Published in final edited form as:

Environ Res. 2018 February ; 161: 562–572. doi:10.1016/j.envres.2017.11.051.

Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004–5

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Abstract

Background—Exposure to bisphenols and phthalates in pregnancy may lead to adverse health effects in women themselves and their offspring.

Objective—To describe first trimester bisphenol and phthalate urine concentrations, including bisphenol and phthalate replacements, and determine nutritional, socio-demographic and lifestyle related determinants.

Methods—In a population-based prospective cohort of 1396 mothers, we measured first trimester bisphenol, phthalate and creatinine urine concentrations (samples collected in 2004–2005, median gestational age 12.9 weeks [inter-quartile range (IQR) 12.1–14.4]). We examined associations of

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Competing financial interests

All authors declare they have no potential competing financial interests.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2017.11.051>.

potential determinants with log-transformed bisphenol and phthalate concentrations. Outcomes were back-transformed. Nutritional analyses were performed in a subgroup of 642 Dutch participants only, as the Food Frequency Questionnaire was aimed at Dutch food patterns.

Results—Bisphenol A, bisphenol S, and bisphenol F were detected in 79.2%, 67.8% and 40.2% of the population, respectively. Mono-*n*-butylphthalate, mono-(2-ethyl-5-hydroxyhexyl)phthalate and monobenzylphthalate were detected in > 90% of the population. Nutritional intake was not associated with bisphenol and phthalate concentrations after correction for multiple testing was applied. Obesity was associated with higher high-molecular-weight phthalate concentrations and the lack of folic acid supplement use with higher di-*n*-octylphthalate concentrations (respective mean differences were 46.73 nmol/l [95% CI 14.56–93.72] and 1.03 nmol/l [0.31–2.06]).

Conclusion—Bisphenol S and F exposure was highly prevalent in pregnant women in the Netherlands as early as 2004–5. Although associations of dietary and other key factors with bisphenol and phthalate concentrations were limited, adverse lifestyle factors including obesity and the lack of folic acid supplement use seem to be associated with higher phthalate concentrations in pregnant women. The major limitation was the availability of only one urine sample per participant. However, since phthalates are reported to be quite stable over time, results concerning determinants of phthalate concentrations are expected to be robust.

Keywords

Bisphenol; Phthalate; Social determinants of health; Life style; Food and beverages

1. Introduction

Bisphenols are used to produce polycarbonate plastics and epoxy resins used in various consumer products, including the lining of metal cans, toys, water pipes and paper products (Liao and Kannan, 2014; Liao et al., 2012b; Vandenberg et al., 2007). Phthalates are frequently added to personal care products and vinyl plastics to impart flexibility, pliability and elasticity (Braun et al., 2013; Sathyanarayana, 2008; Serrano et al., 2014). When ingested, both bisphenols and phthalates undergo a first-pass metabolism consisting of glucuronidation or sulfation and these chemicals have been shown to cross the placenta-blood barrier (Braun et al., 2013; Mattison et al., 2014; Schonfelder et al., 2002; Silva et al., 2004).

During the last few decades, concerns over human exposure and potential health effects from bisphenol A (BPA) and several phthalates including di-2-ethylhexylphthalate (DEHP) have led to regulations on its production and usage in North America and the European Union. However, these governmental embargoes apply mainly to toys and childcare products for oral exposure. In the meantime, this stimulated the use of synthetic bisphenol analogues and DEHP replacements. A shift in phthalate metabolite concentrations has been observed in the first decade of this century (Zota et al., 2014). The European Chemicals Agency (ECHA) reported that the share of phthalate replacements, such as di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP), in total phthalate sales in Europe has increased with over 40% in the years between 2001 and 2010 with concurrently a decline in the share of DEHP (European Chemicals Agency (ECHA), 2013). DEHP replacements have been introduced in

the mid-20th century and have first been identified in human urinary samples from 1988 (Wittassek et al., 2007). Several studies reported the presence of bisphenol analogues in environmental compartments, foods and consumer products in the last decade (Chen et al., 2016). However, bisphenol S (BPS) has not been reported in a human biomonitoring study before 2010 (Liao et al., 2012a). Quantification of bisphenol analogues in those specimens that were collected before the governmental regulations were effective is lacking. Human biomonitoring and association studies have rarely focused on bisphenol analogues and were performed in non-pregnant subjects.

An increasing body of evidence suggests that early life exposure to bisphenols and phthalates may lead to several adverse short and long term health effects (Philips et al., 2016). Diet is considered an important source of bisphenol and phthalate exposure (Lorber et al., 2015; Schecter et al., 2013; Schettler, 2006). Certain food groups such as canned food, fish, meat and poultry have been associated with bisphenol and phthalate levels (Braun et al., 2011; Cantonwine et al., 2014; Trasande et al., 2013b; Watkins et al., 2014). Previous studies among pregnant women generally reported higher levels of bisphenols and phthalates to be associated with lower socio-economic status, younger maternal age and smoking (Arbuckle et al., 2014, 2015; Casas et al., 2013; Valvi et al., 2015), but results are inconsistent (Arbuckle et al., 2014; Berman et al., 2014). Overweight has also been suggested as a determinant of bisphenol and phthalate levels (Valvi et al., 2015). Detailed information on nutritional, socio-demographic and lifestyle related determinants of bisphenol and phthalate concentrations in pregnant women might improve identification of women at risk for higher exposure to these chemicals.

We performed a population-based prospective cohort study among 1396 pregnant women in 2004–2005 to describe first trimester bisphenol and phthalate urinary concentrations and determine nutritional, socio-demographic and lifestyle related determinants.

2. Methods

2.1. Study design and population for analysis

The present study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards (Kooijman et al., 2016). In total, 8879 women were enrolled in pregnancy, of which 76% before a gestational age of 18 weeks. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Centre in Rotterdam. Written consent was obtained from all participating women (World Medical Association, 2013). Bisphenol and phthalate concentrations were measured in a subgroup study among 1431 mothers whose children also participated in postnatal studies. This subgroup included singleton pregnancies only. Thirty-five women without a first trimester urinary sample were excluded, which led to 1396 women included in the analysis. Dietary intake assessment in the Generation R study was aimed at Dutch dietary intake patterns. Therefore, information on maternal dietary intake was only included for Dutch participants, leading to 642 women included in the analysis for nutrition related factors (**Flow chart is given in Fig. 1**).

