.2, 10.9)	0.72 (0.69, 0.75)	0.60 (0.51, 0.67)	0.51 (0.43, 0.59)	0.44 (0.36, 0.52)
Q3	10.7 (9.9, 11.6)	8.9 (8.2, 9.6)	10.0 (9.6, 10.3)	0.68 (0.64, 0.71)	0.55 (0.47, 0.63)	0.50 (0.42, 0.58)	0.42 (0.34, 0.50)
Q4	10.9 (10.0, 11.9)	9.2 (8.4, 10.1)	10.2 (9.8, 10.6)	0.74 (0.70, 0.78)	0.48 (0.38, 0.57)	0.42 (0.33, 0.51)	0.32 (0.24, 0.41)
p, trend	0.57	0.61	0.76	0.62	0.23	0.44	0.50
non-DEHP factor							
Q1	10.8 (10.0, 11.7)	9.0 (8.3, 9.8)	10.2 (9.9, 10.6)	0.71 (0.67, 0.75)	0.58 (0.50, 0.66)	0.53 (0.45, 0.60)	0.42 (0.34, 0.50)
Q2	11.1 (10.2, 12.0)	9.3 (8.5, 10.1)	10.0 (9.8, 10.5)	0.69 (0.66, 0.73)	0.55 (0.47, 0.63)	0.48 (0.40, 0.56)	0.40 (0.32, 0.48)
Q3	11.4 (10.5, 12.3)	9.5 (8.7, 10.3)	10.2 (9.9, 10.6)	0.74 (0.70, 0.77)	0.47 (0.39, 0.55)	0.40 (0.32, 0.48)	0.36 (0.29, 0.44)
Q4	11.3 (10.4, 12.2)	9.3 (8.6, 10.2)	10.2 (9.8, 10.6)	0.71 (0.67, 0.75)	0.58 (0.50, 0.66)	0.50 (0.42, 0.58)	0.37 (0.29, 0.45)
p, trend	0.39	0.52	0.94	0.62	0.68	0.36	0.30
Parabens factor							
Q1	10.8 (9.9, 11.7)	9.1 (8.4, 10.0)	10.2 (9.8, 10.6)	0.70 (0.66, 0.74)	0.53 (0.45, 0.61)	0.45 (0.37, 0.53)	0.37 (0.30, 0.46)
Q2	11.0 (10.1, 11.8)	9.1 (8.4, 9.9)	10.2 (9.7, 10.5)	0.71 (0.68, 0.75)	0.59 (0.51, 0.67)	0.53 (0.45, 0.60)	0.45 (0.37, 0.53)
Q3	11.6 (10.7, 12.6)	9.7 (9.0, 10.5)	10.1 (9.7, 10.5)	0.70 (0.66, 0.73)	0.49 (0.41, 0.57)	0.44 (0.36, 0.52)	0.35 (0.28, 0.43)
Q4	11.2 (10.3, 12.1)	9.2 (8.4, 10.0)	10.4 (10.0, 10.8)	0.74 (0.71, 0.78)	0.57 (0.49, 0.65)	0.49 (0.41, 0.57)	0.38 (0.30, 0.46)
p, trend	0.38	0.72	0.59	0.18	0.93	0.93	0.62

[a] Models were adjusted for maternal age (continuous), body mass index (continuous), year of IVF treatment cycle (continuous), primary SART infertility diagnosis at study entry (female, male, unknown), having had a previous IUI (yes, no) and scores of the other two factors (continuous).

DEHP factor (Table 4, adjusted relative differences in Supplemental Table S5). Specifically, compared to women in the lowest quartile of the DEHP factor, women in the highest quartile had, on average, lower probabilities of implantation (−22% p-value = 0.12), clinical pregnancy (−24% p-value = 0.13), and live birth (−38% p-value = 0.08). Among this group of women who underwent IVF cycles in earlier study years, smaller decreases in live birth were also found across quartiles of non-DEHP factor, while no decreases in implantation, clinical pregnancy and live birth were observed across quartiles of the paraben factor.

