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Association of prenatal exposure to phthalates and synthetic phenols with pubertal development in three European cohorts

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ABSTRACT

Background: There is limited epidemiological evidence on the association of prenatal exposure to phthalates and synthetic phenols with altered pubertal timing.

Objective: To examine the association of prenatal exposure to phthalates, bisphenol A (BPA), parabens, benzophenone 3 (BP-3), and triclosan (TCS) with pubertal development in girls and boys from three European cohorts. **Methods:** Urinary metabolites of six different phthalate diesters (DEP, DiBP, DnBP, BBzP, DEHP, and DiNP), BPA, methyl- (MePB), ethyl- (EtPB), propyl- (PrPB), and butyl-paraben (BuPB), BP-3, and TCS were quantified in one or two (1st and 3rd trimester) urine samples collected during pregnancy (1999–2008) from mothers in three birth cohorts: INMA (Spain), EDEN (France), and MoBa (Norway). Pubertal development of their children was assessed at a single visit at age 7–12 years (579 girls, 644 boys) using the parent-reported Pubertal Development Scale (PDS). Mixed-effect Poisson and g-computation and Bayesian Kernel Machine Regression (BKMR) were employed to examine associations of individual and combined prenatal chemical exposure, respectively, with the probability of overall pubertal onset, adrenarche, and gonadarche (stage 2+) in girls and boys. Effect modification by child body mass index (BMI) was also assessed.

Results: Maternal concentrations of the molar sum of DEHP and of DiNP metabolites were associated with a slightly higher probability of having started puberty in boys (relative risk, RR [95% CI] = 1.13 [0.98–1.30] and 1.20 [1.06–1.34], respectively, for a two-fold increase in concentrations), with a stronger association for DiNP in boys with overweight or obesity. In contrast, BPA, BuPB, EtPB, and PrPB were associated with a lower probability of pubertal onset, adrenarche, and/or gonadarche in all boys (e.g. overall puberty, BPA: RR [95% CI] = 0.93 [0.85–1.01] and BuPB: 0.95 [0.90–1.00], respectively), and the association with BPA was stronger in boys with underweight/normal weight. In girls, MEHP and BPA were associated with delayed gonadarche in those with underweight/normal weight (RR [95% CI] = 0.86 [0.77–0.95] and 0.90 [0.84–0.97], respectively). Most of

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these associations were trimester specific. However, the chemical mixture was not associated with any pubertal outcome in boys or girls.

Conclusions: Prenatal exposure to certain phthalates and synthetic phenols such as BPA may impact the pubertal development of boys, and weight status may modify this effect. BPA may also alter the pubertal development of girls.

1. Introduction

Various studies have described a change in the age at puberty onset over recent decades, reporting a marked secular trend towards younger age at menarche and suggestive trend towards earlier thelarche in girls and a possible trend towards earlier puberty onset in boys (Eckert-Lind et al., 2020; Euling et al., 2008; Papadimitriou, 2016; Sørensen et al., 2012). There is evidence that exposure during prenatal and postnatal developmental windows to endocrine-disrupting chemicals (EDCs), particularly those interfering with estrogen and androgen signaling pathways, may play a role in accelerating or even delaying puberty (Buck Louis et al., 2008; Lopez-Rodriguez et al., 2021; Uldbjerg et al., 2022); however, the impact of mixtures of EDCs remains largely undocumented (Uldbjerg et al., 2022). Both early and late puberty has been linked to adverse health outcomes, including psychological difficulties during adolescence and an increased risk of cardiometabolic disorders and endocrine-related cancers in later life (Golub et al., 2008).

Phthalates and synthetic phenols such as bisphenol A (BPA) and parabens are synthetic EDCs commonly encountered in everyday consumer products (Nakiwala et al., 2020). Phthalates are used as softeners of plastics, primarily polyvinyl chloride (PVC), and as fragrance retainers (Hauser and Calafat, 2005). BPA is used in the production of polycarbonate, a hard plastic present in many consumer products, and it is also found in epoxy resins used for the inner coatings of some metal-based food and beverage cans (Geens et al., 2012); however, its utilization use in food contact materials and other products has recently been drastically restricted in the EU (EFSA, 2023). Parabens commonly serve as preservatives in pharmaceuticals, personal care products, and processed foods (Sanchis et al., 2020). Other synthetic phenols include benzophenone-3 (BP-3), a sunscreen agent added to a wide range of personal care and plastic products (Han et al., 2016), and triclosan (TCS), an antibacterial compound frequently used in personal care, industrial, and household products (Dann and Hontela, 2011). Human biomonitoring data describe the highly frequent detection of these chemicals in urine samples from the general European population, including pregnant women and children (Bräuner et al., 2022; Casas et al., 2013; Haug et al., 2018; Rolland et al., 2020; Sarzo et al., 2022; Vogel et al., 2023).