2.2. Bisphenol and phthalate measurements in urine

Bisphenol and phthalate concentrations were measured in a spot urine sample obtained from each subject during the first trimester measurement (median gestational age 12.9 weeks, inter-quartile range 12.1–14.4 weeks). All urine samples were collected between February 2004 and July 2005. Urine samples were collected between 8 a.m. and 8 p.m. in 100-ml polypropylene urine collection containers, stored at 4 °C and transported within 24 h of receipt to the STAR-MDC laboratory before being distributed manually in 25-ml polypropylene vials to be frozen at – 20 °C. The urine specimens were shipped on dry ice in 4 ml polypropylene vials to the Wadsworth Center, New York State Department of Health, Albany, New York for analysis of bisphenol and phthalate concentrations.

Quantitative detection of phthalate metabolites was achieved utilizing a solid-phase extraction (SPE) method followed by enzymatic deconjugation of the glucuronidated phthalate monoesters coupled with high performance liquid chromatography electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS), as previously used (Asimakopoulos et al., 2016). Assay precision is improved by incorporating $^{13}\text{C}_4$ - or $^2\text{D}_4$ -isotopically-labeled internal standards for each of the phthalate metabolites. This selective method allows for rapid detection of eighteen metabolites of phthalates with the majority of limits of detection (LOD) in the range of 0.008–0.3 ng/ml.

Quantitative detection of bisphenols was achieved utilizing a liquid-liquid extraction (LLE) method followed by enzymatic deconjugation of the glucuronidated bisphenols coupled with high performance liquid chromatography electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). With the use of $^{13}\text{C}_{12}$ -BPA and $^3\text{C}_{12}$ -BPS isotopically labeled internal standards, eight bisphenols were quantified with limits of detection in the range of 0.03 and 0.18 ng/ml, except for bisphenol AF with an LOD of 0.79 ng/ml.

Contamination that arises from laboratory materials and solvents was monitored by the analysis of procedural blanks. All values remained below the LOD and were subtracted. The regression coefficients of the calibration curves were > 98%. For each batch of 25 samples, one procedural blank was analyzed. Throughout the analysis, 1 pre-extraction matrix spike sample was prepared for every 25 samples analyzed by spiking known concentrations (40 ng ml^{-1}) of target analytes and passing them through the entire analytical procedure. In addition, the Standard Reference Materials 3672 (Organic Contaminants in Smokers' Urine) and 3673 (Organic Contaminants in Non-Smokers' Urine) from the National Institute of Standards & Technology (NIST), which contain certified values for 11 phthalate metabolites and BPA, were analyzed with every 50 samples (Schantz et al., 2015). Our results for NIST SRMs were within $\pm 15\%$ of the certified values. A calibration check standard was performed, and methanol was injected after every 25 samples as a check for drift in instrumental sensitivity and carry-over between samples, respectively.

Recoveries of target chemicals passed through the entire analytical procedure ranged between 84.4% and 112.0%, except for mono-(8-methyl-1-nonyl)phthalate (mIDP), phthalic acid (PA), mono-octylphthalate (mOP) and monoisononylphthalate (mINP). Relative recoveries for mINP, PA and mIDP were 77–125% after correction with isotope labeled analogues. Samples were analyzed for creatinine using HPLC-ESI-MS/MS, improved by

incorporating $^2\text{D}_3$ -creatinine. Quantification of calibration check standards resulted in an LOD of 0.30 ng/ml. All identified bisphenol and phthalate urinary biomarkers, their values, detection rates, limits of detection and quantification, and recoveries are shown in Supplementary Table S1. Detailed description of the analytical procedure is shown in Supplementary material S2.

We grouped urinary biomarkers for exposure to phthalates according to their origin and use in product categories. Metabolites were included in the metabolite groups if > 80% of metabolites were above the limit of detection (LOD). We calculated the weighted molar sums for total bisphenols, low molecular weight (LMW) phthalate, high molecular weight (HMW) phthalate, di-2-ethylhexylphthalate (DEHP), and di-*n*-octylphthalate (DNOP) metabolites by using the formula: $((\text{concentration in ng/ml}) * (1/\text{molecular weight}) * (1/10^{-3})) + ((\text{concentration in ng/ml}) * (1/\text{molecular weight}) * (1/10^{-3})) + \text{etc.}$ By using this formula, weighted molar sums are presented in nmol/l. Phthalate groups were constructed based on molecular weight and parent phthalates. LMW phthalate concentration was calculated as the weighted sum of molar concentrations of mono-methyl phthalate (mMP), mono-ethyl phthalate (mEP), mono-*n*-butyl phthalate (mBP), and mono-isobutyl phthalate (mIBP); HMW phthalate concentration as the sum of mono-(2-ethyl-5-carboxypentyl) phthalate (mECP), mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP), mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP), mono(3-carboxypropyl) phthalate (mCPP), monobenzyl phthalate (mBzP), mono-hexylphthalate (mHxP), and mono-2-heptylphthalate (mHpP). DEHP concentration was calculated by adding the molarities of mECP, mEHHP, mEOHP, and mCMHP. DNOP concentrations was calculated as the molarity of mono(3-carboxypropyl) phthalate (mCPP). Phthalic acid (PA) was analyzed separately as a proxy for total phthalate exposure. Bisphenols with > 50% of the samples above the LOD were analyzed separately. For bisphenol and phthalate concentrations below the LOD we substituted values with a LOD value divided by the square root of 2 ($\text{LOD}/\sqrt{2}$), as performed earlier (Hornung and Reed, 1990).

2.3. Maternal nutrition, socio-demographic, and lifestyle related factors

2.3.1. Nutrition related factors—Dietary determinants included maternal daily caloric intake and 23 food groups. Maternal daily dietary intake was assessed at enrollment using a modified version of the validated semi-quantitative food-frequency questionnaire (FFQ) of Klipstein-Grobusch et al. (1998). The FFQ covered the average dietary intake over the previous three months, covering the dietary intake in the first trimester of pregnancy (Tielemans et al., 2016). The Dutch food composition table 2006 was used for calculating daily intake of nutritional values (Netherlands-Nutrition-Centre, 2006). Maternal daily caloric intake was categorized in four groups (< 1600 kcal, 1600–1999 kcal, 2000–2399 kcal, and ≥ 2400 kcal).

