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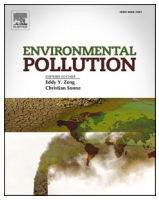

Associations of maternal urinary bisphenol and phthalate concentrations with offspring reproductive development☆

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ABSTRACT

Fetal exposure to bisphenols and phthalates may influence development of the reproductive system. In a population-based, prospective cohort study of 1059 mother-child pairs, we examined the associations of maternal gestational urinary bisphenols and phthalates concentrations with offspring reproductive development from infancy until 13 years. We measured urinary bisphenol and phthalate concentrations in each trimester. We obtained information on cryptorchidism or hypospadias after birth from medical records. At 9.7 years, we measured testicular and ovarian volume by MRI. At 13.5 years, we measured child Tanner stages and menstruation through questionnaire. We performed linear or logistic regression models for boys and girls to assess the associations of maternal urinary average and trimester-specific bisphenols and phthalates with child reproductive outcomes. Next, to further explore potential synergistic or additive effects of exposures together, we performed mixed exposure models using a quantile g computation approach. Models were adjusted for maternal age, ethnicity, body-mass index, education, parity, energy intake, smoking and alcohol use, and child's gestational age at birth, birthweight and body-mass index. In boys, no associations of maternal gestational phthalate or bisphenol with offspring cryptorchidism and hypospadias were found. Higher maternal high-molecular-weight phthalate and total bisphenol, but not phthalic acid or low-molecular-weight phthalate, were associated with larger child testicular volume at 10 years. Higher maternal phthalic acid and total bisphenol were associated with earlier genital and pubic hair development at 13 years, respectively (p-values<0.05). In girls, we found no associations of maternal urinary bisphenol and phthalate with ovarian volume or menstrual age. Only higher maternal urinary high-molecular-weight phthalate was associated with earlier pubic hair development at 13 years (p-values <0.05). Higher mixture exposure was associated with earlier pubic hair development in both sexes. In conclusion, higher maternal gestational urinary bisphenol and phthalate concentrations were associated with alterations in offspring reproductive development, mainly in boys.

1. Introduction

Worldwide, the age of pubertal onset is decreasing in girls and to the lesser extent in boys, and the incidence of precocious puberty is increasing (Sørensen et al., 2012; Eckert-Lind et al., 2020). Pubertal onset depends on the development of the reproductive neuroendocrine system from fetal life onwards (Ho et al., 2017). Fetal exposure to endocrine-disrupting chemicals (EDCs) influences the steroid-dependent development of the reproductive system and might cause reproductive

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disorders at several stages of sexual maturation, such as crytorchidism, hypospadia and altered timing of puberty (Ho et al., 2017; Cargnelutti et al., 2021). The EDCs bisphenols and phthalates are used in a variety of common plastic products, possess estrogenic and anti-androgenic potentials and are able to freely cross the placenta (Philips et al., 2017; Mose et al., 2007; Nahar et al., 2015). In mice, fetal exposure to bisphenols and phthalates has been associated with reproductive developmental abnormalities including cryptorchidism and hypospadias, and with delayed preputial separation and precocious or delayed vaginal opening, which are indices of pubertal onset (Cargnelutti et al., 2021). In human populations, studies on the effect of fetal exposure to phthalates and bisphenols on reproductive development are scarce. No consistent associations with infant cryptorchidism or hypospadias have been found (Kilcoyne and Mitchell, 2019). Higher exposure to phthalates has been associated with lower ovarian follicle counts in women undergoing fertility treatment, and both higher bisphenol A (BPA) and phthalate have been associated with polycystic ovarian syndrome, potentially imparing ovarian function (Karwacka et al., 2019; Jin et al., 2019; Palioura and Diamanti-Kandarakis, 2015). However, the effect of fetal exposure on ovarian development has not yet been studied. On pubertal development, a longitudinal cohort study from California among ~340 participants showed associations of higher fetal exposure to BPA, and high-molecular-weight phthalates (HMWP) di-2-ethylhexylphthalate (DEHP) and monobenzylphthalate (mBzP) with later pubertal onset in girls. Only higher fetal exposure to the low-molecular weight phthalate (LMWP) monoethylphthalate (mEP) was associated with earlier pubarche (Berger et al., 2018; Harley et al., 2019). In boys, higher fetal exposure to BPA and the HMWPs mono (3-carboxypropyl)phthalate (mCPP) and mono(carboxy-isooctyl) phthalate was associated with earlier onset of puberty (Berger et al., 2018; Harley et al., 2019). A prospective cohort study among ~200 participants from Mexico showed that in girls, higher fetal exposure to DEHP and to the LMWPs mBzP and mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP) were associated with earlier onset but slower progression of breast development, and higher mono-2-ethylhexyl phthalate was associated with earlier pubic hair development (Cathey et al., 2020; Watkins et al., 2017a; Watkins et al., 2017b; Watkins et al., 2014; Ferguson et al., 2014). In boys, higher exposure to the LMWP mono-n-butylphthalate (mBP) and to mBzP was associated with later pubic hair development, but no associations with testicular development were present (Cathey et al., 2020; Watkins et al., 2017a; Watkins et al., 2017b; Watkins et al., 2014; Ferguson et al., 2014). We hypothesized that higher fetal exposure to bisphenols and phthalates is associated with altered offspring reproductive development, reflected by reproductive tract abnormalities, altered testicular and ovarian development and altered onset of puberty. Based on animal and human studies, these effects may be sex-specific.

Nomenclature

Abbreviations

BMI	body mass index
BP	total bisphenol
BPA	bisphenol A
BPF	bisphenol F
BPS	bisphenol S
DAG	direct acyclic graph
DEHP	di-2-ethylhexylphthalate
DNOP	di-n-octyl phthalate
EDC	endocrine-disrupting chemical
HMWP	high-molecular-weight phthalate
IQR	interquartile range
LMWP	low-molecular -weight phthalate
LOD	limit of detection
mBP	mono-n-butylphthalate
mBzBP	monobenzylphthalate
mCMHP	mono[(2-carboxymethyl)-hexyl]phthalate
mCPP	mono(3-carboxypropyl)phthalate
mECPP	mono-(2-ethyl-5-carboxypentyl)phthalate
mEHHP	mono-(2-ethyl-5-hydroxyhexyl)phthalate
mEOHP	mono-(2-ethyl-5-oxohexyl)phthalate
mEP	monoethylphthalate
mHpP	mono-2-heptylphthalate
mHxP	mono-hexylphthalate
mMP	monomethylphthalate
MRI	magnetic resonance imaging
PA	phthalic acid
SD	standard deviation

In a population-based prospective cohort of 1272 mothers-child pairs, we aimed to identify critical bisphenol and phthalate exposures and critical periods of exposure in fetal reproductive development. We assessed the associations of maternal urinary bisphenol and phthalate concentrations in pregnancy with reproductive development from infancy to 13 years among boys and girls. We assessed infant reproductive tract abnormalities, ovarian and testicular volume at 10 years using Magnetic Resonance Imaging (MRI) and pubertal development and onset of menstruation at 13 years.