Experimental studies have demonstrated that phthalates and synthetic phenols disrupt hormone action via anti-androgenic and/or estrogenic mechanisms (Duh-Leong et al., 2023). In rodents, *in utero* or early life exposure to diethylhexyl phthalate (DEHP) was associated with advanced ovarian development and estrous cycle in female rats (Ma et al., 2006; Wang et al., 2016) and either advanced or delayed pubertal onset in male rats (Ge et al., 2007; Guerra et al., 2023; Noriega et al., 2009). Prenatal, neonatal, and postnatal exposure to high BPA doses was associated with earlier vaginal opening in females (Dong et al., 2022; Lusa-Ward et al., 2012; Wang et al., 2023) but with delayed preputial separation in males (Tan et al., 2003; Tyl et al., 2002). Other studies in female rats observed delayed vaginal opening after prepubertal exposure to parabens (Vo et al., 2010) and either advanced or delayed (Rodriguez and Sanchez, 2010; Stoker et al., 2010) vaginal opening after prepubertal exposure to TCS.

In humans, various studies have addressed the association of exposure to phthalates with pubertal timing (Uldbjerg et al., 2022), but few have examined the impact of prenatal exposures, with conflicting results. Specifically, earlier onset of menarche, breast development (thelarche), and/or pubic hair growth (pubarche) in girls was related to

prenatal exposure to DEHP and other phthalates (Cathey et al., 2020; Harley et al., 2019; Hart et al., 2014; Watkins et al., 2014, 2017a), although some authors reported opposite or null associations (Berman et al., 2021; Berger et al., 2018). Among boys, studies of prenatal phthalate exposure variously reported either earlier gonadal development (gonadarche) and pubarche (Berger et al., 2018), delayed adrenal development (adrenarche) or pubarche (Ferguson et al., 2014; Watkins et al., 2017b), or null associations (Cathey et al., 2020; Harley et al., 2019). Prenatal BPA exposure was previously shown to be associated with delayed thelarche, pubarche, and menarche in girls (Berger et al., 2018), earlier gonadarche and pubarche in boys (Berger et al., 2018), and delayed adrenarche in boys (Ferguson et al., 2014), while other researchers found no association with pubertal markers in either girls or boys (Watkins et al., 2014, 2017a, 2017b). Regarding other synthetic phenols, only one study addressed the association between prenatal exposure and puberty, reporting a relationship between *in utero* TCS exposure and earlier menarche but no association with parabens and BP-3 exposure in either sex (Harley et al., 2019). Pre/peripubertal exposure of girls to parabens, TCS, and BP-3 was associated with either earlier (Binder et al., 2018; Giannini et al., 2022; Harley et al., 2019; Zhou et al., 2023) or delayed puberty (Wolff et al., 2010, 2014, 2017) and peripubertal exposure of boys to BP-3 or parabens with delayed puberty (Zhou et al., 2023), whereas one group found no association between childhood exposure to BP-3 or parabens with puberty timing in either sex (Harley et al., 2019).

Our group previously addressed the association between prenatal phthalate exposure and pubertal development in Spanish children. We observed an association between maternal urinary metabolites of individual phthalates (including di-n-butyl phthalate [DnBP] and DEHP) and earlier puberty onset, especially in boys of normal weight, but no association between the phthalate mixture and advanced or delayed puberty in either boys or girls (Freire et al., 2022). The aim of the present investigation in children from three European cohorts was to extend our previous research by assessing the potential effect on pubertal development of prenatal exposure to phthalates, BPA, parabens, BP-3, and TCS, considered both individually and as a mixture. A secondary objective was to examine the role of weight status in this association, given that obesity is a strong predictor of pubertal development (Kaplowitz, 2008; Reinehr and Roth, 2019) and has been reported to modify the association between phthalates/phenols and puberty (Berger et al., 2018; Binder et al., 2018; Wolff et al., 2014), and because child weight status may be on the causal pathway (Berman et al., 2021; Güil-Oumrait et al., 2022; Harley et al., 2017).

2. Material and methods

2.1. Study population

Participants were selected from three ongoing longitudinal population-based birth cohorts established in three European countries: INMA (Environment and Childhood) in Spain (Guxens et al., 2012); Étude des Déterminants Pré et Postnatals du Développement et de la Santé de l'Enfant (EDEN) in France (Heude et al., 2016); and Norwegian Mother, Father, and Child Cohort Study (MoBa) in Norway (Magnus et al., 2016); these cohorts cover singleton deliveries from 1999 to 2009 (Table 1). Maternal urinary phthalate and environmental phenol biomarkers were assessed in the EDEN and MoBa cohorts and in Gipuzkoa, Sabadell, and Valencia INMA subcohorts. A total of 207 children in

Table 1
Characteristics of mother-child pairs from three European birth cohorts.