2.3.2. Socio-demographic related factors—Information on maternal age at enrollment (< 25 years, 25–29.9 years, 30–34.9 years, and ≥ 35 years), parity (nulliparity/multiparity), educational level (low/high) and maternal ethnicity (Dutch or European/Non-European) was obtained from the first questionnaire at enrollment. Low educational level

was defined as no education, or finished primary or secondary education. High educational level was defined as higher education finished.

2.3.3. Lifestyle related factors—Information on pre-pregnancy weight (kg) and use of folic acid supplementation (yes/no) was obtained from the first questionnaire at enrollment. Maternal height (cm) was measured at enrollment and used to calculate pre-pregnancy body mass index (BMI) ($< 20 \text{ kg/m}^2$, $20\text{--}24.9 \text{ kg/m}^2$, $25\text{--}29.9 \text{ kg/m}^2$, and $\geq 30 \text{ kg/m}^2$). Information on smoking (yes/no) was assessed by questionnaires in each trimester.

2.4. Statistical analysis

First, we performed descriptive statistics to examine all identified bisphenol and phthalate urinary biomarkers, including their values and detection rates, and general subject characteristics.

Second, we examined correlations between first trimester bisphenol and phthalate urine concentrations using Spearman's correlation coefficients, taking the skewed distribution of bisphenols and phthalates into account.

Third, we investigated the associations of maternal nutrition, sociodemographic and lifestyle related determinants with differences in maternal first trimester bisphenol and phthalate urine concentrations using basic and multivariable linear regression models. For all regression models, all bisphenol and phthalate urinary metabolite concentrations were log-transformed to account for right skewness in the distribution and to prevent for negative numbers. Outcomes were back-transformed by using the formula: $(e^{\text{constant}} + e^{\text{coef}}) - (e^{\text{constant}})$.

We examined the associations of dietary food groups and daily dietary caloric intake in Dutch women with differences in bisphenol and phthalate urinary concentrations. Non-Dutch women were excluded from nutritional analyses, since dietary intake assessment was aimed at Dutch dietary intake patterns. Food groups were dichotomized for the amount of consumption by using the 90th percentile as a cutoff. Daily dietary caloric intake was categorized to account for potential non-linear effects, with the largest group as the reference group.

We examined socio-demographic and lifestyle related factors in the larger, entire study population with differences in bisphenol and phthalate urinary concentrations. To account for non-linear effects of potential determinants and confounders, all were included as categorical variables. The largest group was used as the reference.

Sub-analyses of individual bisphenols or phthalate metabolites were performed for all significant models to determine which metabolites were driving the association. All basic models were adjusted for urinary creatinine to adjust for dilution. Multivariable models were additionally adjusted for maternal age, parity, educational level, maternal ethnicity, pre-pregnancy BMI, smoking and use of folic acid supplementation. Multivariable models investigating nutrition related determinants were additionally adjusted for daily dietary caloric intake. Owing to the fact that nutrition related determinants were derived from a food

frequency questionnaire (FFQ) aimed at Dutch food consumption patterns, nutrition related determinants were not imputed. Missing data of the potential sociodemographic and lifestyle related determinants and covariates were imputed using multiple imputation. Five imputed data sets were created and pooled for analyses. Imputed socio-demographic and lifestyle related determinants were used for both the univariate and multivariable models to prevent exclusion of incomplete cases. The percentage of missing values within the population for analysis was 15%, except for folic acid supplement use (20.1%). We used the Bonferroni correction to account for multiple testing at $P < 0.05/k$ (k: the number of hypotheses by means of the number of potential determinants tested summed by the two main metabolite groups of bisphenols and phthalates). All analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Participant characteristics and correlations

Table 1 shows the subject characteristics of included women. Table 2 shows all the metabolites that were included in all separate groups as abovementioned, their values and detection rates in urine. Supplementary Table S1 shows all identified bisphenol and phthalate urinary biomarkers.

Measurable urinary concentrations of BPA, bisphenol S (BPS), and bisphenol F (BPF) were found in 79.2%, 67.8% and 40.2% of the samples, respectively. Bisphenol Z (BPZ), bisphenol B (BPB), bisphenol AP (BPAP) and bisphenol P (BPP) were detected in < 15% of samples. We did not detect BPAF in any sample. In the detected samples, median concentrations of BPA, BPS and BPF were 1.66 ng/ml (IQR 0.72–3.56), 0.36 ng/ml (IQR 0.17–1.08), and 0.57 (IQR 0.30, 1.29), respectively. LMW phthalate, and DEHP metabolites were detected in > 98% of the population. Mono-hexylphthalate (mHxP) and mono-2-heptylphthalate (mHpP) were detected in a considerable number of samples; however a significant part of values was detected in the range between the limit of detection (LOD) and the limit of quantification (LOQ). DEHP replacements including di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) had detection rates below 15%.

Table 3 shows Spearman's correlation coefficients of bisphenol and phthalate concentrations, including separately BPA, BPS and PA. Bisphenol and phthalate concentrations were strongly correlated (Spearman's correlation coefficient for the correlation between total bisphenols and PA metabolites 0.37, P value < 0.001). Phthalate groups were strongly correlated, ranging from 0.46 for the correlation between LMW phthalate and DNOP metabolites to 0.98 for the correlation between HMW phthalate and DEHP metabolites (all have a P value < 0.001). Among the separate bisphenols, except for correlations of BPA with BPS, BPF and BPAP, bisphenols are not correlated (Supplementary Table S3). For the separate phthalate metabolites, PA is strongly correlated with all other phthalates with > 20% of samples above LOD (Spearman's correlation coefficient ranging from 0.34 to 0.69 (P value < 0.001)) (Supplementary Table S4). All in the analyses included phthalate metabolites with > 20% of samples above LOD are strongly correlated (P value < 0.001). Among the DEHP metabolites correlation is very high, ranging from 0.77 to 0.98 (P value < 0.001).

3.2. Maternal dietary determinants

Urinary bisphenol and phthalate concentrations did not vary by intake frequencies of examined food groups or daily dietary caloric intake after correction for multiple testing, both in basic and multivariable models. Basic models showed high consumption of grains, cakes, coffee, soft drinks, alcoholic drinks, soups and bouillon and daily dietary caloric intake to be associated with differences in bisphenol or phthalate urine concentrations (Supplementary Table S5).