Consistent with the overall null results for the PCA-derived factor scores in the adjusted main analysis (Table 3), the BKRM analyses did not identify any associations of urinary concentrations of BPA, parabens, and phthalate metabolites with IVF outcomes while accounting for correlation between exposure biomarkers (data not shown). Among

Table 4
Adjusted[a] mean differences in probability of implantation, clinical pregnancy, live birth and principal components of SG-adjusted urinary bisphenol A, paraben and phthalate metabolites among 290 women undergoing 432 IVF cycles in the EARTH Study between 2006 and 2012.

	Implantation (probability)	Clinical pregnancy (probability)	Live birth (probability)
DEHP factor			
Q1	0.65 (0.50, 0.77)	0.59 (0.44, 0.72)	0.50 (0.36, 0.64)
Q2	0.62 (0.51, 0.72)	0.52 (0.41, 0.63)	0.45 (0.34, 0.57)
Q3	0.57 (0.47, 0.66)	0.53 (0.43, 0.62)	0.44 (0.35, 0.54)
Q4	0.51 (0.42, 0.60)	0.45 (0.36, 0.54)	0.34 (0.26, 0.43)
p, trend	0.08	0.14	0.06
non-DEHP factor			
Q1	0.62 (0.52, 0.71)	0.57 (0.47, 0.66)	0.47 (0.37, 0.57)
Q2	0.57 (0.46, 0.66)	0.51 (0.41, 0.61)	0.42 (0.32, 0.52)
Q3	0.49 (0.39, 0.59)	0.43 (0.33, 0.53)	0.41 (0.31, 0.51)
Q4	0.60 (0.49, 0.69)	0.52 (0.41, 0.62)	0.37 (0.27, 0.47)
p, trend	0.50	0.26	0.17
parabens factor			
Q1	0.56 (0.44, 0.67)	0.48 (0.37, 0.59)	0.35 (0.25, 0.47)
Q2	0.63 (0.53, 0.73)	0.58 (0.48, 0.68)	0.51 (0.41, 0.62)
Q3	0.48 (0.39, 0.58)	0.43 (0.34, 0.53)	0.36 (0.26, 0.49)
Q4	0.60 (0.50, 0.68)	0.52 (0.43, 0.62)	0.43 (0.33, 0.53)
p, trend	0.91	0.93	0.88

[a] Models were adjusted for maternal age (continuous), body mass index (continuous), primary SART infertility diagnosis at study entry (female, male, unknown), having had a previous IUI (yes, no) and scores of the other two factors (continuous).

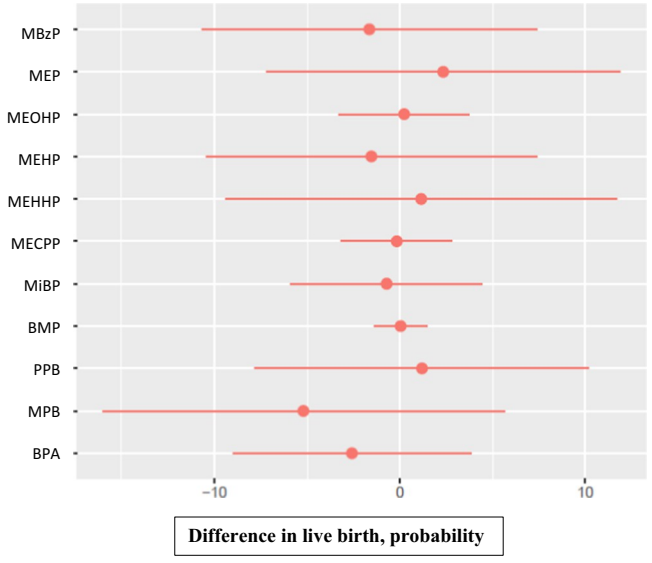

Fig. 1. Adjusted mean differences in probability of clinical pregnancy (estimates and 95% CI) as a function of urinary bisphenol A, paraben and phthalate metabolite concentrations among 290 women undergoing 432 IVF cycles in the EARTH Study between 2006 and 2012. Point estimates show the difference in mean live birth probability (%) when each chemical was increased from the 25th to the 75th percentile of its distribution, while fixing other chemical at their median concentrations. The results were estimated by Bayesian Kernel Machine Regression, adjusting for age (continuous), body mass index (continuous), year of IVF treatment cycle (continuous), having had a previous IUI (yes/no), primary SART infertility diagnosis at study entry (female, male, unknown), and scores of the other two factors (continuous).

women who underwent IVF cycles between 2006 and 2012, BKMR results also did not identify a single, large contributor to the decreased probabilities of live birth across the DEHP and non-DEHP factors (Table 4, Fig. 1). Results yielded wide confidence intervals and were not significant.