Characteristics	n (%) or mean \pm SD		
	All (N = 1223)	Girls (N = 579)	Boys (N = 644)
Cohort (recruitment years, age at puberty assessment)			
INMA-Gipuzkoa (2006–2008, 7.7–8.5 years)	231 (18.9)	114 (19.7)	117 (18.2)
INMA-Sabadell (2004–2006, 7.8–10.7 years)	318 (26.0)	149 (25.7)	169 (26.2)
INMA-Valencia (2004–2005, 8.5–9.8 years)	229 (18.7)	110 (19.0)	119 (18.5)
EDEN (2003–2006, 9.3–12.1 years)	193 (15.8)	83 (14.3)	110 (17.1)
MoBa (1999–2009, 8.0–9.8 years)	252 (20.6)	123 (21.2)	129 (20.0)
Pregnancy and birth characteristics			
Mother's educational attainment during pregnancy			
Up to primary	178 (14.6)	82 (14.2)	96 (14.9)
Secondary	425 (34.8)	195 (33.7)	230 (35.7)
University	620 (50.7)	302 (52.2)	318 (49.4)
Parity at delivery			
None	657 (53.7)	311 (53.7)	346 (53.7)
1	453 (37.0)	209 (36.1)	244 (37.9)
2 or more	113 (9.2)	59 (10.2)	54 (8.4)
Maternal pre-pregnancy BMI			
Underweight	68 (5.6)	28 (4.8)	40 (6.2)
Normal weight	855 (69.9)	419 (72.4)	436 (67.7)
Overweight/obese	300 (24.5)	132 (22.8)	168 (26.1)
Birth weight (g)	3335 \pm 477	3259 \pm 447	3404 \pm 493
Low birth weight (< 2500 g)	45 (3.7)	26 (4.5)	19 (3.0)
Gestational age (weeks)	39.8 \pm 1.6	39.8 \pm 1.5	39.8 \pm 1.6
Preterm birth (< 37 weeks)	44 (3.6)	18 (3.1)	26 (4.0)
Breastfeeding duration (weeks)			
0	221 (18.1)	98 (16.9)	123 (19.1)
>0-16	312 (25.5)	158 (27.3)	154 (23.9)
>16-24	344 (28.1)	159 (27.5)	185 (28.7)
>24	346 (28.3)	164 (28.3)	182 (28.3)
Child/mother characteristics at puberty assessment			
Child age (years)			
7	9.0 \pm 1.0	8.9 \pm 1.0	9.0 \pm 1.0
8	227 (18.6)	112 (19.3)	116 (18.0)
9	467 (38.2)	229 (39.6)	236 (36.7)
≥ 10	316 (25.8)	149 (25.7)	170 (26.4)
≥ 10	213 (17.4)	89 (15.4)	122 (18.9)
Mother lives with child's father	1047 (85.6)	558 (86.6)	489 (85.4)
Child weight status^a			
Overweight/normal weight	803 (65.7)	381 (66.8)	416 (64.6)
Overweight	201 (16.4)	96 (16.6)	105 (16.3)
Obese	219 (17.9)	96 (16.6)	123 (19.1)

^a Obtained by converting body mass index (BMI) to z-score for age and sex based on WHO reference curves for children (5–19 years): normal weight (± 1 standard deviation), overweight/obese ($> +1$ standard deviation, equivalent to $\text{BMI} \geq 25 \text{ kg/m}^2$ at 19 years).

EDEN (11% of births), 272 children in MoBa (2.5%), and 1298 children in INMA subcohorts (58%) participated, as part of the HELIX Project, in a harmonized clinical examination at their respective study centers during 2014–2015 (Maitre et al., 2018), evaluating their pubertal development at 7–12 years of age (INMA-Gipuzkoa: 7.7–8.5 years; INMA-Sabadell: 7.8–10.7 years; INMA-Valencia: 8.5–9.8 years; EDEN: 9.3–12.1 years; MoBa: 8.0–9.8 years). A full description of the follow-up methods and study population, including eligibility criteria and sample size calculations, are available in Agier et al. (2019) and Quanjer et al. (2012). The current study included all children in these cohorts with available data on maternal phthalate and/or phenol biomarkers and pubertal development (N = 1223, 579 girls and 644 boys); “girls” refers to children assigned female at birth and “boys” refers to children assigned male at birth. An informed consent was signed by all mothers during pregnancy and by child guardians at clinical examination, and the research protocol was approved by the Ethic Committees of each country/region.

2.2. Exposure assessment

Maternal urinary concentrations of phthalate metabolites and environmental phenols served as surrogate biomarkers of prenatal exposure. Exposure biomarkers were measured in two spot urine samples collected from INMA-Sabadell mothers at the 1st and 3rd trimesters, in pooled samples (equal volumes of samples from 1st and 3rd trimester) from INMA-Gipuzkoa mothers, and in two spot urine samples (phenols) or pooled samples (phthalates) from mothers in INMA-Valencia (INMA subcohorts: 1st trimester: mean \pm standard deviation [SD] = 13.2 ± 1.5 weeks, 3rd trimester: mean \pm SD = 33.1 ± 2.0 weeks). In EDEN and MoBa cohorts, exposure biomarkers were measured in one spot urine sample collected at 2nd trimester of pregnancy (EDEN: mean \pm SD = 26.1 ± 1.24 weeks, MoBa: mean \pm SD = 18.8 ± 0.92 weeks). Urine samples were collected under non-fasting conditions in 100-mL polypropylene containers and then aliquoted in 10 mL polyethylene tubes and stored at -20°C until analysis. In INMA-Sabadell (phthalates and phenols) and INMA-Valencia (phenols) subcohorts, the mean value of the two urine samples was used as biomarker of *in utero* exposure.