2. Methods

2.1. Study design and population

This study is embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood in Rotterdam, the Netherlands (Kooijman et al., 2016). Study approval was obtained by the Medical Ethical Committee of the Erasmus Medical Center, University Medical Center, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all mothers. In total, 1379 pregnant women had bisphenol and phthalate measurements in each trimester of pregnancy. Of those, 696 delivered singleton life-born boys and 683 singleton life-born girls. In boys, 673 mother-son pairs had information on at least one reproductive outcome, of whom 639 mother-son pairs had data on infant reproductive abnormalities, 355 had child MRI measurements and 320 had information on puberty characteristics. In girls, 524 mother-daughter pairs had information on at least one reproductive outcome, of whom 359 mother-daughter pairs had child MRI measurements, 453 had information on puberty characteristics and 286 had information on first menstruation (Flowchart shown in Fig. 1). Reasons for missing data were that children did not participate at the 10 or 13 year visit or that they did not fill in the questions on pubertal development.

2.2. Maternal bisphenol and phthalate analysis

Maternal bisphenol and phthalate concentrations were measured in spot urine samples obtained from each woman at three time points during pregnancy (median 12.9 weeks of gestation (interquartile range (IQR) 12.1–14.4)); median 20.4 weeks of gestation (IQR 19.9–20.9); median 30.2 weeks of gestation (IQR 29.9–30.8)). Details on bisphenol, phthalate and creatinine analysis are described in detail previously (Philips et al., 2018). Analysis were performed at Wadsworth Center,

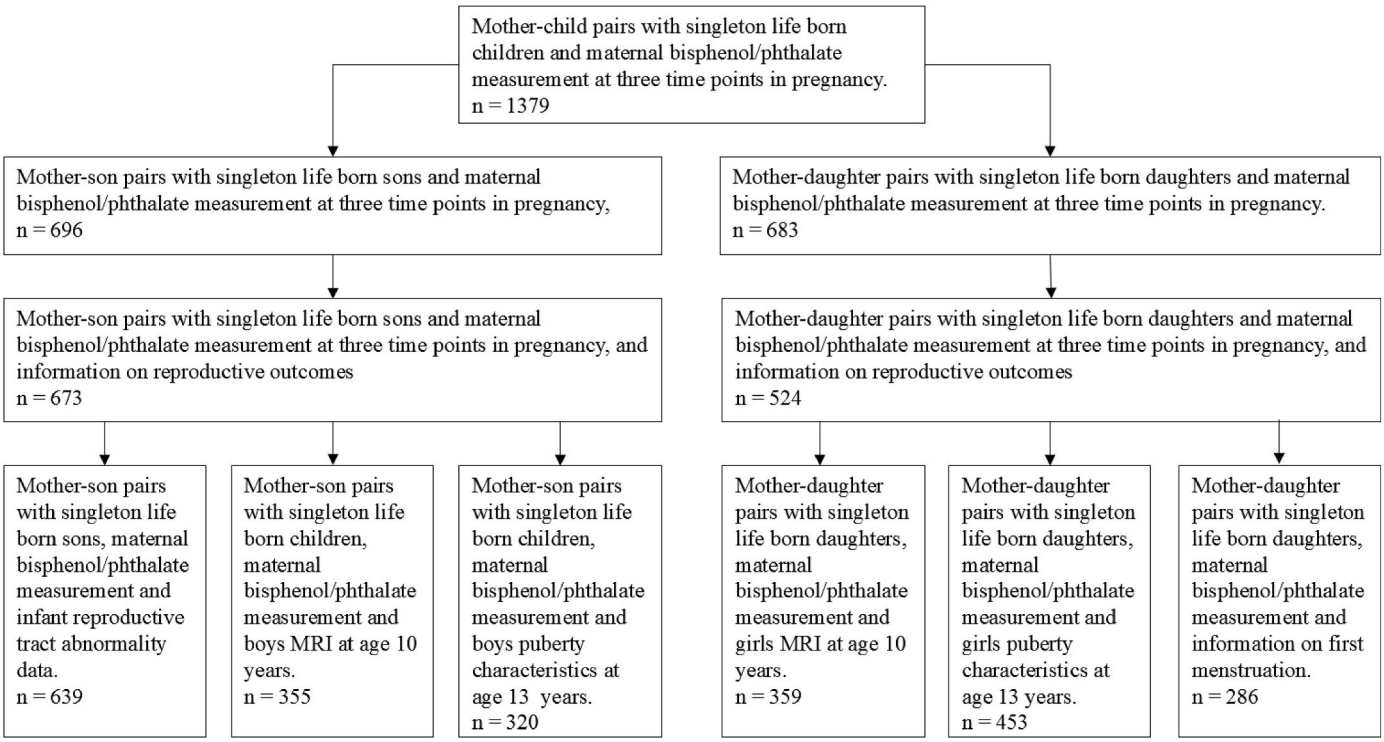

Fig. 1. Flowchart of participants included in the study.

New York State Department of Health, and New York University School of Medicine in New York, USA.

Metabolites were assessed individually and included in metabolite groups when ≥20% of the samples was above the limit of detection (LOD) (Table S1). We selected the LOD cut-off of 20% because this cut-off enabled us to include the maximum number of participants in the analyses with adequate variability in the data to detect associations and to perform analysis across the full exposure range. This approach is in line with previous studies in the field (Philips et al., 2018; Sol et al., 2020; van den Dries Michiel et al. et al.). We grouped the urinary biomarkers of exposure to phthalates according to their chemical structure into LMWP and HMWP, which included DEHP and di-n-octyl phthalate (DNOP). We used this grouping of phthalate related biomarkers as we hypothesized that chemicals with similar chemical structures have similar potential biological effects on fetal development. The LMWP group in all three trimesters consisted of mEP, mBP, mono-methylphthalate (mMP) and mono-isobutylphthalate (mIBP). The HMWP group in first trimester consisted of the DEHP group, the DNOP group, mono-hexylphthalate (mHxP), mono-2-heptylphthalate (mHpP) and mBzP. The HMWP group in second and third trimester consisted of the DEHP group, the DNOP group and mBzP. The DEHP group in all three trimesters consisted of mEOHP, mono-(2-ethyl-5-carboxypentyl) phthalate (mECPP), mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP) and mono[(2-carboxymethyl)-hexyl]phthalate (mCMHP). The DNOP group in all three trimesters consisted of mCPP. Phthalic acid (PA) was analyzed as a proxy for total phthalate exposure. Individual bisphenols were grouped as a proxy for total bisphenol (BP) exposure. The BP group in first and third trimester consisted of BPA, bisphenol S (BPS) and bisphenol F (BPF). The BP group in second trimester consisted of BPA and BPS, because BPF in second trimester did not fulfill the inclusion criteria of >20% of samples above the limit of detection. We calculated the weighted molar sums for BP, LMWP, HMWP, DEHP and DNOP. Concentrations below the LOD were imputed by the LOD of that compound divided by the square root of 2 ($LOD/\sqrt{2}$), which has shown to provide an accurate estimation of the mean and standard deviation of exposure values in data that are not highly skewed (Hornung and Reed, 1990). The descriptive statistics of the bisphenols and phthalates are shown in Table S2. To account for urinary dilutions, metabolite concentrations were converted to μmol/g creatinine. To reduce the potential for exposure misclassification due to temporal variability, we calculated the average exposure during pregnancy by calculating mean of three measurements taken logitudinally.