Table 4 shows multivariable models for nutritional related factors with a nominal p-value < 0.05. At nominal level, pregnant women in the upper 10% of consumption of vegetables had higher HMW phthalate, DEHP and DNOP metabolite concentrations (respective mean differences 38.17 nmol/l (95% CI 7.18, 90.7), 31.4 nmol/l (95% CI 5.16, 77.3) and 1.14 nmol/l (95% CI 0.12, 2.98)). In a subanalysis of individual phthalate metabolites, the association between vegetable intake and HMW phthalates was mainly driven by DEHP metabolites, however associations did not remain after correction for multiple testing (Supplementary Table S6). We observed lower concentrations of bisphenol S (BPS) and PA in pregnant women who had eaten a large quantity of grains in the past three months, while for women with a high consumption of fish and shellfish higher total bisphenol or bisphenol A (BPA) concentrations were found. Women who consumed a large amount of soft drinks had higher LMW phthalate concentrations, while women with a frequent intake of soups and bouillon had lower LMW phthalate concentrations. Both associations were mainly driven by mono-ethylphthalate (mEP) metabolites, but attenuated to non-significance after Bonferroni correction (Supplementary Table S6). As compared to pregnant women who consumed 2000–2399 kcal per day, women who consumed somewhat less or more kcal per day had lower BPS concentrations.

3.3. Maternal socio-demographic and lifestyle determinants

After correction for multiple testing was applied, all investigated determinants were associated with differences in bisphenol or phthalate urine concentrations in basic models (Supplementary Table S7). Table 5 shows multivariable associations of socio-demographic and lifestyle related factors with urinary bisphenol and phthalate concentrations. After Bonferroni correction, pre-pregnancy obesity was associated with higher HMW phthalate concentrations (mean difference 46.73 nmol/l (95% CI 14.56, 93.72)) and the lack of folic acid supplement use with higher DNOP concentrations (mean difference 1.03 nmol/l (95% CI 0.31, 2.06)). In a subanalysis of individual HMW phthalate metabolites, the association of pre-pregnancy BMI was driven by mono-(2-ethyl-5-carboxypentyl)phthalate (mECP), monobenzylphthalate (mBzP) and mono-2-heptylphthalate (mHpP) metabolites (Supplementary Table S8).

Maternal age was not associated with bisphenol or phthalate concentrations. At nominal level, multiparous women had higher DNOP concentrations (mean difference 0.58 ng/ml [95% CI 0.11, 1.24]). Pregnant women with a low educational level had higher PA and LMW phthalate concentrations. Among pregnant women with non-European descent, we observed lower urinary BPS concentrations, but higher HMW phthalate concentrations which was driven by mBzP metabolites. The association with mBzP metabolites remained

significant after correction for multiple testing. Pregnant women with a higher pre-pregnancy BMI, who smoked during pregnancy and did not use folic acid supplementation had higher first trimester bisphenol and phthalate urine concentrations.

4. Discussion

4.1. Main findings

One of the major findings of this study is that bisphenol exposures other than BPA were widely prevalent in pregnant women in a population-based cohort in the Netherlands between 2004 and 2005. The second major finding was that first trimester bisphenol and phthalate urine concentrations were mainly influenced by lifestyle related factors such as high pre-pregnancy BMI and the lack of folic acid supplement use. Nutritional factors were not associated with bisphenol and phthalate concentrations when correction for multiple testing was applied.

4.2. Interpretation of findings

To our knowledge, this is the first study to describe BPS and BPF in samples collected as early as 2004–2005. Generally, comparisons of values need to be restricted to the time and place of sampling, because bisphenol and phthalate use changes over the years due to governmental embargoes (Zota et al., 2014). Urinary concentrations of BPA and phthalate metabolites found in our study population were generally somewhat lower than concentrations in other Western studies performed in the same time period. Detection rates were comparable or slightly lower (Casas et al., 2013; Harley et al., 2017; Philippat et al., 2014; Valvi et al., 2013; Woodruff et al., 2011).

Except substitution of BPA by BPS and BPF in man-made consumer products as an explanation for our findings, we cannot exclude BPS and BPF exposure through natural sources. A recent study found BPF in mustard made from seeds of *Sinapis alba* (Zoller et al., 2016). Contamination was ruled out. The authors explain this finding as reaction product of a breakdown pathway. This finding suggest potential other natural sources that could explain BPF and also BPS exposure.

Since the year 1999 certain phthalates, including DEHP, dibutylphthalate (DBP), butylbenzylphthalate (BBP), DINP, DIDP and DNOP, have been banned from toys and childcare products in the European Union (Byrne, 1999). We observed high detection rates for DEHP metabolites, metabolites of DBP and BBP (respectively mBP and mBzP) and mCPP, which is a DNOP metabolite. DINP and DIDP metabolites were scarcely detected in our study population. A recent reevaluation concluded that there was no evidence for further exposure bans on DINP and DIDP. However, the other banned phthalates have not been included in this assessment (European Chemicals Agency (ECHA), 2013). Although, since February 2015 (REACH sunset date), several phthalates, including DEHP, BBP, DBP and diisobutylphthalate (DIBP), have been listed on the Authorisation List of the European Chemicals Agency (ECHA) (2017). Our findings suggest that DEHP, DBP, BBP and DNOP were widely used in the early 2000s. Due to recent regulations DEHP, DBP and BBP

exposure should be limited nowadays. DNOP has not been included in these regulations and is therefore a cause for concern.

In the general population, diet has been considered the major source of phthalate exposure (Schechter et al., 2013; Schettler, 2006). DEHP seems to be of major concern, which is widespread in food packaging (Serrano et al., 2014). Also bisphenols are potentially transferred into food items from food packaging, including the lining of metal cans (Liao and Kannan, 2013; Philips et al., 2016). In previous studies, high consumption of canned vegetables and canned fish was associated with higher BPA concentrations in pregnant women (Braun et al., 2011; Casas et al., 2013). For phthalates, frequent consumption of poultry, meat and fish were associated with higher DEHP and HMW phthalate concentrations, while soy consumption was inversely associated with DEHP concentrations (Cantonwine et al., 2014; Trasande et al., 2013b).

In contrast to our hypothesis and previous findings, none of the nutrition related factors was associated with bisphenol and phthalate urine concentrations when corrections for multiple testing were applied. The absence of multiple testing correction and the use of short-term diet recall data (24/48 h) in previous studies might be responsible for this discrepancy. In contrast to previous studies, we have used a FFQ for the average intake in the past three months. Both bisphenols and phthalates have short biological half-lives (less than 24 h) (Braun et al., 2013; Mattison et al., 2014). The trends we have observed for high consumption of certain food groups, though not significant, may therefore rather be a proxy for healthy or unhealthy food consumption patterns of lifestyle than that those food groups actually contain these compounds. However, we cannot explain why women with a high intake of vegetables have higher concentrations of HMW phthalate metabolites.