Urinary monoester metabolites of three low-molecular-weight phthalates (diethyl phthalate [DEP], di-iso-butyl phthalate [DiBP], and DnBP) and two high-molecular-weight phthalates (butyl-benzyl phthalate [BBzP] and DEHP) were measured in the mothers of all 1223 children, including: mono-ethyl phthalate (MEP), metabolite of DEP; mono-iso-butyl phthalate (MiBP), metabolite of DiBP; mono-n-butyl phthalate (MnBP), metabolite of DnBP; mono-benzyl phthalate (MBzP), metabolite of BBzP; and mono-(2-ethyl-hexyl) phthalate (MEHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono (2-ethyl-5-carboxypentyl)phthalate (MECPP), primary metabolites of DEHP. Mono-hydroxy-isononyl phthalate (OH-MiNP) and mono-oxo-isononyl phthalate (oxo-MiNP), metabolites of the high-molecular-weight phthalate di-iso-nonyl phthalate (DiNP), were also measured in maternal urine from INMA-Gipuzkoa, INMA-Valencia, EDEN, and MoBa cohorts (N = 905). Phthalate measurements in urine samples from Gipuzkoa, Valencia, EDEN, and MoBa were performed at the Norwegian Institute of Public Health (NIPH) (Oslo, Norway) by liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) (Sabaredzovic et al., 2015). The integration of DiNP metabolites in Gipuzkoa and Valencia differed from that in EDEN and MoBa, with all isomers integrated together in INMA samples but only single isomers integrated in EDEN and MoBa samples. Samples from Sabadell were analyzed at the Hospital del Mar Medical Research Institute (IMIM, Barcelona, Spain) by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) according to Valvi et al. (2015). Limits of detection (LODs) ranged from 0.07 to 1.0 $\mu\text{g/L}$ (Freire et al., 2022). Measurements of urinary phthalate metabolite concentrations were highly correlated (Spearman coefficient ranging from $r = 0.69$ to 0.97) between the laboratories, as previously reported (Tamayo-Uria et al., 2019).

Parabens (methyl paraben [MePB], ethyl paraben [EtPB], propyl paraben [PrPB], and butyl paraben [BuPB]), BPA, and BP-3 were measured in pooled samples from INMA-Gipuzkoa at the Instituto de Investigación Biosanitaria IBS GRANADA (Granada, Spain) using dispersive liquid-liquid microextraction and UHPLC-MS/MS, with LODs ranging from 0.04 to 0.12 $\mu\text{g/L}$ (Vela-Soria et al., 2014). TCS was not measured in samples from Gipuzkoa. Parabens, BPA, BP-3, and TCS were measured in samples from INMA-Sabadell, INMA-Valencia, and MoBa at NIPH using online solid-phase extraction followed by UHPLC-MS/MS (Sakhi et al., 2018), with LODs ranging from 0.03 to 0.07 $\mu\text{g/L}$. BPA was measured in samples from INMA-Sabadell mothers by the Department of Analytical Chemistry of the University of Cordoba (Spain) using HPLC-MS/MS (Casas et al., 2013), with a LOD of 0.10 $\mu\text{g/L}$. MePB and EtPB were not measured in samples from MoBa, because these chemicals were added as preservatives in the collection vials used for this cohort. Phenols were measured in samples from the EDEN cohort at the CDC's National Center for Environmental Health laboratory in

Washington DC (LODs: 0.2–2.3 µg/L) (Philippat et al., 2012).

The Jaffé method was used to measure urine creatinine in the samples from all cohorts. Dilution was based on the mean creatinine level of 1st and 3rd trimester samples from Sabadell (phthalates and phenols) and Valencia (phenols). The mean of the two individual creatinine measurements was used for pooled samples. Measurements in 20 urine samples from Valencia showed a high correlation between the creatinine level in the pool and the mean level of the two independent urine samples ($r = 0.97$).

2.3. Puberty assessment

During the clinical examination at the age of 7–12 years, parents (98% mothers) rated the pubertal status of their children using the Peterson's Pubertal Development Scale (PDS), widely applied as an alternative to physician rating measures (Petersen et al., 1988). The PDS has five items that are common to both sexes on growth spurt (height), pubic hair onset, and skin changes (pimples), two items for boys on facial hair growth and voice deepening and two for girls on breast development and menarche. The parent responds on a four-point scale from 1 (no development started) to 4 (development appears complete). Following the criteria proposed by Carskadon and Acebo (1993) and Shirtcliff et al. (2009), the continuous PDS score was converted into a 5-point ordinal scale (1-pre-puberty, 2-early puberty, 3-mid-puberty, 4-late puberty, and 5-post-puberty). The Carskadon and Acebo (1993) algorithm calculates the pubertal development from the item on body hair growth and the two sex-specific items. The Shirtcliff et al. (2009) algorithm is based on all five PDS items and differentially captures gonadal and adrenal indicators of physical development, associating breast development, menarche, and growth spurt with gonadal hormonal changes in girls and testicular enlargement, growth spurt, voice deepening, and facial hair growth with these changes in boys. In both sexes, pubic/body hair and skin changes are associated with adrenal hormonal changes (Shirtcliff et al., 2009). Three outcomes were examined in the present study: overall pubertal development by Carskadon and Acebo algorithm and adrenal development (adrenarche) and gonadal development (gonadarche) by Shirtcliff et al. algorithm, categorizing each outcome as stage 1 (pre-puberty) or stage 2+ (puberty initiated).