For all analyses, urinary bisphenol and phthalate concentrations were natural log-transformed to obtain normal distributions and further standardized by the IQR to allow interpretation of the effect estimates.

2.3. Reproductive development in boys

The presence of cryptorchidism and hypospadias was assessed during routine screening performed in child health centers, as described previously (Snijder et al., 2012). As soon as live births are registered in the municipal birth register, child health care centers invite all parents to participate in a national preventive child healthcare programme, free of charge. Genital inspection and manipulation of the testis were performed by trained physicians at 5 visits (mean age 1.1 months (SD 0.2), 2.0 months (SD 0.2), 3.4 months (SD 0.4), 4.4 months (SD 0.3) and 6.2 months (SD 0.7)) (Pierik et al., 2005). Boys were diagnosed as cryptorchid if one or both testes could not be manipulated to a stable scrotal position.

At the age of 10 years, testicular volume was measured by MRI, as described previously (Kooijman et al., 2016). Larger testicular volume represents more advanced genital and pubertal development (Marshall and Tanner, 1970). MRI has been described as an accurate and reproduceable technique for imaging testicular volume (Kabay et al., 2009). All children were scanned using a 3.0 T MRI (MR 750w, GE Healthcare, Milwaukee, WI, USA) using standard imaging and positioning protocols. Testicular volume was scored by trained research assistants. Individual mean testicular volumes were square root transformed and standard deviation scores (SDS) were calculated.

At the age of 13 years, stage of pubertal development was assessed through questionnaires using clinical Tanner staging. Tanner staging is a common clinical marker of pubertal development using a 5-point

categorical scale (Marshall and Tanner, 1970; Marshall and Tanner, 1969). Children were first explained upon the Tanner stages by a researche nurse, and then filled out digital questionnaires in a private room at the research center. Pictures and descriptions of each Tanner stage were shown, and children were asked which stage was most applicable to them. We obtained stage of genital development (ranging from G1 (prepubertal) to G5 (adult)) and pubic hair development (PH1 to PH5) (Marshall and Tanner, 1970; Marshall and Tanner, 1969). As Tanner Stages are dependent on age, for analysis we made a classification using the expected Tanner stage for that age as reference category. For analysis, boys were categorized in the following three groups: Tanner stage conform expected for their age, Tanner stage lower than expected for their age and Tanner stage higher than expected for their age. We used this categorization because of the low number of participants in the lowest and highest Tanner stages. Tanner stages conform the age of our sample (mean 13.5 years (SD ± 0.3)) were stage G3 and G4 for genital development and stage PH3 for pubic hair development (Marshall and Tanner, 1970; Marshall and Tanner, 1969; Karpati et al., 2002).

2.4. Reproductive development in girls

Ovarian volume was measured by MRI at 10 years (Kooijman et al., 2016). MRI has been described as an accurate and reproduceable technique for imaging ovarian volume (Leonhardt et al., 2014). All children were scanned using a 3.0 T MRI (MR 750w, GE Healthcare, Milwaukee, WI, USA) using standard imaging and positioning protocols. Ovarian volume was scored by trained research. Smaller ovaria might represent either lower ovarian follicle counts or slower development of the ovaria. Individual mean ovarian volumes were square root transformed and SDS were calculated.

At the age of 13 years, Tanner stages and age at first mensturation were assessed through questionnaires. Tanner staging is a common clinical marker of pubertal development using a 5-point categorical scale (Marshall and Tanner, 1970; Marshall and Tanner, 1969). Children were first explained upon the Tanner stages by a researche nurse, and then filled out digital questionnaires in a private room at the research center. Pictures and descriptions of each Tanner stage were shown, and children were asked which stage was most applicable to them. We obtained stage of breast development (ranging from B1 (prepubertal) to B5 (adult)) and pubic hair development (PH1 to PH5). For analysis, girls were categorized in the following three groups: Tanner stage conform expected for their age, Tanner stage lower than expected for their age and Tanner stage higher than expected for their age. We used this categorization because of the low number of participants in the lowest and highest Tanner stages. Tanner stages conform the age of our sample (mean 13.5 years (SD ± 0.3)) were stage B3 or B4 for breast development and stage PH3 or PH4 for pubic hair development (Marshall and Tanner, 1970; Marshall and Tanner, 1969). In girls that had already menstruated, the age at first menstruation was asked through questionnaire.

2.5. Covariates

Information on maternal age, ethnicity (European or non-European), pre-pregnancy body-mass index (BMI), educational level (no education, finished primary school, secondary school or higher), total energy intake in kilocalories and parity (nulli- or multiparous) was obtained at enrollment through questionnaires (Kooijman et al., 2016). We assessed maternal smoking (never smoked in pregnancy, smoked until pregnancy was known, continued smoking in pregnancy) and alcohol consumption (never alcohol in pregnancy, alcohol until pregnancy was known, alcohol continued in pregnancy) in each trimester of pregnancy. Child birth characteristics were obtained from midwife and hospital records. Information on breastfeeding was assessed through questionnaires after birth. At the age of 10 and 13 years, we measured children their weight and height without shoes and calculated age-adjusted BMI.

2.6. Statistical analysis

First, we performed a non-response analysis comparing our study sample to all women with singleton life-born children within the study cohort. Based on previous literature, we hypothesized sex-specific effects, and therefore conducted analyses for boys and girls separately (Berger et al., 2018; Harley et al., 2019; Cathey et al., 2020; Watkins et al., 2017a; Watkins et al., 2017b; Watkins et al., 2014; Ferguson et al., 2014). Second, we explored the correlation structure of the exposures by calculating pairwise Pearson's correlation coefficients between all individual phthalates and bisphenols in each trimester. We visualized these correlation using heath maps (R package ggplot2).

In boys, first we examined the associations of maternal urinary concentrations of bisphenol and phthalate groups and subgroups (the exposure groups), with risks of reproductive developmental abnormalities (cryptorichidism or hypospadias) in infancy using logistic regression models. Second, we examined the associations of the exposure groups with testicular volume at 10 years using linear regression models. Third, we examined the associations of the exposure groups with Tanner categories for genital and pubic hair development at 13 years using multinomial logistic regression models. Using a similar approach in girls, we examined the associations of the exposure groups with ovarian volume at 10 years using linear regression models and with pubertal development in Tanner categories at 13 years using multinomial logistic regression models. We also examined the associations of the exposure groups with the age of first menstruation using linear regression models. In our basic models, we adjusted for child age at the time of measurement. Subsequently, we additionally adjusted for maternal and child sociodemographic, lifestyle and physical factors that were selected based on existing literature and Directed Acyclic Graph (DAG) analysis (DAG shown in Figure S1). Maternal confounders age, ethnicity, pre-pregnancy BMI educational level, parity, total energy intake, smoking status and alcohol use (Philips et al., 2018; Euling et al., 2008; Aghaee et al., 2019a). Child confounders were gestational age at birth, birthweight and being breastfed (Euling et al., 2008; Hvidt et al., 2019; Li et al., 2017; Aghaee et al., 2019b). We considered child's BMI a potential mediator and therefore corrected for child's BMI z-scores according to Fredriks in the adjusted model. For comparison, we also performed analysis adjusting for BMI instead of BMI z-scores. Missing values of all other covariates were imputed using multiple imputation by the fully conditional specification method, and pooled results from five imputed datasets were reported (Buuren, 2018). We used a nominal 2-sided significance threshold of <0.05. To correct for multiple testing we also considered a p-value threshold defined as 0.05 devided by the effective number of independent tests estimated based on the correlation structure between the exposures (p-value of 0.0098) (Sol et al., 2020; Li et al., 2012).