4. Discussion

Applying PCA and BKMR methods, we investigated whether urinary concentrations of BPA, parabens, and phthalate metabolites were associated with reproductive and pregnancy outcomes among women undergoing IVF. Three main factors were identified using PCA: the DEHP factor, characterized by relatively high urinary concentrations of four DEHP metabolites: MEHP, MEHHP, MEOHP and MECPP; the non-DEHP factor, with high urinary concentrations of MBP, MiBP, and MBzP; and the paraben factor, with relatively high urinary concentrations of methyl-paraben, propyl-paraben and MEP. None of these three factors were associated with oocyte counts, endometrial thickness, fertilization rate and probabilities of implantation, clinical pregnancy and live birth. Similarly, BKMR results did not identify any specific biomarker to be associated with any reproductive outcome in the main analyses. However, decreased implantation, clinical pregnancy or live birth across quartiles of the DEHP factor were found in a sensitivity analysis among women who underwent IVF cycles during earlier study years (2006 and 2012) and when exposure was observed to be the highest in this cohort. BKRM results did not identify any specific biomarker to drive these decreases among this subgroup of women.

The current PCA identified similar factors as in a recent publication from our group on phthalate metabolite mixtures and birth weight in the same study cohort (Chiu et al., 2018) and also in other pregnancy cohorts (Maresca et al., 2016), in which two main phthalate factors, the DEHP and the non-DEHP, were identified. DEHP is commonly added to plastics to make them flexible and is found in consumer products, flooring and wall coverings, food contact applications, and medical devices. Metabolites of DEHP include MEHP, MEHHP, MEOHP and MECPP. Other phthalate metabolites such as MBP and MiBP may have common sources and their precursors are used as solvents and plasticizers for cellulose acetate, varnishes and coatings, and some are also found in personal care products (Braun et al., 2014; Hauser and Calafat, 2005). Although BPA was not characteristic of any of the factors derived from the PCA, it partially contributed to the DEHP factor. BPA can be used in the manufacture of polycarbonate plastics, epoxy resin liners of canned foods, some dental sealants and composites, and thermal receipts (Minguez-Alarcon et al., 2016c). The paraben factor was characterized by relatively high urinary concentrations of both parabens (methyl-paraben and propyl-paraben), as well as MEP. While diethyl phthalate, the parent compound of MEP, can be found in personal care products, parabens are used as preservatives in cosmetics, personal care products, pharmaceuticals, and food (Andersen, 2008). Since PCA converts a set of observed variables into principal components based on the collinearity between the exposure variables rather than the underlying biological effects of a given mixture on the outcomes, the identified principal components reflected such exposure patterns.

While we did not find an association of any of the three factors with any reproductive outcome in the main analysis, decreases in implantation, clinical pregnancy and live birth were observed across quartiles of the DEHP factor in a sensitivity analysis restricted to women who underwent IVF cycles during earlier years of study (2006–2012). We have previously investigated whether urinary concentrations of BPA, parabens, and phthalate metabolites are individually related to reproductive outcomes in a smaller group of women from the same study cohort who underwent an IVF cycle between 2004 and 2012 (Hauser et al., 2016; Minguez-Alarcon et al., 2015, 2016a). In line with the main results in the current manuscript and also with the sensitivity analysis during earlier years of study, no differences in IVF outcomes were observed across quartiles of urinary concentrations of BPA (Minguez-Alarcon et al., 2015) or parabens (Minguez-Alarcon et al., 2016a). Decreased oocyte counts and probabilities of clinical pregnancy and live birth were observed (Hauser et al., 2016), as well as increased pregnancy loss (Messerlian et al., 2016) across quartiles of urinary DEHP metabolite concentrations,