Several studies have examined potential determinants of BPA and phthalate concentrations during pregnancy using various methodologies, which complicates comparisons. In general, previous studies identified maternal younger age, lower education, overweight and smoking as determinants of higher BPA and LMW phthalate concentrations during pregnancy (Arbuckle et al., 2014, 2015; Cantonwine et al., 2014; Casas et al., 2013; Valvi et al., 2015). However, for HMW phthalates maternal older age, higher education and nonsmoking have been associated with higher concentrations (Arbuckle et al., 2014; Cantonwine et al., 2014). In our study, maternal age was not associated with bisphenol or phthalate concentrations. Correction for multiple testing has not been applied in previous studies. In agreement with previous studies, we found an association at nominal level of lower education with higher LMW phthalate concentrations. In contrast to our finding that multiparity was associated with higher DNOP metabolite concentrations at nominal level, previous studies did not find consistent associations between parity and phthalate concentrations (Arbuckle et al., 2014; Cantonwine et al., 2014). Nulliparity, however, has been identified as a determinant of higher BPA concentrations in a Spanish birth cohort study (Casas et al., 2013).

To some extent in line with our findings, a cohort study in 350 pregnant women reported that non-Hispanic black and Hispanic women had higher concentrations of LMW phthalate metabolites, mBzP and mono-(2-ethylhexyl)phthalate (mEHP) metabolites in early pregnancy than other ethnic groups (James-Todd et al., 2017). Although, the role of ethnicity

as a determinant of bisphenol and phthalate concentrations has been scarcely studied, several association studies have identified race/ethnicity-specific associations of bisphenols and phthalates with childhood BMI and diabetes risks (Huang et al., 2014; Trasande et al., 2012, 2013a). Consequently, determinants underlying these ethnic differences need to be further studied.

Previous studies have shown associations of higher maternal BMI and smoking during pregnancy with higher BPA and LMW phthalate concentrations, whereas inversed associations or no associations were found for HMW phthalate concentrations (Arbuckle et al., 2014; Cantonwine et al., 2014; Lewin et al., 2017; Valvi et al., 2015). Conversely, in our study we found strong associations between pre-pregnancy BMI in the obesity range and higher HMW phthalate concentrations. Phthalates, and bisphenols to some extent, are lipophilic chemicals (Philips et al., 2016; Wang et al., 2015). Even though we cannot rule out that obese women are more exposed to HMW phthalates, it seems metabolically feasible that obese women have greater adipose stores of lipophilic chemicals. In line with previous studies we found higher total bisphenol, PA and LMW phthalate concentrations in smokers than in non-smokers, driven by BPA and mEP (Arbuckle et al., 2014; Valvi et al., 2015). Although the associations with smoking might reflect lifestyle habits, in- and exhaled tobacco smoke may contain BPA and PA because BPA and di-2-methoxyethyl phthalate are used in some cigarette filters (Jackson and Darnell, 1985).

To our knowledge, this is the first study to examine folic acid supplement use as a determinant of bisphenol and phthalate concentrations. In our study, we found the lack of folic acid supplement use to be associated with higher DNOP metabolite concentrations. Although epigenetic effects of bisphenols and phthalates are not yet clear, methyl donors like folate have been proposed as a potential point of engagement to prevent potential epigenetic effects (Dolinoy et al., 2007). Effects of folic acid supplement use on epigenetic changes due to bisphenol and phthalate exposure need to be further investigated.

In line with our hypothesis, in this population-based sample in the Netherlands adverse lifestyle related factors seem to be associated with higher bisphenol and phthalate urine concentrations rather than sociodemographic factors. Many research groups have raised their concerns about confounding effects on bisphenol and phthalate concentrations from nutrition related factors. As a sensitivity analysis, we included maternal daily dietary caloric intake as a covariate in models for potential socio-demographic and lifestyle related determinants. In these analysis, maternal daily dietary caloric intake has been included as a categorical variable with a separate missing group to prevent exclusion of incomplete cases. Since maternal daily dietary caloric intake was only included for Dutch mothers and the missing category was highly correlated with non-European descent, effects on ethnicity estimates could not be compared. Conclusions for all other socio-demographic and lifestyle related determinants did not change.

All adverse lifestyle related factors assessed in this study were associated with higher bisphenol and phthalate urine concentrations at nominal level. However, despite of the scarce associations found with certain food groups in Dutch mothers, we cannot rule out that lifestyle related factors are a proxy for unmeasured food consumption patterns. Several

studies have found associations of phthalate concentrations with the use of personal care products, household products and materials used for flooring and walls (Buckley et al., 2012; Valvi et al., 2015). The adverse lifestyle related factors identified in this study might therefore be a proxy for harmful product exposure. Altogether, we cannot exclude that the associations we have observed are rather a proxy for unhealthy lifestyle habits in general with consequently higher exposure levels than that these factors actually increase exposure or urinary excretion.

4.3. Strengths and limitations

Strengths of this study were the prospective data collection from early pregnancy onwards in a multi-ethnic population, the relatively large sample size, and large amount of bisphenols and phthalate metabolites analyzed. Analyses for the current study are performed in a subgroup from the Generation R Study. Non-response analyses showed similar distributions and values of potential socio-demographic and lifestyle determinants (Supplementary Table S9). The response rate at baseline was 61% (Kooijman et al., 2016). It seems unlikely that this level of non-response would lead to biased effect estimates, since selection bias in large cohort studies arises mainly from loss to follow up rather than from a non-response at baseline (Nohr et al., 2006).

For this study, a large amount of bisphenols and phthalate metabolites have been analyzed. The analytical techniques as used for this study allowed for detection of both unconjugated (free) compounds, as well as glucuronidated and sulfated bisphenol and phthalate conjugates. Assay precision has been improved by incorporating isotopically-labeled internal standards and confirmed with regularly proficiency testing and reference maternal analysis.