As a secondary exploratory analyses, we repeated all analyses using trimester-specific maternal urinary BP, PA, LMWP and HMWP concentrations as exposure to identify potential sensitive windows in fetal development for exposure to bisphenols and phthalates. Also, as a sensitivity analysis we adjusted for creatinine by refitting average exposure models, with the exposure groups expressed in nanomol per liter urine and creatinine concentration in microgram per milliliters added as an additional covariate. We used this approach to assure that estimates from our main analysis were not driven by non-dilution factors related to creatinine excretion (O'Brien Katie et al., 2016). Next, we performed a sensitivity analysis to estimate the joint effect of the phthalate metabolites and bisphenols on the reproductive outcomes, because restricting analysis to single pollutants may ignore synergistic or additive health effects which can be detected by assessing chemical mixtures as a whole (Bopp et al., 2018). We used the quantile-based g-computation approach (R package qgcomp). (Keil Alexander, Buckley Jessie, O'Brien Katie, Ferguson Kelly, Zhao, White Alexandra)

Quantile-based g-computation quantifies the expected change in the outcome, give a one quantile increase in all exposures simultaneously. We estimated the joint effect of the overall maternal urinary mixture including average bisphenol and phthalates metabolites in pregnancy on 1) odds on reproductive developmental abnormalities in infancy, 2) child testicular or ovarian volume on the MRI at 10 years and 3) child pubertal development at 13 years. Each analysis was adjusted for the potential confounders. All analyses were performed using the Statistical Package for the Social Sciences version 25.0 (IBM Corp, Armonk, New York, USA) and R Statistical software version 4.1.0.

3. Results

3.1. Participant characteristics

The mean age of mothers in our sample was 30.8 years (±SD 4.7), and median BMI was 22.7 (IQR 20.8, 25.3) (Table 1). Most women were European (63.4%), nulliparous (61.4%), highly educated (52.3%) and never smoked in pregnancy (76.5%). Alcohol use in pregnancy was high (40.6%). As compared to all women within the Generation R study without bisphenol and phthalates measurements, women with these measurements were older, more often European, nulliparous and highly educated, smoked less often and continued drinking alcohol more often in pregnancy (non-response analysis shown in Table S3). The correlation structure of the exposure showed that the correlation between the individual metabolites from different exposure groups was <0.5 in each trimester, which is considered very low or low (Figure S2 to S4). Metabolites that were high or very high correlated (correlation >0.7) were the LMWPs mMP, mEP and mBP in second trimester, and the HMWPs mEHHP, mECPP and mEOHP in first and third trimester.

3.2. Maternal bisphenol and phthalate urinary concentrations and reproductive development in boys

Cryptorchidism was present in 21 (3.3%) and hypospadia in 2 (0.4%) boys (Table 2). At 10 years, mean testicular volume was 0.99 mL (IQR 0.76, 1.30 mL). At 13 years, most boys had genital Tanner stages G3 (42.2%) or G4 (31.1%), and pubic hair stages PH3 (37.5%) or PH4 (31.1%).

In infants, we found no associations of maternal urinary average phthalate and bisphenol concentrations during pregnancy with the presence of cryptorchidism or hypospadias (Table S4). Table 3 shows that higher maternal urinary average HMWP concentrations, specifically DEHP and DNOP, were associated with larger testicular volume in boys at 10 years (differences 0.09 SDS (95% CI 0.01, 0.16), 0.08 SDS (95% CI 0.01, 0.15) and 0.07 SDS (95%CI 0.01, 0.14) per IQR increase in HMWP, DEHP and DNOP). Also, higher average BP concentrations were associated with larger testicular volume in boys at 10 years (difference 0.08 SDS (95% CI 0.00, 0.16) per IQR increase in BP). We found no association of PA or LMWP with testicular volume. We found no association of average maternal urinary PA, LMWP, HMWP or subgroups concentrations during pregnancy with offspring pubertal genital or pubic hair development (Table 4). Higher maternal average urinary BP concentrations during pregnancy, and specifically BPA concentrations, were associated with lower odds of later pubertal pubic hair development (OR 0.54 (95% CI 0.36, 0.82) and OR 0.58 (95% CI 0.39, 0.87)), but not with genital development (Table 4). Considering multiple

Table 1
General characteristics of the study population, stratified for boys and girls.

Maternal characteristics	Boys (n = 673)	Girls (n = 524)
Maternal age in years, mean (±SD)	30.7 (4.8)	30.9 (4.6)
Ethnicity, n (%)		
European	422 (63.2)	333 (63.8)
Non-European	246 (36.8)	189 (36.2)
Parity, n (%)		
Nullipara	413 (61.4)	321 (61.3)
Multipara	260 (38.6)	203 (38.7)
Pre-pregnancy body mass index, median (IQR)	22.7 (20.8, 25.4)	22.7 (20.9, 25.2)
Smoking, n (%)		
Never smoked during pregnancy	451 (75.7)	376 (77.0)
Smoked until pregnancy was known	60 (10.1)	52 (10.7)
Continued smoking in pregnancy	85 (14.3)	60 (12.3)
Alcohol use, n (%)		
Never alcohol in pregnancy	237 (39.9)	209 (43.1)
Alcohol until pregnancy was known	109 (18.4)	86 (17.7)
Alcohol continued in pregnancy	248 (41.8)	190 (39.2)
Highest education finished, n (%)		
No education	1 (0.1)	0 (0.0)
Primary	44 (6.8)	32 (6.3)
Secondary	263 (40.8)	210 (41.3)
Higher	336 (52.2)	267 (52.5)
Child characteristics – Birth		
Gestational age at birth in weeks, median (IQR)	40.3 (39.3, 41.1)	40.3 (39.4, 41.0)
Premature birth (<37 weeks), n(%)	17 (2.5)	13 (2.5)
Birthweight in grams, mean (±SD)	3531.7 (499.3)	3378.0 (470.7)
Macrosomia (>4000gr), n (%)	107 (15.9)	15 (2.9)
Low birthweight (<2500gr), n (%)	18 (2.7)	15 (2.9)
Breastfeeding at age of 6 months	214 (35.2)	152 (31.9)
Child characteristics – 10 year visit	Boys (N = 355)	Girls (N = 359)
Age child at visit in years, mean (±SD)	9.7 (0.2)	9.7 (0.2)
Body mass index, median (IQR)	16.5 (15.3, 18.1)	17.0 (15.6, 19.0)
Child characteristics – 13 year visit	Boys (N = 314)	Girls (N = 453)
Age child at visit in years, mean (±SD)	13.5 (0.3)	13.5 (0.3)
Body mass index, median (IQR)	18.2 (16.8, 20.2)	19.6 (17.9, 21.7)

Values presented as mean (± standard deviation (SD), median (interquartile range (IQR)) or number of participants (valid %).