which is also consistent with our sensitivity analysis including women who underwent an IVF cycle between 2006 and 2012. The main explanation for differences between the main null results in this study and our earlier publications is the significant decrease in urinary concentrations of these EDCs after 2012, which has also been shown among U.S. women of the general population (CDC, 2018). Nevertheless, BKRM results did not identify any specific biomarker which drove decreases in implantation, pregnancy and live birth across quartiles of DEHP factor among this subgroup of women in earlier years of study (sensitivity analysis including IVF cycles before 2012), which is not consistent with associations between some individual urinary phthalate metabolite concentrations and pregnancy outcomes in our earlier publication (Hauser et al., 2016). This may be explained by the fact that in the current manuscript, compared to our earlier publication, we are evaluating related but not identical hypotheses. The motivation to perform the current analysis, and one of the main strengths, is to minimize residual confounding due to exposure to other EDCs. BKMR methods account for correlation between chemicals, which in this manuscript includes BPA, parabens and phthalates; these correlations were not considered in prior analyses and co-exposure was not accounted for (Hauser et al., 2016). Other epidemiological studies evaluating phthalates in relation to IVF outcomes include one recent study conducted in Israel (Machtinger et al., 2018). Machtinger and coworkers have found some detrimental effect of certain phthalates on oocyte counts and fertilization rate. However, they reported no associations with implantation, clinical pregnancy, and live birth among Israeli women undergoing IVF who had similar urinary phthalate metabolite concentrations compared to women in the current analysis. Experimental studies have shown the endocrine disrupting activity of phthalates as reproductive toxicants (Davis et al., 1994; Ema et al., 2000; Lovekamp-Swan and Davis, 2003). Both BPA and parabens have shown weak estrogenic activity through binding with estrogen receptors α and β in vitro studies (Kuiper et al., 1998; Shaw and deCatanzaro, 2009). While there is sufficient experimental evidence reporting that BPA has a detrimental effect on female reproduction (Peretz et al., 2014), animal evidence for parabens has been limited (SCCS, 2011; Vo et al., 2010). However, exposures in these experimental studies are higher and not comparable with human studies in the field.

The present study has several limitations. First, it may not be possible to generalize our findings to couples from the overall population of couples attempting conception. Nevertheless, approximately 15% to 25% of couples trying to achieve pregnancy are diagnosed with infertility (Slama et al., 2012; Thoma et al., 2013), which make our study findings applicable to a significant proportion of couples seeking treatment. In the United States in 2015 alone, couples underwent approximately 209,000 assisted reproductive technology (ART) treatments cycles (SART, 2015), a substantial increase from 60,000 ART cycles in 1995 (Anonymous, 1998). However, women in our study population are mostly white and well educated, which may differ from other women attending other fertility centers. Second, exposure misclassification is possible given the short biological half-lives of these non-persistent chemicals and their episodic exposure (Braun et al., 2012). However, the two urine samples, collected for the vast majority of the participants would partially reduce exposure misclassification. Third, residual confounding is still possible because other EDCs (e.g., phenols, phthalates), which may be correlated with those included in the current analysis were not measured or were excluded from this analysis because of relatively low sample size or low detection frequencies (e.g., triclosan, bisphenol F). Fourth, due to the complexity of this analysis we did not consider male partner's exposure. However, including paternal data would not change results for female outcomes such as oocyte yield and endometrial thickness. The biggest strength of this study is the use of novel and sophisticated methods to analyze mixtures of exposure biomarkers in relation to reproductive endpoints. Other strengths include the prospective design, which minimizes the risk of reverse causation; complete follow-up of participants, and comprehensive adjustment for other reproductive and lifestyle factors that could result in residual confounding.

In conclusion, these results confirm the need to consider mixtures of chemicals when evaluating reproductive and pregnancy outcomes. While this study showed no overall significant associations between mixtures of urinary concentrations of BPA, parabens, and phthalates in relation to reproductive outcomes among women attending a fertility center, diminished pregnancy success with certain phthalate metabolites, as a mixture, were found among women in earlier years of study who had higher urinary concentrations of these EDCs.