2.4. Statistical analysis

Phthalate metabolite and phenol values below the LOD (<5%) were imputed using a quantile regression approach for left-censored missing data. The molar-weight (mw) adjusted sum of the urinary metabolites of DEHP was calculated by dividing each metabolite concentration by its molecular weight and summing, *i.e.*, the sum of DEHP metabolites ($\sum \text{DEHP}$) was $(\text{MEHP}/\text{mw}) + (\text{MEHHP}/\text{mw}) + (\text{MEOHP}/\text{mw}) + (\text{MECPP}/\text{mw})$. The same procedure was applied for the two metabolites of DiNP, *i.e.*, $\sum \text{DiNP} = (\text{OH-MiNP}/\text{mw}) + (\text{oxo-MiNP}/\text{mw})$. The sum of concentrations was expressed in µg/L by multiplying the molar sums with the molecular weight of the respective parent compound. Spearman correlation coefficients were used to examine correlations between chemicals. Missing data on covariates (<5%) were imputed (simple imputation) by using the mode for categorical variables and the median for continuous variables.

The association of each chemical biomarker (individual phthalate metabolites, $\sum \text{DEHP}$, $\sum \text{DiNP}$, and phenolic compounds) with the probability of stage 2+ classification (single-exposure associations) was assessed for each pubertal outcome, separately in girls and boys, by applying mixed-effect Poisson regression analysis with robust error variance (Zou, 2004); the cohort of origin was entered as random variable to correct for potential heterogeneity across study centers, with exposure biomarkers and the remaining covariates as fixed variables. Concentrations of phthalate metabolites and phenols were right-skewed and therefore natural log-transformed before inclusion in models.

Confounders were selected *a priori* using a directed acyclic graph (DAG) and included cohort, maternal schooling (up to primary, secondary, university), and maternal pre-pregnancy BMI (continuous, in kg/m²) (Fig. S1) (Berger et al., 2018; Harley et al., 2019). Models also included child age at puberty assessment (continuous, in years) and natural log-transformed concentrations of maternal urinary creatinine to correct for urine dilution (O'Brien et al., 2016). Covariates were entered in the main model (*i.e.*, single exposure non-stratified model for the association of each chemical biomarker with overall pubertal development) in two steps: model 1 included child age, cohort, and maternal urinary creatinine; and model 2 (main model) also included maternal schooling and pre-pregnancy BMI. The BMI z-score of the child (continuous, standardized for age and sex according to the World Health Organization [WHO, 2023]) was further included in the model (model 3) to assess possible mediation by BMI and to estimate the direct effect of phthalates/phenols on pubertal development. Regression estimates were transformed to represent the ratio of the probability (relative risk, RR) of being in stage 2+ for a two-fold increase in prenatal urinary phthalate metabolite or phenol concentrations with corresponding 95% confidence interval (CI). In a secondary analysis, associations with overall pubertal progression were examined in girls, considering three categories (stage 1, 2, and 3+) and using multinomial logistic regression. This analysis was not conducted for boys due to the small number in stage 3+.

The chemical mixture effect was assessed by quantile g-computation and Bayesian Kernel Machine Regression (BKMR) (Bobb et al., 2015) using the 'qgcomp' (<https://cran.r-project.org/web/packages/qgcomp/index.html>) and the 'bkmr' package (<https://cran.r-project.org/web/packages/bkmr/index.html>) in R, respectively. Mixture models assessed the effect of the mixture of 12 chemicals determined in all of the girls ($N = 579$) and boys ($N = 644$) (*i.e.*, MEP, MiBP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, BuPB, PrPB, BPA, and BP-3). G-computation regression was based on 1000 bootstraps, categorizing urinary phthalate and phenol exposure biomarkers in quartiles. Each exposure biomarker was assigned a weight representing its relative contribution to the estimated effect in a positive or negative direction (Keil et al., 2020). If the individual chemical showed a different direction of the effect, the weight was interpreted as the proportion of the partial effect in the negative or positive direction. G-computation regression estimates were expressed as the RR of puberty onset per quartile increase in mixture concentration with 95% CI. Utilizing the BKMR approach, pubertal outcomes were regressed on a flexible function of the mixture components specified by a kernel function, using the Markov chain Monte Carlo method with 10,000 iterations. In all models, the posterior inclusion probability (PIP) was calculated for each chemical as well as the overall effect of the mixture on each pubertal outcome. The latter was calculated by comparing the estimated flexible function of the chemicals (denoted by h) when all chemicals are at a particular percentile with the h value when they are all at 50th percentile. Visual inspection was used to assess convergence. Both g-computation and BKMR models were adjusted for the same covariates as those in single-exposure model 2.