Table 2
Reproductive characteristics of the study population, stratified for boys and girls.

Boys	
Infants (n = 639)	
Cryptorchidism, n (%)	21 (3.3)
Hypospadia, n (%)	2 (0.4)
Age 10 years (n = 355)	
Testicular volume in mL, median (IQR)	0.99 (0.76, 1.30)
Age 13 years (n = 320)	
Tanner stage genital development (n = 275), n (%)	
Stage G1	5 (1.8)
Stage G2	48 (17.5)
Stage G3	116 (42.2)
Stage G4	86 (31.1)
Stage G5	20 (7.3)
Tanner stage pubic hair development (n = 314), n (%)	
Stage PH1	21 (6.7)
Stage PH2	63 (20.0)
Stage PH3	118 (37.5)
Stage PH4	98 (31.1)
Stage PH5	15 (4.8)
Girls	
Age 10 years (n = 359)	
Ovarian volume in mL, median (IQR)	1.31 (0.86, 2.00)
Age 13 years (n = 453)	
Tanner stage breast development (n = 359), n (%)	
Stage B1	4 (1.1)
Stage B2	44 (12.3)
Stage B3	144 (40.1)
Stage B4	141 (39.3)
Stage B5	26 (7.2)
Tanner stage pubic hair development (n = 322), n (%)	
Stage PH1	3 (0.9)
Stage PH2	27 (8.4)
Stage PH3	100 (31.1)
Stage PH4	137 (42.5)
Stage PH5	55 (17.1)
Age at first menstruation (n = 286), mean (±SD)	11.9 (0.9)

Values presented as mean (±standard deviation (SD)), median (interquartile range (IQR)) or number of participants (valid %).

Table 3
Associations of maternal average urinary concentrations of bisphenol and phthalate groups with child's testicular volume at the age of 10 years.

Exposure	SDS difference in testicular volume	95% CI	P-value
Phthalic acid	−0.01	−0.09, 0.07	0.803
Low-molecular-weight phthalate	−0.04	−0.14, 0.05	0.393
High-molecular-weight phthalate	0.09	0.01, 0.16	0.021*
Di-2-ethylhexylphthalate	0.08	0.01, 0.15	0.033*
Di-n-octylphthalate	0.08	0.01, 0.14	0.028*
Total bisphenol	0.08	0.00, 0.16	0.042*
Bisphenol A	0.06	−0.02, 0.13	0.170
Bisphenol S	0.04	−0.04, 0.12	0.334

Values represent regression coefficients (95% confidence interval) and corresponding p-values of the regression models that reflect the difference in testicular volume for an interquartile range increase in maternal urinary phthalate and bisphenol groups (in μmol/g creatinine).
Models include maternal age, ethnicity, pre-pregnancy body-mass index, education level, parity, intake, smoking and alcohol use, breastfeeding, and child's gestational age adjusted birthweight, and age and age-adjusted body-mass index at time of measurement.

testing, only the associations of higher BP and BPA with earlier pubic hair development remained significant (p-value <0.013).

Trimester specific analysis showed that higher maternal urinary BP concentrations in second trimester were associated with larger testicular volume at age 10 years (p-value <0.05) (Table S5). At age 13 years, higher maternal urinary second trimester PA and first and third trimester BP concentrations were associated with lower odds on later pubertal development, and higher third trimester BP with lower odds on earlier pubic hair development (p-values <0.05) (Tables S6 and S7). The results of the basic model were largely in line with the main model, adjusted for maternal and child's sociodemographic and lifestyle factors (Tables S4 to S7). Adjusting for BMI in general yielded similar effects as adjusting for BMI z-scores (results not shown). Adding maternal average urinary creatinine as separate covariate to the models in general yielded similar effects in boys, except for the associations of higher maternal HMWP and BP with testicular at 10 years, which attenuated towards zero (Table S12 to S15).

Higher concentrations of the overall exposure mixture were not significantly associated with the odds on cryptorchidism or hypospadias (OR 1.12 (95% CI 0.54, 2.32) per quantile increase in all individual exposure) or with testicular volume at 10 years (difference 0.05 (95% CI -0.13, 0.22)). The exposure mixture effects on pubertal genital development were neither significant (OR lower genital Tanner Stage than expected 0.63 (95% CI 0.35, 1.14), OR higher genital Tanner Stage 1.41 (95%CI 0.58, 3.43). Higher urinary exposure mixture was associated with lower odds on being in a lower pubic hair Tanner Stage (OR 0.55 (95% CI 0.32, 0.95), but not with being in a higher pubic hair Tanner Stage (OR 0.05 (95%CI 0.45, 1.23). The contributions of the individual metabolites on the total mixture effect for all outcomes is shown in Figure S5.

3.3. Maternal bisphenol and phthalate urinary concentrations and reproductive development in girls

Mean ovarian volume was 1.31 mL (IQR 0.86, 2.00 mL) (Table 2). At 13 years, most girls had Tanner stages B3 (40.1%) or B4 (39.3%) for breast development and stages PH3 (31.1%) or PH4 (42.5%) for pubic hair development. Mean age at first menstruation was 11.9 years (±SD 0.9).

We found no associations of maternal average urinary concentrations of PA, LMWP, HMWP, BP and subgroups during pregnancy with ovarian volume at 10 years (Table 5). At 13 years, higher maternal average HMWP, and specifically DEHP, concentration during pregnancy, were associated with lower odds on later pubic hair development (OR 0.50 (95%CI 0.29, 0.87) and OR 0.54 (95%CI 0.31, 0.95) per IQR increase in

Table 4
Association of maternal average urinary concentrations of bisphenol and phthalate groups and subgroups with Tanner stages for genital and pubic hair development in boys at the age of 13 years.