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Competing financial interests

None of the authors has any conflicts of interest to declare.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC), the US Government, the Department of Health and Human Services (DHHS) or the National Institutes of Health (NIH). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or DHHS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.02.025.

References

Aker, A.M., Watkins, D.J., Johns, L.E., Ferguson, K.K., Soldin, O.P., Anzalota Del Toro, L.V., et al., 2016. Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women. Environ. Res. 151, 30–37.

Andersen, F.A., 2008. Final amended report on the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben, and benzylparaben as used in cosmetic products. Int. J. Toxicol. 27 Suppl. 4, 1–82.

Anonymous, 1998. Assisted reproductive technology in the United States and Canada: 1995 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil. Steril. 69, 389–398.

Attina, T.M., Hauser, R., Sathyanarayana, S., Hunt, P.A., Bourguignon, J.P., Myers, J.P., et al., 2016. Exposure to endocrine-disrupting chemicals in the USA: a population-based disease burden and cost analysis. The Lancet Diabetes & Endocrinology 4, 996–1003.

Birnbaum, L.S., 2012. Niehs's new strategic plan. Environ. Health Perspect. 120, a298.

Bloom, M.S., Kim, D., Vom Saal, F.S., Taylor, J.A., Cheng, G., Lamb, J.D., et al., 2011. Bisphenol a exposure reduces the estradiol response to gonadotropin stimulation during in vitro fertilization. Fertility and Sterility 96 e672, 672–677.

Bobb JF. 2017. Introduction to bayesian kernel machine regression and the bkmr r package. Available at: Https://jenfb.Github.Io/bkmr/overview.Html [accessed september, 2018].

Bobb, J.F., Valeri, L., Claus Henn, B., Christiani, D.C., Wright, R.O., Mazumdar, M., et al., 2015. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics (Oxford, England) 16, 493–508.

Braun, J.M., Smith, K.W., Williams, P.L., Calafat, A.M., Berry, K., Ehrlich, S., et al., 2012. Variability of urinary phthalate metabolite and bisphenol a concentrations before and during pregnancy. Environ. Health Perspect. 120, 739–745.

Braun, J.M., Just, A.C., Williams, P.L., Smith, K.W., Calafat, A.M., Hauser, R., 2014. Personal care product use and urinary phthalate metabolite and paraben