Urinary concentrations of bisphenols and phthalates were based on a single spot urine in the first trimester of pregnancy. Both bisphenols and phthalates have short biological half-lives (less than 24 h) (Braun et al., 2013; Mattison et al., 2014). Despite of the reported short biological half-lives, it has been suggested that one single urine sample for phthalate concentrations reasonably reflects exposure for up to three months or even longer (Hauser et al., 2004; Townsend et al., 2013). Within-person variability of BPA urinary concentrations is reported to be high, constraining the value of one single urine sample for bisphenol measurement (Pollack et al., 2016). We can therefore not exclude that the results of our study with regards to the bisphenols would be different when multiple samples were included. However, for the phthalates, we expect the results to be robust.

Samples have been stored at -20°C for approximately 10 years. Therefore, we cannot exclude that there has been some biological activity during the storage period. It has been suggested that -80°C would be a more optimal storage temperature. A study investigating effects of storage time and temperature on bisphenol and phthalate levels found that samples stored at room temperature (20°C) had lower concentrations of phthalate metabolites and BPA than samples stored at -80°C after a time period of 8 weeks (Guo et al., 2013). Studies investigating effects of storage temperature over a longer period are lacking.

Some studies measure mEHP concentrations as a metabolite of DEHP. Based on literature, we decided not to measure mEHP concentrations. MEHP only represents a small percentage (< 1%) of DEHP metabolites and is not considered a reliable biomarker for human DEHP exposure (Silva et al., 2006). MEHP occurs naturally from the hydrolysis of DEHP and is ubiquitous in the environment, explaining the high background that tempers our analysis. The other DEHP metabolites, such as mECPP, mEHHP, mCMHP and mEOHP are oxidative metabolites that are produced only in vivo and therefore reliable biomarkers (Koch et al., 2006).

The FFQ as used in this study was aimed at Dutch dietary intake patterns, making this assessment less reliable for non-Dutch participants. We therefore excluded non-Dutch participants from this analysis. As mentioned before, ethnicity might play a key factor in bisphenol and phthalate metabolism and effects. Therefore, the lack of ethnic variance results in non-generalizable effect estimates of dietary determinants. As abovementioned, we have used a FFQ for the average intake in the past three months instead of the last 24 h while both bisphenols and phthalates have short biological half-lives, making it difficult to draw firm conclusions about food groups as sources of exposure.

In our study, information on nutritional and several lifestyle related determinants was self-reported, in which underreport may have led to misclassification and consequently underestimation of effects. However, the prevalence of lifestyle related factors corresponds with national figures for that time period (de Walle and de Jong-van den Berg, 2002; Lanting et al., 2015). Previous studies reported time of sampling, fasting time and seasonality as potential determinants for bisphenol and phthalate urine concentrations (Aylward et al., 2011; Hoepner et al., 2013). As mentioned above, personal care products and household products have been identified as determinants of phthalate concentrations (Buckley et al., 2012; Valvi et al., 2015). In our study, this information was not available.

5. Conclusion

BPS and BPF exposure was highly prevalent in pregnant women in the Netherlands as early as 2004–2005. This finding questions the assumption that BPS and BPF exposure was limited in the early 2000s. Associations attributed to BPA in this time-period may be therefore confounded. DEHP replacements were scarcely detected in our population. However, high detection rates were found of DEHP itself and other phthalates that already were banned from toys childcare products, suggesting continued exposure through consumer products. We identified limited associations of dietary and other key determinants with bisphenol and phthalate concentrations. Adverse lifestyle factors including obesity and the lack of folic acid supplement use seem to be associated with higher phthalate concentrations in pregnant women. Notwithstanding the associations found between obesity and higher HMW phthalate concentrations, associations were limited suggesting a lower likelihood of confounding of phthalate body mass contaminant relationships which we plan to examine in forthcoming work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Generation R Study is conducted by the Erasmus Medical Center in close collaborations with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, Rotterdam, the Rotterdam Homecare Foundation, Rotterdam and the Stichting Trombosedienst and Artsenlaboratorium Rijnmond (STAR-MDC), Rotterdam. We gratefully acknowledge the contribution of children and parents, general practitioners, hospitals, midwives and pharmacies in Rotterdam.

Sources of support and funding

The general design of the Generation R Study is made possible by financial support from the Erasmus MC, University Medical Center, Rotterdam, the Netherlands, the Organization for Health Research and Development (ZonMw) 166010001 and the Ministry of Health, Welfare and Sport. This study was supported by Grant ES022972 from the National Institutes of Health, USA. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health. VWVJ received additional grants from the Netherlands Organization for Health Research and Development (VIDI 016.136.361), and the European Research Council (ERC Consolidator Grant, ERC-2014-CoG-64916).

Abbreviations

BMI	body mass index
BPA	bisphenol A
BPS	bisphenol S
DEHP	di-2-ethylhexylphthalate
DNOP	di- <i>n</i> -octylphthalate
EDC	endocrine-disrupting chemical
FFQ	food-frequency questionnaire
HPLC-ESI-MS/MS	high performance liquid chromatography electrospray ionization-tandem mass spectrometry
mCPP	mono(3-carboxypropyl)phthalate
mIDP	mono-(8-methyl-1-nonyl)phthalate
mINP	monoisononylphthalate
mOP	monooctylphthalate
LOD	limit of detection
LOQ	limit of quantification
LMW	low molecular weight
HMW	high molecular weight
PA	phthalic acid

SPE

solid-phase extraction

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Fig. 1.
Flowchart.

Table 1Subject characteristics (n = 1396)^a.

	Value
Maternal age (years)	30.6 (4.8)
Pre-pregnancy body mass index (kg/m ²) ^b	22.7 (18.5, 35.0)
Parity (% nulliparous)	61.0
Child sex (% males)	50.5
Maternal ethnicity (%)	
Dutch/European	61.9
Non-European	38.1
Highest completed education (%)	
Low education	49.6
High education	50.4
Daily dietary caloric intake (kcal)	2077 (509)
Maternal smoking during pregnancy (%)	
No	75.2
Yes	24.8
Folic acid supplement use (%)	
No	19.4
Yes	80.6
Creatinine (µg/ml) ^b	1011 (153, 3435)

^aValues represent means (standard deviation) or valid percentages.^bMedian (95% range).

Table 2

Bisphenol and phthalate urinary concentrations (n = 1396).