Exposure	Tanner Stage	Genital development			Pubic hair development		
		OR	95%CI	P-value	OR	95% CI	P-value
Phthalic acid	Lower than expected for age	0.64	0.40, 1.01	0.052	0.75	0.50, 1.11	0.152
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.41	0.75, 2.65	0.288	1.04	0.71, 1.51	0.846
Low-molecular-weight phthalate	Lower than expected for age	0.83	0.51, 1.34	0.442	0.76	0.49, 1.19	0.235
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.43	0.72, 2.83	0.306	0.85	0.56, 1.29	0.446
High-molecular-weight phthalate	Lower than expected for age	0.91	0.60, 1.37	0.638	0.99	0.68, 1.44	0.947
	Conform expected for age	Reference			Reference		
	Higher than expected for age	0.91	0.52, 1.59	0.731	1.12	0.80, 1.59	0.517
Di-2-ethylhexylphthalate	Lower than expected for age	0.86	0.57, 1.30	0.475	1.05	0.73, 1.51	0.799
	Conform expected for age	Reference			Reference		
	Higher than expected for age	0.81	0.46, 1.42	0.459	1.08	0.77, 1.51	0.668
Di-n-octylphthalate	Lower than expected for age	1.11	0.78, 1.58	0.576	0.81	0.57, 1.15	0.235
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.07	0.64, 1.79	0.785	1.19	0.87, 1.63	0.265
Total bisphenol	Lower than expected for age	0.67	0.43, 1.05	0.081	0.55	0.37, 0.83	0.005*
	Conform expected for age	Reference			Reference		
	Higher than expected for age	0.99	0.53, 1.85	0.973	0.74	0.52, 1.06	0.101
Bisphenol A	Lower than expected for age	0.65	0.42, 1.03	0.065	0.59	0.39, 0.89	0.011*
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.11	0.60, 2.06	0.743	0.81	0.56, 1.15	0.240
Bisphenol S	Lower than expected for age	0.86	0.55, 1.37	0.531	0.89	0.60, 1.34	0.587
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.33	0.74, 2.39	0.337	0.74	0.51, 1.08	0.119

Values represent odds ratio (OR) (95% confidence interval (CI)) and corresponding p-values of the regression models that reflect the odds on a higher or lower Tanner stage than expected for age for an interquartile range increase in maternal urinary phthalate and bisphenol groups (in μmol/g creatinine). Model includes maternal age, ethnicity, pre-pregnancy body-mass index, education level, parity, intake, smoking and alcohol use, breastfeeding, and child's gestational age adjusted birthweight, and age and age adjusted body-mass index at time of measurement.

Table 5
Association of maternal average urinary concentrations of bisphenol and phthalate groups with ovarian volume (age 10 years) and age at first menstruation.

Exposure	SDS difference in ovarian volume	95% CI	P-value	Difference in age at first mensturation in years	95% CI	P-value
Phthalic Acid	0.02	−0.12, 0.15	0.816	0.03	−0.11, 0.17	0.678
Low-molecular-weight phthalates	0.06	−0.07, 0.19	0.336	−0.07	−0.22, 0.08	0.378
High-molecular-weight phthalates	0.05	−0.05, 0.16	0.321	−0.02	−0.13, 0.09	0.684
Di-2-ethylhexylphthalate	0.05	−0.06, 0.15	0.378	−0.02	−0.13, 0.09	0.741
Di-n-octylphthalate	0.02	−0.09, 0.12	0.761	0.14	0.02, 0.26	0.022*
Total bisphenol	0.05	−0.06, 0.16	0.353	0.05	−0.08, 0.18	0.429
Bisphenol A	0.07	−0.04, 0.18	0.198	0.04	−0.08, 0.17	0.471
Bisphenol S	−0.03	−0.16, 0.09	0.622	0.17	0.02, 0.31	0.025*

Values represent regression coefficients (95% confidence interval (CI)) and corresponding p-values of the regression models that reflect 1) the difference (standard deviation score (SDS)) in ovarian volume or 2) difference (years) in age at first menstruation for an interquartile range increase in maternal urinary phthalate and bisphenol groups and subgroups (in μmol/g creatinine). Model includes maternal age, ethnicity, pre-pregnancy body-mass index, education level, parity, intake, smoking and alcohol use, breastfeeding, and child's gestational age adjusted birthweight, and age and age adjusted body-mass index at time of measurement.

maternal urinary HMWP and DEHP), but not with breast development (Table 6). No associations of average concentrations of other phthalate or bisphenol groups during pregnancy with pubertal breast or pubic hair development were present. Higher average maternal urinary concentrations of DNOP and BPS, but not of main phthalate or bisphenol groups, were associated with an older age at first menstruation (differences 0.14 years (95%CI 0.02, 0.26) and 0.17 years (95%CI 0.02, 0.31)).

Timester-specific analysis showed no associations of higher maternal urinary phthalates and bisphenols with ovarian volume at age 10 (Table S8). At age 13 years, higher first trimester HMWP was associated with earlier pubic hair development (Tables S9 and S10). The results of the basic model were largely in line with the main model (Tables S8 – S11). Adjusting for BMI in general yielded similar effects as adjusting for BMI z-scores (results not shown). Adding maternal average creatinine as separate covariate to the models did not significantly change any associations in girls (Table S16 - S19).

Higher concentrations of the overall exposure mixture were not associated with ovarian volume at 10 years (difference 0.14 (95% CI -0.03, 0.31) per one quantile increase in all individual exposures), nor with breast Tanner Stages (OR lower than expected 0.89 (95% CI 0.50, 1.59), OR higher than expected 0.82 (95% CI 0.33, 1.99)). Higher urinary exposure mixture was associated with lower odds on being in a lower pubic hair Tanner Stage (OR 0.40 (95% CI 0.18, 0.88), but not with being in a higher pubic hair Tanner Stage (OR 0.94 (95%CI 0.53, 1.66) or with age at first menstruation (difference 0.05 (95% CI -0.32, 0.41)). The contributions of the individual metabolites on the total mixture effect for all outcomes is shown in Figure S6.

4. Discussion

In this population-based prospective cohort study, we observed sex-dependent associations of maternal urinary bisphenol and phthalate concentrations in pregnancy with offspring reproductive development, with the strongest effect among boys. In boys, higher maternal urinary phthalate and bisphenol concentrations throughout pregnancy were associated with larger testicular volume and earlier pubertal development, with the strongest effects for HMWP and BP. Mixture exposure was associated with earlier pubic hair development only. No effects on

Table 6
Association of maternal average urinary concentrations of bisphenol and phthalate groups with Tanner stages for breast and pubic hair development in girls at the age of 13 years.

Exposure	Tanner Stage	Breast development			Pubic hair development		
		OR	95% CI	P-value	OR	95% CI	P-value
Phthalic acid	Lower than expected for age	1.12	0.72, 1.75	0.611	0.98	0.57, 1.71	0.950
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.08	0.57, 2.05	0.824	1.17	0.76, 1.80	0.487
Low-molecular-weight phthalates	Lower than expected for age	0.70	0.42, 1.17	0.172	0.71	0.39, 1.30	0.271
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.12	0.54, 2.32	0.763	0.83	0.50, 1.36	0.453
High-molecular-weight phthalates	Lower than expected for age	1.06	0.70, 1.61	0.769	0.50	0.29, 0.87	0.017*
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.02	0.57, 1.83	0.946	1.03	0.71, 1.50	0.869
Di-2-ehtylhexylphthalate	Lower than expected for age	0.99	0.66, 1.50	0.967	0.54	0.31, 0.95	0.031*
	Conform expected for age	Reference			Reference		
	Higher than expected for age	1.05	0.60, 1.85	0.865	0.97	0.67, 1.40	0.868
Di-n-octylphthalate	Lower than expected for age	1.13	0.76, 1.68	0.542	0.66	0.39, 1.10	0.111
	Conform expected for age	Reference			Reference		
	Higher than expected for age	0.75	0.41, 1.37	0.341	0.89	0.60, 1.32	0.545
Total bisphenol	Lower than expected for age	0.97	0.66, 1.42	0.867	0.74	0.45, 1.22	0.242
	Conform expected for age	Reference			Reference		
	Higher than expected for age	0.72	0.39, 1.33	0.294	1.22	0.85, 1.76	0.290
Bisphenol A	Lower than expected for age	1.06	0.74, 1.53	0.742	0.87	0.54, 1.40	0.560
	Conform expected for age	Reference			Reference		
	Higher than expected for age	0.71	0.38, 1.33	0.278	1.41	0.98, 2.03	0.061
Bisphenol S	Lower than expected for age	0.86	0.53, 1.39	0.526	0.75	0.43, 1.31	0.319
	Conform expected for age	Reference			Reference		
	Higher than expected for age	0.80	0.42, 1.50	0.484	0.88	0.57, 1.35	0.546