concentrations during pregnancy among women from a fertility clinic. Journal of Exposure Science & Environmental Epidemiology 24, 459–466.
CDC. 2018. Centers for disease control and prevention. Fourth report on human exposure to environmental chemicals, updated tables, (March 2018). Atlanta, GA: U.S. Department of health and human services, centers for disease control and prevention. Available at https://www.Cdc.Gov/exposurereport/ (accessed july 2018).
Chavarro, J.E., Minguez-Alarcon, L., Chiu, Y.H., Gaskins, A.J., Souter, I., Williams, P.L., et al., 2016. Soy intake modifies the relation between urinary bisphenol a concentrations and pregnancy outcomes among women undergoing assisted reproduction. J. Clin. Endocrinol. Metab. 101, 1082–1090.
Chiu, Y.H., Bellavia, A., James-Todd, T., Correia, K.F., Valeri, L., Messerlian, C., et al., 2018. Evaluating effects of prenatal exposure to phthalate mixtures on birth weight: a comparison of three statistical approaches. Environ. Int. 113, 231–239.
Davis, B.J., Maronpot, R.R., Heindel, J.J., 1994. Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats. Toxicol. Appl. Pharmacol. 128, 216–223.
Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., et al., 2009. Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr. Rev. 30, 293–342.
Ema, M., Miyawaki, E., Kawashima, K., 2000. Effects of dibutyl phthalate on reproductive function in pregnant and pseudopregnant rats. Reproductive toxicology (Elmsford, NY) 14, 13–19.
Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., et al., 2015. Edc-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, E1–e150.
Hauser, R., Calafat, A.M., 2005. Phthalates and human health. Occup. Environ. Med. 62, 806–818.
Hauser, R., Gaskins, A.J., Souter, I., Smith, K.W., Dodge, L.E., Ehrlich, S., et al., 2016. Urinary phthalate metabolite concentrations and reproductive outcomes among women undergoing in vitro fertilization: results from the earth study. Environ. Health Perspect. 124, 831–839.
Inhorn, M.C., Patrizio, P., 2015. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Hum. Reprod. Update 21, 411–426.
Kuiper, G.G., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H., van der Saag, P.T., et al., 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 139, 4252–4263.
Lovekamp-Swan, T., Davis, B.J., 2003. Mechanisms of phthalate ester toxicity in the female reproductive system. Environ. Health Perspect. 111, 139–145.
Machtinger, R., Gaskins, A.J., Racowsky, C., Mansur, A., Adir, M., Baccarelli, A.A., et al., 2018. Urinary concentrations of biomarkers of phthalates and phthalate alternatives and ivf outcomes. Environ. Int. 111, 23–31.
Maresca, M.M., Hoepner, L.A., Hassoun, A., Oberfield, S.E., Mooney, S.J., Calafat, A.M., et al., 2016. Prenatal exposure to phthalates and childhood body size in an urban cohort. Environ. Health Perspect. 124, 514–520.
Mascarenhas, M.N., Flaxman, S.R., Boerma, T., Vanderpoel, S., Stevens, G.A., 2012. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 9, e1001356.
Meeker, J.D., Ehrlich, S., Toth, T.L., Wright, D.L., Calafat, A.M., Trisini, A.T., et al., 2010. Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reproductive toxicology (Elmsford, NY) 30, 532–539.
Messerlian, C., Wylie, B.J., Minguez-Alarcon, L., Williams, P.L., Ford, J.B., Souter, I.C., et al., 2016. Urinary concentrations of phthalate metabolites and pregnancy loss among women conceiving with medically assisted reproduction. Epidemiology (Cambridge, Mass) 27, 879–888.
Messerlian, C., Williams, P.L., Ford, J.B., Chavarro, J.E., Minguez-Alarcon, L., Dadd, R., et al., 2018. The environment and reproductive health (earth) study: a prospective preconception cohort. Human Reproduction Open 2018.
Minguez-Alarcon, L., Gaskins, A.J., 2017. Female exposure to endocrine disrupting chemicals and fecundity: a review. Current Opinion in Obstetrics & Gynecology 29, 202–211.
Minguez-Alarcon, L., Gaskins, A.J., Chiu, Y.H., Williams, P.L., Ehrlich, S., Chavarro, J.E., et al., 2015. Urinary bisphenol a concentrations and association with in vitro fertilization outcomes among women from a fertility clinic. Human Reproduction (Oxford, England) 30, 2120–2128.
Minguez-Alarcon, L., Chiu, Y.H., Messerlian, C., Williams, P.L., Sabatini, M.E., Toth, T.L., et al., 2016a. Urinary paraben concentrations and in vitro fertilization outcomes among women from a fertility clinic. Fertil. Steril. 105, 714–721.
Minguez-Alarcon, L., Gaskins, A.J., Chiu, Y.H., Souter, I., Williams, P.L., Calafat, A.M., et al., 2016b. Dietary folate intake and modification of the association of urinary bisphenol a concentrations with in vitro fertilization outcomes among women from a fertility clinic. Reproductive toxicology (Elmsford, NY) 65, 104–112.