	Median (IQR) (nmol/l) ^a	Median (IQR) (ng/ml) ^b	Percentage of values below the limit of detection (LOD)	Percentage of values above the limit of quantification (LOQ)
Total bisphenols	9.24 (3.54, 20.25)			
Bisphenol A (BPA)		1.66 (0.72, 3.56)	20.8	66.0
Bisphenol S (BPS)		0.36 (0.17, 1.08)	32.2	52.7
Bisphenol F (BPF)		0.57 (0.30, 1.29)	59.8	19.6
Phthalic acid (PA) metabolites		57.08 (30.77, 121.91)	0.3	99.1
Low molecular weight (LMW) metabolites	1087.27 (429.24, 2934.47)			
Monomethylphthalate (mMP)		5.43 (2.75, 9.88)	0.1	99.9
Monoethylphthalate (mEP)		138.03 (41.22, 486.71)	0.1	99.9
Mono-isobutylphthalate (mIBP)		21.55 (9.55, 45.91)	0.1	99.8
Mono- <i>n</i> -butylphthalate (mBP)		16.21 (7.01, 31.22)	0.7	98.9
High molecular weight (HMW) metabolites	221.94 (112.98, 407.84)			
Di-2-ethylhexylphthalate (DEHP) metabolites	174.28 (89.26, 325.95)			
Mono-(2-ethyl-5-carboxypentyl)phthalate (mECP)		16.43 (8.26, 31.82)	0.1	99.3
Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)		12.02 (5.83, 23.21)	0.1	99.6
Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)		7.81 (3.53, 15.48)	0.1	99.1
Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)		14.17 (7.60, 26.69)	0.1	99.9
Di-<i>n</i>-octylphthalate (DNOP) metabolites	5.81 (3.11, 11.07)			
Mono(3-carboxypropyl)phthalate (mCPP)		1.46 (0.78, 2.78)	0.2	99.8
Other high molecular weight metabolites				
Monobenzylphthalate (mBzP)		6.59 (3.07, 12.91)	8.2	90.4
Mono-hexylphthalate (mHxP)		0.33 (0.16, 0.63)	23.6	53.9
Mono-2-heptylphthalate (mHpP)		1.10 (0.60, 2.34)	35.5	35.2

^aWeighted molar sums of total bisphenols, low molecular weight (LMW) phthalate, high molecular weight (HMW) phthalate, di-2-ethylhexylphthalate (DEHP), and di-*n*-octylphthalate (DNOP) metabolites in nmol/l with non-detectable levels of separate compounds imputed as LOD/sqr(2). Separate metabolites are included only if more than 80% of values was above the LOD.

^bValues represent the median (IQR) of detected values.

Table 3

Spearman's correlation coefficients of bisphenols and phthalates (N = 1396).

Bisphenols			Phthalates				
Total bisphenols	Bisphenol A (BPA)	Bisphenol S (BPS)	Phthalic acid (PA) metabolites	Low molecular weight (LMW) phthalate metabolites	High molecular weight (HMW) phthalate metabolites	Di-2-ethylhexyl phthalate (DEHP) metabolites	Di- <i>n</i> -octylphthalate (DNOP) metabolites
Total bisphenols	—	—	—	—	—	—	—
BPA	1.00	—	—	—	—	—	—
BPS	0.16 [*]	1.00	—	—	—	—	—
PA metabolites	0.37 [*]	0.15 [*]	1.00	—	—	—	—
LMW phthalate metabolites	0.27 [*]	0.12 [*]	0.75 [*]	1.00	—	—	—
HMW phthalate metabolites	0.37 [*]	0.16 [*]	0.60 [*]	0.50 [*]	1.00	—	—
DEHP metabolites	0.37 [*]	0.16 [*]	0.58 [*]	0.47 [*]	0.98 [*]	1.00	—
DNOP metabolites	0.34 [*]	0.18 [*]	0.57 [*]	0.46 [*]	0.77 [*]	0.74 [*]	1.00

Spearman's correlation coefficients of bisphenol and phthalate groups, included separately BPA, BPS, PA and mCPP. For both the groups and separate components, values below the limit of detection (LOD) are imputed by LOD/sqrt(2).

* P-value<0.001.

Table 4

Multivariable associations between nutritional related factors and urinary bisphenol and phthalate concentrations in Dutch women (N = 642).

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenol (nmol/l) [95% CI]	Bisphenol A (BPA) (ng/ml) [95% CI]	Bisphenol S (BPS) (ng/ml) [95% CI]	Phthalic acid (PA) (ng/ml) [95% CI]	Low Molecular Weight (LMW) Phthalate (nmol/l) [95% CI]	High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]	Di-2-ethylhexylphthalate (DEHP) (nmol/l) [95% CI]	Di- <i>n</i> -octylphthalate (DNOP) (nmol/l) [95% CI]
Food groups^a								
Vegetables	–	–	–	–	–	38.07 [7.18, 90.72] [*]	31.40 [5.16, 77.28] [*]	1.14 [0.12, 2.98] [*]
Grains	–	–	–0.07 [–0.08, –0.01] [*]	–8.47 [–11.50, –0.24] [*]	–	–	–	–
Fish and shellfish	2.02 [0.08, 5.99] [*]	0.23 [0.02, 0.74] [*]	–	–	–	–	–	–
Soft drinks	–	–	–	–	211.65 [6.58, 659.22] [*]	–	–	–
Soups and bouillon	–	–	–	–	–159.60 [–196.07, –25.59] [*]	–	–	–
Daily dietary caloric intake^b								
< 1600 kcal (<i>n</i> = 85)	–	–	–0.05 [–0.07, 0.03]	–	–	–	–	–
1600–1999 kcal (<i>n</i> = 180)	–	–	–0.06 [–0.07, –0.01] [*]	–	–	–	–	–
2000–2399 kcal (<i>n</i> = 199)	–	–	Reference	–	–	–	–	–
2400 kcal (<i>n</i> = 178)	–	–	–0.06 [–0.07, –0.01] [*]	–	–	–	–	–

^{*} P-value < 0.05.

^a Values are regression coefficients (95% confidence intervals) from multivariable linear regression models that reflect the difference in bisphenol and phthalate urine concentrations in ng/ml or nmol/l in women in the upper 10% of consumption of that particular food group or dietary pattern compared to the first 90%.

^b Values are regression coefficients (95% confidence intervals) from linear regression models that reflect the difference in bisphenol and phthalate concentrations in ng/ml or nmol/l compared with the reference. Significance levels are accounted for multiple testing using Bonferroni correction. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, daily dietary caloric intake, maternal highest finished education, smoking, folic acid supplement use and creatinine.