Values represent odds ratio (OR) (95% confidence interval (CI)) and corresponding p-values of the regression models that reflect the odds on a higher or lower Tanner stage than expected for age for an interquartile range increase in maternal urinary phthalate and bisphenol groups (in μmol/g creatinine). Model includes maternal age, ethnicity, pre-pregnancy body-mass index, education level, parity, intake, smoking and alcohol use, breastfeeding, and child's gestational age adjusted birthweight, and age and age adjusted body-mass index at time of measurement.

cryptorchidism or hypospadias were found. In girls, no consistent associations of maternal phthalates or bisphenols throughout pregnancy with offspring ovarian volume, breast development or menstrual age were present. Higher maternal HMWP concentrations were associated with earlier pubic hair development only. Mixture exposure was associated with earlier pubic hair development only.

4.1. Interpretation of main findings

Due to the extensive use of bisphenols and phthalates in everyday products, pregnant women are frequently exposed and so are their fetuses, as bisphenols and phthalates freely cross the placenta (Mose et al., 2007; Ye et al., 2008). Fetal exposure to these EDCs might influence the development of reproductive system and subsequently adversely affect the reproductive organ systems and puberty (Ho et al., 2017). Most studies investigating the effects of fetal bisphenol and phthalates exposure on reproductive development involve rodents and suggest sex-specific effects. Although these studies provide valuable information, important differences in germ cell development and steroidgenesis between rodents and humans exist (McKinnell et al., 2013; Scott et al., 2009). However, only few studies on fetal bisphenol and phthalate exposure and reproductive development in humans have been conducted and results remain controversial (Berger et al., 2018; Harley et al., 2019; Cathey et al., 2020; Watkins et al., 2017a; Watkins et al., 2017b; Watkins et al., 2014; Ferguson et al., 2014).

Exposure to phthalates in pregnant rodents has been shown to reduce fetal testosterone production in male offspring resulting in a high indicidence of cryptorchidism and hypospadias, but in humans no consistent associations have been found (Kilcoyne and Mitchell, 2019). Most studies on male reproductive development in human populations have focused on pubertal development (Berger et al., 2018; Harley et al., 2019; Cathey et al., 2020; Watkins et al., 2017a; Watkins et al., 2017b; Watkins et al., 2014; Ferguson et al., 2014). In boys aged 9–13 years, a study from California with ~150 mother-son pairs found that higher average maternal urinary concentrations of HMWP metabolites and BPA were associated with earlier onset of genital and pubic hair development (Berger et al., 2018; Harley et al., 2019). This study did not measure PA or LMWP, and HMWP and BP were only measured twice in pregnancy. In contrast, a study from Mexico with ~100 mother-son pairs found that higher maternal phthalate was not associated with genital development in boys aged 8–14 years, but higher LMWP and HMWP metabolites were associated with later onset of pubic hair development (Cathey et al., 2020; Watkins et al., 2017b; Ferguson et al., 2014).

In the present study, we found no associations of fetal bisphenol and phthalate exposure with cryptorchidism or hypospadias. This result should be interpreted with caution, as the incidence of cryptorchidism and hypospadias was low and associations might have been missed due to lack of power. Nontheless, as the majority of previous studies had only one maternal urinary measurement in pregnancy, our study might add valuable information, as we had repeated exposure measurements reducing the risk of exposure misclassification due to temporal variability, as bisphenols and phthalates are non-persistent. Also, our study is the first investigating the association between maternal phthalate and bisphenol urinary concentrations with testicular volume using imaging techniques in boys of (pre)pubertal age (Cargnelutti et al., 2021). Measuring testicular volume by MRI might be an important step in understanding the underlying mechanisms of altered testicular development, and has high additional value as it takes away the potential measurement errors of self-reported testicular volume or the orchidometer. It might be useful in more accurate analysis of the effect of environmental exopsures on the hypothalamic-pituitary-gonadal axis. Higher fetal exposure to HMWP and BP were associated with larger testicular volume, indicating faster genital development (Kabay et al., 2009; Leonhardt et al., 2014). On pubertal development, partly in line with the Californian study we observed that higher maternal urinary PA and BP concentrations and specifically BPA during pregnancy were associated with earlier genital pubertal development (Berger et al., 2018; Harley et al., 2019). Thus, our findings suggest that fetal exposure to phthalates, especially HMWP, and bisphenols, especially BPA, is related to early pubertal development in boys.

In girls, human studies on the association of fetal exposure to bisphenols and phthalates with offspring ovarian function and volume are lacking, but animal studies show that fetal EDC exposure is associated with alterations in ovarian development, impaired function of the ovaries and polycystic-ovary disease (Barrett and Sobolewski, 2014; Hannon and Flaws, 2015). On pubertal development, the Californian study found that in ~170 mother-daughter pairs, higher maternal HMWP and BPA were associated with generally later offspring breast and pubic hair development (Berger et al., 2018; Harley et al., 2019). On the contrary, the Mexican study of ~120 mother-daughter pairs, found that higher maternal LMWP during pregnancy was associated with higher initial stages but slower progression of breast development, and higher maternal BPA during pregnancy with higher initial stages of breast development only (Cathey et al., 2020; Watkins et al., 2017a). On the effect of fetal bisphenol and phthalate exposure on menstrual development, literature is inconclusive. Higher fetal phthalate exposure has been associated with both earlier, later and unchanged age at first menstruation and higher fetal bisphenol exposure with unchanged age at first menstruation (Berger et al., 2018; Watkins et al., 2017a; Watkins et al., 2014).