Effect modification by child weight status was explored by using interaction terms in single exposure models and stratifying single and multiple (g-computation and BKMR) exposure models according to the BMI (underweight/normal weight vs. overweight/obese). A sensitivity analysis was conducted by analyzing trimester-specific associations (1st and 3rd trimester) with phthalates except for DiNP (INMA-Sabadell) and synthetic phenols (INMA-Sabadell and INMA-Valencia). Because the integration of OH-MiNP and oxo-MiNP in Gipuzkoa and Valencia was different from that in EDEN and MoBa, the association with DiNP biomarkers was further analyzed separately for INMA (Freire et al., 2022) and EDEN/MoBa, also using g-computation regression to conduct mixture-effect analysis separately for INMA and EDEN/MoBa. SPSS version 28 (IBM, Armonk, NY) and R version 4.3.0 were used for statistical analyses.

3. Results

Among all 1223 children, 231 belonged to INMA-Gipuzkoa, 318 to INMA-Sabadell, 229 to INMA-Valencia, 193 to EDEN, and 252 to MoBa cohorts. Half of the mothers had a university education, and the majority were primiparous, had a normal weight before pregnancy, and breastfed the index child for at least 4 months, with almost all of them delivering a normal-weight full-term baby. At the clinical examination, 83% of the children were 7–9 years old and only 6% were 11 years or older; 34% were overweight or obese; and >85% of them lived with both parents (Table 1). Mothers from EDEN and MoBa had a higher education level, and their children were less likely to be overweight in comparison to those from INMA, while children from MoBa were breastfed for a longer period of time (Table S1).

All phthalate metabolites and phenols under study were detected in more than 95% of urine samples, and the majority were detected in 98–100% of samples (Table 2). MEP, metabolite of the phthalate DEP, and the paraben congener MePB showed the highest urinary concentrations (median = 150.93 and 148.13 µg/L, respectively), followed by PrPB (median = 32.98 µg/L), MECPP (median = 29.90 µg/L), MiBP (median = 28.40 µg/L), and MnBP (median = 25.98 µg/L), while the lowest concentrations were for DiNP metabolites (Table 2). Urinary concentrations of phthalate metabolites (except OH-MiNP and oxo-MiNP: 3- to 5-fold lower in EDEN and MoBa samples due to the distinct integration approach) and phenols were higher in samples from INMA-Sabadell, INMA-Valencia, and EDEN mothers than in those from INMA-Gipuzkoa and MoBa mothers, observing especially high phenol concentrations in samples from the INMA-Sabadell and INMA-Valencia mothers (Table S2). In general, moderate positive correlations ($r = 0.20$ – 0.60) were observed between phthalate biomarkers, while slight to moderate positive correlations ($r = 0.10$ – 0.50) were observed between phenols and between phthalates and phenols. However, non-significant correlations were observed between TCS and both BPA and BuPB,

Table 2

Concentrations (µg/L) of phthalate metabolites and phenols in maternal urine^a of mothers from three European cohorts.

Exposure biomarkers	Total sample of girls and boys (N = 1223)			
	%>LOD	25th percentile	Median	75th percentile
Phthalates				
MEP	100	66.36	150.93	393.46
MiBP	100	15.45	28.40	53.53
MnBP	99.9	12.96	25.98	48.39
MBzP	100	3.99	9.87	21.68
MEHP	99.9	4.23	7.38	13.14
MEHHP	100	10.66	19.70	36.67
MEOHP	100	7.51	13.95	25.68
MECPP	100	18.24	29.90	51.36
∑DEHP	–	49.07	87.28	152.53
OH-MiNP ^b	97.5	0.72	1.46	3.19
oxo-MiNP ^b	98.5	0.72	1.53	3.40
∑DiNP ^b	–	2.00	4.15	9.02
Synthetic phenols				
BPA	96.3	1.68	3.11	5.63
MePB ^b	100	52.39	148.13	414.63
EtPB ^b	99.6	2.90	10.39	30.61
PrPB	99.6	9.32	32.98	97.06
BuPB	96.2	0.69	2.95	9.10
BP-3	99.4	1.15	3.76	21.33
TCS ^b	98.8	2.43	16.64	92.87

LOD: Limit of detection.

^a Average concentration in two urine samples collected during pregnancy in Sabadell (1st and 3rd trimester); concentration in pooled samples (1st and 3rd trimester) for mothers in Gipuzkoa; average concentration in two urine samples (phenols) or concentration in pooled samples (phthalates) for mothers in Valencia; or concentration in urine samples from the 2nd trimester (EDEN and MoBa).

^b Sub-sample: DiNP metabolites, N = 905; MePB and EtPB, N = 980; TCS, N = 992.

between parabens and most phthalates, between BP-3 and both DEHP and DiNP metabolites, and between BPA and DiNP metabolites (Table S3).

Among girls, 42% had started puberty, and 31% and 40% had reached adrenarche and gonadarche, respectively. Among boys: 26%, 14%, and 24%, respectively (Table 3). Few children were in stages 3–5 of overall pubertal, adrenal, and gonadal development, although 20% of girls were in stage 3 of overall pubertal development (Table 3). As expected, cohorts with older children (EDEN, INMA-Sabadell, INMA-Valencia), especially the girls, were more likely to have started puberty (Fig. 1; Table S4).