Table 5

Multivariable associations of socio-demographic and lifestyle related factors with urinary bisphenol and phthalate concentrations (N = 1396).

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenols (nmol/l) [95% CI]	Bisphenol A (BPA) (ng/ml) [95% CI]	Bisphenol S (BPS) (ng/ml) [95% CI]	Phthalic acid (PA) (ng/ml) [95% CI]	Low Molecular Weight (LMW) Phthalate (nmol/l) [95% CI]	High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]	Di-2-ethylhexylphthalate (DEHP) (nmol/l) [95% CI]	Di- <i>n</i> -octylphthalate (DNOP) (nmol/l) [95% CI]
Maternal age								
<25 years (<i>n</i> = 190)	0.44 [− 0.68, 2.26]	0.07 [− 0.05, 0.29]	0.02 [− 0.03, 0.11]	2.14 [− 4.06, 11.80]	98.91 [− 45.28, 344.55]	16.56 [− 6.04, 49.79]	11.37 [− 6.85, 38.59]	0.31 (− 0.32, 1.26)
25–29.9 years (<i>n</i> = 374)	0.15 [0.61, 1.33]	− 0.01 [− 0.08, 0.11]	0.00 [− 0.03, 0.05]	0.61 [− 3.65, 6.93]	47.71 [− 49.27, 202.18]	1.21 [− 12.56, 20.82]	− 0.57 [− 11.66, 15.47]	0.02 (− 0.39, 0.60)
30–34.9 years (<i>n</i> = 598)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
35 years (<i>n</i> = 234)	− 0.07 [− 0.86, 1.19]	0.03 [− 0.06, 0.18]	0.02 [− 0.02, 0.09]	2.00 [− 3.12, 9.69]	2.73 [− 90.23, 158.95]	0.62 [− 14.68, 22.80]	0.68 [− 12.07, 19.40]	0.48 (− 0.08, 1.29)
Parity								
Primiparous (<i>n</i> = 849)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Multiparous (<i>n</i> = 547)	0.49 [− 0.27, 1.62]	0.02 [− 0.05, 0.14]	0.00 [− 0.03, 0.05]	− 3.98 [− 6.70, 0.15]	− 41.66 [− 102.77, 59.17]	8.52 [− 5.02, 27.25]	8.14 [− 3.37, 24.24]	0.58 (0.11, 1.24) *
Educational level								
Low (<i>n</i> = 709)	0.50 [− 0.34, 1.77]	0.09 [− 0.01, 0.25]	− 0.01 [− 0.03, 0.04]	5.83 [0.57, 13.37] *	132.74 [17.43, 311.57] *	8.67 [− 6.17, 29.46]	4.25 [− 7.38, 20.80]	− 0.08 (− 0.46, 0.60)
High (<i>n</i> = 687)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Maternal ethnicity								
Dutch/European (<i>n</i> = 860)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Non-European (<i>n</i> = 536)	− 0.73 [− 1.23, 0.09]	− 0.05 [− 0.10, 0.05]	− 0.04 [− 0.05, − 0.00] *	− 0.35 [− 4.24, 5.42]	75.51 [− 24.74, 233.95]	18.45 [1.65, 41.70] *	12.95 [− 0.63, 32.01]	0.03 (− 0.36, 0.60)
Pre-pregnancy body mass index (kg/m ²)								
<20 kg/m ² (<i>n</i> = 203)	0.05 [− 0.80, 1.41]	− 0.01 [− 0.09, 0.14]	− 0.02 [− 0.05, 0.04]	0.03 [− 4.72, 7.27]	− 62.33 [139.45, 73.68]	− 1.20 [− 17.37, 22.62]	0.52 [− 13.53, 21.52]	− 0.23 (− 0.66, 0.42)
20–24.9 kg/m ² (<i>n</i> = 785)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
25–29.9 kg/m ² (<i>n</i> = 290)	0.77 [− 0.30, 2.45]	0.07 [− 0.05, 0.28]	0.01 [− 0.02, 0.07]	7.64 [0.88, 17.63] *	58.78 [− 44.09, 225.04]	7.46 [− 9.00, 30.99]	5.63 [− 7.88, 25.20]	− 0.02 (− 0.49, 0.68)
30 kg/m ² (<i>n</i> = 118)	1.88 [0.13, 4.78] *	0.17 [− 0.00, 0.49]	0.10 [0.01, 0.27] *	13.16 [2.51, 29.86] *	195.31 [− 12.50, 564.77]	46.73 [14.56, 93.72] **†	32.34 [6.90, 70.25] *	0.79 (− 0.07, 2.12)
Smoking during pregnancy								
No (<i>n</i> = 1051)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Yes (<i>n</i> = 345)	1.27 [0.20, 2.89] *	0.15 [0.03, 0.33] *	0.01 [− 0.02, 0.07]	5.32 [0.21, 12.66] *	90.37 [− 12.13, 250.89]	6.80 [− 7.70, 27.18]	6.45 [− 5.67, 23.64]	0.07 (− 0.34, 0.65)
Folic acid supplement use								
No (<i>n</i> = 290)	1.04 [− 0.05, 2.74]	0.16 [0.03, 0.38] *	0.03 [− 0.01, 0.11]	2.34 [− 3.27, 10.86]	63.92 [− 52.75, 257.51]	27.90 [6.29, 59.28] *	19.75 [2.53, 44.31] *	1.03 (0.31, 2.06) **†

Difference in bisphenol concentrations					Difference in phthalate concentrations				
Total Bisphenols (nmol/l) [95% CI]	Bisphenol A (BPA) (ng/ml) [95% CI]	Bisphenol S (BPS) (ng/ml) [95% CI]	Phthalic acid (PA) (ng/ml) [95% CI]	Low Molecular Weight (LMW) Phthalate (nmol/l) [95% CI]	High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]	Di-2-ethylhexylphthalate (DEHP) (nmol/l) [95% CI]	Di- <i>n</i> -octylphthalate (DNOP) (nmol/l) [95% CI]		
Yes (<i>n</i> = 1106)	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]

Values are regression coefficients (95% confidence intervals) from multivariable linear regression models that reflect differences in urinary concentrations of bisphenol and phthalate concentrations, compared with the reference. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, maternal ethnicity, maternal highest finished education, smoking, folic acid supplement use and creatinine.

* P-value < 0.05,

[‡] Significant using Bonferroni. Numbers are after multiple imputation.