In the present study, we found no associations of fetal bisphenol and phthalate exposure with offspring ovarian volume. Ovarian volume might be representative for anteral follicle count or the presence of polycystic ovarian syndrome, however it does not represent the ovarian function. Further studies should additionally focus on follicle counts, ovarian architecture, the occurrence of hirsutism and possibly steroid levels such as testosterone. On pubertal development, in contrast with previous literature, we only found an association of higher maternal HMWP and of mixture exposure with earlier offspring pubic hair development. Additionally, higher maternal urinary concentrations of DNOP, but not of the main phthalate and bisphenol subgroups, were associated with a later age at first menstruation. Discrepancies in the association of maternal exposure to bisphenols and phthalates and offspring pubertal development between our and previous studies might be due to our relatively healthy population with only 18 percent of overweight children, different levels of maternal bisphenol and phthalate exposure, and differences in study design, for instance timing of phthalate and bisphenol measurement and assessment of puberty characteristics. Thus, our findings suggest that fetal exposure to phthalates, but not bisphenols, might be related to pubertal development in girls, but this effect is weaker compared to boys. Although previous literature suggested that mainly in girls pubertal development is influenced by BMI, no associations were explained by sociodemographic or lifestyle characteristics such as BMI, substantiating the independent effect of bisphenols and phthalates (Li et al., 2017). This also suggests that BMI is not significantly operating as an effect modifier in the association between maternal bisphenol and phthalate exposure in pregnancy and offspring reproductive development.

To the best of our knowledge, our study is the first to assess maternal mixed exposure effects of bisphenols and phthalates together on child reproductive outcomes. Assessing the effects of individual exposures is important from an etiological perspective, but might ignore health effects which can be detected assessing the effect of chemical mixtures as a whole (Bopp et al., 2018). Chemicals might have unexpected synergistic or additive effects, and concurrent exposure to multiple exposures may have adverse health effects, even when individual exposures are below concentrations considered harmfull (Carpenter et al., 2002). This might explain the differences between the associations found for individual exposures and mixture exposure in the present study; higher pregnancy exposure to the mixture of bisphenols and phthalates was associated with earlier pubertal pubic hair development in offspring boys and girls only.

The mechanisms underlyling these associations remain to be elucidated. Phthalates and bisphenols have estrogenic and anti-androgenic potentials (Philips et al., 2017). The association of higher fetal exposure to bisphenols and phthalates and earlier testicular development in boys and pubic hair development in boys and girls might be explained through their known anti-androgenic capacities (Philips et al., 2017). Testosterone causes genital development in boys and pubic hair development in both sexes (Hiort, 2002). Increased exposure to the anti-androgenic chemicals might disturb the hypothalamic-pituitary-gonadal axis, potentially causing a reverse effect in puberty. We saw little effect on the estrogen-related development, being mainly breast development and menses, potentially because bisphenol and phthalates are known to have only weak estrogenic capacities (Philips et al., 2017). Also, evidence that fetal exposure to bisphenols and phthalates might cause epigenetic changes has accumulated, a mechanism through which bisphenols and phthalates might interfere with the development of the reproductive endocrine axes (Jacobs et al., 2017). The critical windows of fetal reproductive development toxicity for bisphenols and phthalates are uncertain. In the present study, although associations were the strongest in second and third trimester, exposure to bisphenols or phthalates in all three trimesters was associated with changed genital or pubic hair development in boys aged 10 to 13, suggesting that the entire pregnancy is a sensitive window for exposure. This might not be surprising. Pubertal development is dependent on the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axis. Therefore, alterations in neurodevelopment, adrenal development and gonadal development might influence puberty. Fetal adrenal development begins at ~4 weeks of gestation and gonadal at ~6 weeks, and both continue until after birth (Mesiano and Jaffe, 1997). Likewise, neurodevelopment including the hypothalamic and pituitary gland continues throughout the whole pregnancy. Further studies are needed to evaluate the potential mechanisms of effect using direct measurements of sex-steroids and repeated measurement of pubertal development, potentially using imaging techniques such as MRI, allowing analysis of the progression of puberty.

In our study, associations of bisphenol or phthalate exposure with reproductive development were not limited to the extremes but were present across the full range of exposure. In general, urinary concentrations of BPA and phthalate metabolites within the Generation R population are somewhat lower than concentrations in other Western studies, and detection rates are comparable or slightly lower (Harley et al., 2019; Cathey et al., 2020; Philips et al., 2018). The identified associations might therefore be representative for or be even stronger in the general population. As we aimed to identify critical exposures and critical periods of these exposures for offspring reproductive development, we analyzed different maternal urinary bisphenol and phthalate exposure groups we comparable chemical structures based on their potential biological effects. We are among the first assessing the association of these exposures with reproductive development in humans, which is important from an etiological perspective. Correlation between the different bisphenol and phtahlates groups within each trimester was low, which supports the separate analysis of these exposures. However, as mothers are exposed to a mixture of exposures in real-life, our results need to be taken forward to further studies assessing the impact of a mixture of exposures and potential cumulative effects of multiple exposures together.

Our study suggest that maternal phthalate and bisphenol exposure might contribute to earlier puberty in boys and to the lesser extend in girls, which may contribute to the recent global trend towards earlier puberty (Sørensen et al., 2012; Eckert-Lind et al., 2020). Early pubertal onset is associated with adverse long-term health outcomes such as metabolic syndrome, breast cancer and testicular cancer (Golub et al., 2008). Preventive measures reducing maternal exposure to phthalates and bisphenols might contribute to improving long-term health outcomes in the offspring.

4.2. Methodological considerations

The prospective nature of our study from early pregnancy onwards allowed repeated bisphenol and phthalate measurements throughout the entire pregnancy, including less common substitutes such as BPS and BPF. Our population is relatively high-educated and healthy, which might have affected the generazability of our results. Also, despite the short biological half-lives of bisphenol and phthalates, urinary concentrations were only measured once per trimester. Furthermore, replacement of the phthalate and bipshenol compounds below LOD by the LOD$\sqrt{2}$ might have reduced variability and the ability to detect associations for compounds with a high percentage below LOD. Overall, the percentages of metabolites below LOD were reasonable (<35%), except for bisphenol S and F. Phthalate and bisphenol concentration in our study cohort are comparable to exposure in the common population (Philips et al., 2018; Colorado-Yohar et al., 2021; U.S. Environmental Protection, 2021; Lehmler et al., 2018). Therefore, the results of our study are representative for the associations in the common population. Due to the low numbers of cryptorchidism and hypospadias in our study population, we were not able to analyze these reproductive developmental abnormalities separately. We did not assess reproductive developmental abnormalities in female infants as these are not measured by default at the child health centers and have less short-term consequences as compared to male infant abnomalities. Previous studies on self-reported Tanner Stages have shown that children in earlier stages of puberty tend to overestimate their pubertal stage, while children in later stages tend to underestimate their pubertal stage (Schlossberger et al., 1992; Jean-Claude et al., 2006). We aimed to reduce this bias by creating a private environment and by clear explanation, but this might have led to misclassification bias of the outcome. Last, although we corrected for many potential confounders, residual confounding due to the observational nature of the study might have occurred, for instance by other endocrine-disrupting environmental exposures.

5. Conclusion

Higher maternal urinary concentrations of phthalates and bisphenols throughout pregnancy are associated with alterations in offspring reproductive development, with the strongest effecs among boys. These effects are independent of maternal and child's socio-demographic and lifestyle characteristics. Further studies are needed to replicate our findings and to evaluate the potential mechanisms. Preventive measures reducing maternal exposure to phthalates and bisphenols might contribute to improving long-term health ouctomes in offspring, as earlier pubertal development is related to higher risks for others metabolic syndrome, cardiovascular disease and breast and testicular cancers.

Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119745.

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