Single exposure models for overall puberty development are exhibited in Fig. 2 and Table S5. Among girls, urinary OH-MiNP, oxo-MiNP, and ∑DiNP were associated with a slightly higher probability of having started puberty in the basic model (model 1), but these associations were progressively attenuated after adjustment for maternal education and pre-pregnancy BMI (model 2) and child BMI (model 3) (Table S5); in addition, no significant associations with overall puberty progression were observed in girls in the three-category analysis (Table S6). Among boys, ∑DiNP and ∑DEHP were associated with a slightly higher probability of having started puberty (model 2: RR [95% CI] = 1.20 [1.06–1.34] and 1.13 [0.98–1.30], respectively), and BPA and BuPB with a slightly lower probability of puberty (model 2: RR [95% CI] = 0.93 [0.85–1.01] and 0.95 [0.90–1.00], respectively) (Fig. 2). Some associations in boys were slightly attenuated after the inclusion of child BMI (model 3), but results remained essentially unchanged (Table S5). The association with ∑DiNP was slightly stronger in boys with overweight/obesity vs. underweight/normal weight (RR [95% CI] = 1.24 [1.02–1.48] vs. 1.14 [0.99–1.34], $P_{int} = 0.20$), while the association with BPA was stronger in boys with underweight/normal weight (RR [95% CI] = 0.89 [0.79–0.99] vs. 1.00 [0.87–1.16], $P_{int} = 0.48$) (Fig. S2). BMI-stratified models also showed a significant interaction ($P_{int} = 0.008$) between TCS and weight status, such that TCS was associated with a slightly higher probability of pubertal onset in boys with overweight/obesity (RR [95% CI] = 1.08 [1.01–1.15]) but not in those with underweight/normal weight (RR [95% CI] = 0.89 [0.90–1.06]), and a marginally significant association between BP-3 and lower probability of puberty was only observed in boys with

Table 3

Pubertal development^a of children from three European cohorts at age 7–12 years.

Pubertal outcomes	Girls (n = 579)		Boys (N = 644)	
	N	%	N	%
Pubertal onset (stage 2+)	242	41.8	168	26.1
Overall pubertal development				
Stage 1	337	58.2	476	73.9
Stage 2	120	20.7	150	23.3
Stage 3	118	20.4	18	2.8
Stage 4	4	0.7	0	0
Adrenarche onset (stage 2+)	177	30.6	88	13.7
Adrenal development				
Stage 1	402	69.4	556	86.3
Stage 2	112	19.3	71	11.0
Stage 3	56	9.7	14	2.2
Stage 4	9	1.6	3	0.5
Gonadarche onset (stage 2+)	231	39.9	156	24.2
Gonadal development				
Stage 1	348	60.1	488	75.8
Stage 2	157	27.1	123	19.1
Stage 3	71	12.3	32	5.0
Stage 4	2	0.3	1	0.2
Stage 5	1	0.2	0	0

Stage 1: pre-puberty; stage 2: early puberty; stage 3: mid-puberty, stage 4: late puberty; stage 5: post-puberty.

^a Based on the Petersen's Pubertal Development Scale (PDS): overall pubertal development (Carskadon and Acebo algorithm); adrenal and gonadal development (Shirtcliff et al. algorithm).