Minguez-Alarcon, L., Hauser, R., Gaskins, A.J., 2016c. Effects of bisphenol a on male and couple reproductive health: a review. Fertil. Steril. 106, 864–870.
Mok-Lin, E., Ehrlich, S., Williams, P.L., Petrozza, J., Wright, D.L., Calafat, A.M., et al., 2010. Urinary bisphenol a concentrations and ovarian response among women undergoing ivf. Int. J. Androl. 33, 385–393.
Nishihama, Y., Yoshinaga, J., Iida, A., Konishi, S., Imai, H., Yoneyama, M., et al., 2016. Association between paraben exposure and menstrual cycle in female university students in Japan. Reproductive toxicology (Elmsford, NY) 63, 107–113.
O'Rourke, N., Hatcher, L., 2013. A Step-by-step Approach to Using Sas for Factor Analysis and Structural Equation Modeling. (Sas Institute).
Peretz, J., Vrooman, L., Ricke, W.A., Hunt, P.A., Ehrlich, S., Hauser, R., et al., 2014. Bisphenol a and reproductive health: update of experimental and human evidence, 2007–2013. Environ. Health Perspect. 122, 775–786.
Philips, E.M., Kahn, L.G., Jaddoe, V.W.V., Shao, Y., Asimakopoulos, A.G., Kannan, K., et al., 2018. First trimester urinary bisphenol and phthalate concentrations and time to pregnancy: a population-based cohort analysis. J. Clin. Endocrinol. Metab. 103, 3540–3547.
Pollack, A.Z., Mumford, S.L., Krall, J.R., Carmichael, A.E., Sjaarda, L.A., Perkins, N.J., et al., 2018. Exposure to bisphenol a, chlorophenols, benzophenones, and parabens in relation to reproductive hormones in healthy women: a chemical mixture approach. Environ. Int. 120, 137–144.
Rooney, K.L., Domar, A.D., 2014. The impact of lifestyle behaviors on infertility treatment outcome. Current opinion in obstetrics & gynecology 26, 181–185.
SART, 2015. Preliminary Sart Clinic Summary Report: Sart (Societry for Assisted Reproductive Technologies), (vol. 2017).
SCCS. 2011. Scientific committee on consumer safety. Opinion on parabens sccs/1348/10. Available at: Http://ec.Europa.Eu/health/scientific_committees/consumer_safety/docs/sccs_o_041.Pdf. (accessed august, 2018).
Searle, S.R., Speed, F.M., Milliken, G.A., 1980. Population marginal means in the linear model: an alternative to leasts quare means. AmStat 34, 216–221.
Sharma, R., Biedenharn, K.R., Fedor, J.M., Agarwal, A., 2013. Lifestyle factors and reproductive health: taking control of your fertility. Reprod. Biol. Endocrinol. 11, 66.
Shaw, J., deCatanzaro, D., 2009. Estrogenicity of parabens revisited: impact of parabens on early pregnancy and an uterotrophic assay in mice. Reproductive toxicology (Elmsford, NY) 28, 26–31.
Silva, M.J., Samandar, E., Preau Jr., J.L., Reidy, J.A., Needham, L.L., Calafat, A.M., 2007. Quantification of 22 phthalate metabolites in human urine. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 860, 106–112.
Slama, R., Hansen, O.K., Ducot, B., Bohet, A., Sorensen, D., Giorgis Allemand, L., et al., 2012. Estimation of the frequency of involuntary infertility on a nation-wide basis. Human Reproduction (Oxford, England) 27, 1489–1498.
Smith, K.W., Braun, J.M., Williams, P.L., Ehrlich, S., Correia, K.F., Calafat, A.M., et al., 2012. Predictors and variability of urinary paraben concentrations in men and women, including before and during pregnancy. Environ. Health Perspect. 120, 1538–1543.
Thoma, M.E., McLain, A.C., Louis, J.F., King, R.B., Trumble, A.C., Sundaram, R., et al., 2013. Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. Fertil. Steril. 99, 1324–1331.e1321.
Trasande, L., Zoeller, R.T., Hass, U., Kortenkamp, A., Grandjean, P., Myers, J.P., et al., 2015. Estimating burden and disease costs of exposure to endocrine-disrupting chemicals in the European Union. J. Clin. Endocrinol. Metab. 100, 1245–1255.
Valeri, L., Mazumdar, M.M., Bobb, J.F., Claus Henn, B., Rodrigues, E., Sharif, O.I.A., et al., 2017. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20–40 months of age: evidence from rural Bangladesh. Environ. Health Perspect. 125, 067015.
Vo, T.T., Yoo, Y.M., Choi, K.C., Jeung, E.B., 2010. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. Reproductive toxicology (Elmsford, NY) 29, 306–316.
WHO, 2012. World health organization. State of the science of endocrine disrupting chemicals - 2012. Available at: http://www.Who.Int/ceh/publications/endocrine/en/, Accessed date: September 2018.
Ye, X., Kuklenyik, Z., Needham, L.L., Calafat, A.M., 2005. Automated on-line column-switching hplc-ms/ms method with peak focusing for the determination of nine environmental phenols in urine. Anal. Chem. 77, 5407–5413.