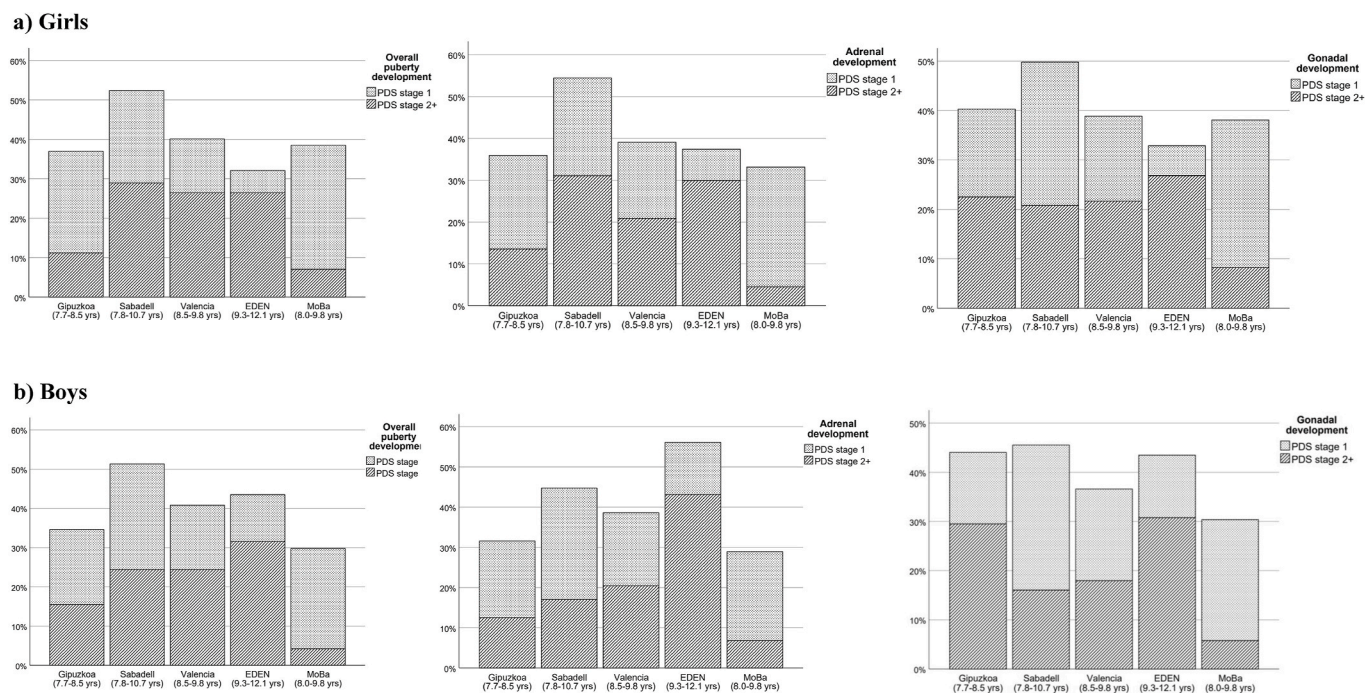


Fig. 1. Title: Percentage of children in stage 2+ per study center.

underweight/normal weight (RR [95% CI] = 0.94 [0.87–1.01]) (Fig. S2).

Separate analysis of adrenal and gonadal development (Fig. 3) revealed the following significant or marginally significant associations in models adjusted for child BMI: between MEP and lower probability of gonadarche in girls and boys; between MiBP and higher probability of adrenarche; between MnBP and lower probability of gonadarche in boys; between MEHP and lower probability of gonadarche in girls and adrenarche in boys; between BPA and lower probability of gonadarche in girls; between TCS and small increase in the probability of adrenarche in girls; and between BPA, EtPB, PrPB, BuPB, and BP-3 and lower probability of adrenarche and/or gonadarche in boys. Associations in girls were stronger in those with underweight/normal weight, and associations of phenols with adrenal and/or gonadal development in boys were generally stronger in those with underweight/normal weight, whereas associations with phthalate metabolites appeared to be stronger in boys with overweight/obesity (Fig. S3).

Despite the limited sample size, results for DiNP metabolites and pubertal development in children from EDEN/MoBa cohorts alone were in the same general direction as observed in the main analysis, showing a marginal association of OH-MiNP with higher probability of gonadarche in boys (RR [95%CI] = 1.17 [0.97–1.40]) (Table S7) and of \sum DiNP with higher probability of puberty onset in boys with higher BMI (Table S8). In INMA cohorts, \sum DiNP was also marginally associated with higher probability of puberty in boys (Freire et al., 2022).

In the trimester-specific analysis using data from Sabadell (phthalates and phenols) and Valencia (phenols), associations of MEHP and BPA with delayed gonadarche in girls were only observed for exposures in the 1st trimester, while 1st but not 3rd trimester concentrations of BuPB and TCS were associated with earlier puberty in girls. In boys, however, associations between DEHP metabolites, \sum DEHP, MiBP, and MnBP and earlier puberty and between phenols (BPA, MePB, BuPB) and delayed puberty were only observed for exposures in the 3rd trimester (Tables S9 and S10).

G-computation analysis did not reveal any significant association between the chemical mixture and pubertal development in boys or girls or in the BMI-stratified analysis (Fig. 4). In BKMR models, PIPs were low for all chemicals with the exception of PrPB and gonadal development in

boys (Fig. S4), and the results evidenced no significant effect of the mixture on any pubertal outcome in the main analysis (Fig. 5) or in the BMI-stratified analysis (Figs. S5 and S6). The separate mixture analysis of INMA and EDEN/MoBa also showed no significant results (Table S11).

4. Discussion

In this study of European birth cohorts, prenatal concentrations of urinary biomarkers of certain phthalates were associated with earlier pubertal development and biomarkers of synthetic phenols with delayed adrenarche and/or gonadarche in boys. Although these associations might not be mediated by child BMI, some were modified by child weight status, finding that earlier puberty was more strongly associated with DiNP exposure in boys with overweight/obese but more strongly associated with BPA and other phenols in those with underweight/normal weight. As exceptions, MnBP and MEHP were associated with delayed gonadarche and adrenarche, respectively, in boys in both weight categories, while TCS was associated with earlier puberty in boys with underweight/normal weight alone. In girls, MEHP and BPA were associated with delayed gonadarche in those with lower BMI. In a subsample of children, associations were mostly seen for exposures in the 1st trimester in girls and in the 3rd trimester in boys, suggesting difference between the sexes in vulnerable developmental windows. However, the mixture analysis suggested no overall effect on pubertal development in boys or girls.

The association of DEHP and DiNP with overall pubertal development in boys observed in single exposure models is in partial agreement with our previous study in the INMA cohorts (Freire et al., 2022) and with findings in the CHAMACOS cohort. This Californian study reported that mean maternal urinary concentrations of MCOP (metabolite of DiNP) and \sum DEHP measured at 1st and 3rd trimesters of pregnancy were associated with earlier gonadarche and pubarche in all boys (aged 9–13 years) in the case of MCOP and in boys with higher BMI in the case of MCOP and \sum DEHP (Berger et al., 2018). In our previous study, \sum DEHP was only associated with earlier gonadarche and adrenarche in normal weight boys (Freire et al., 2022); in the present study, however, \sum DiNP was not associated with adrenarche/gonadarche, \sum DEHP was

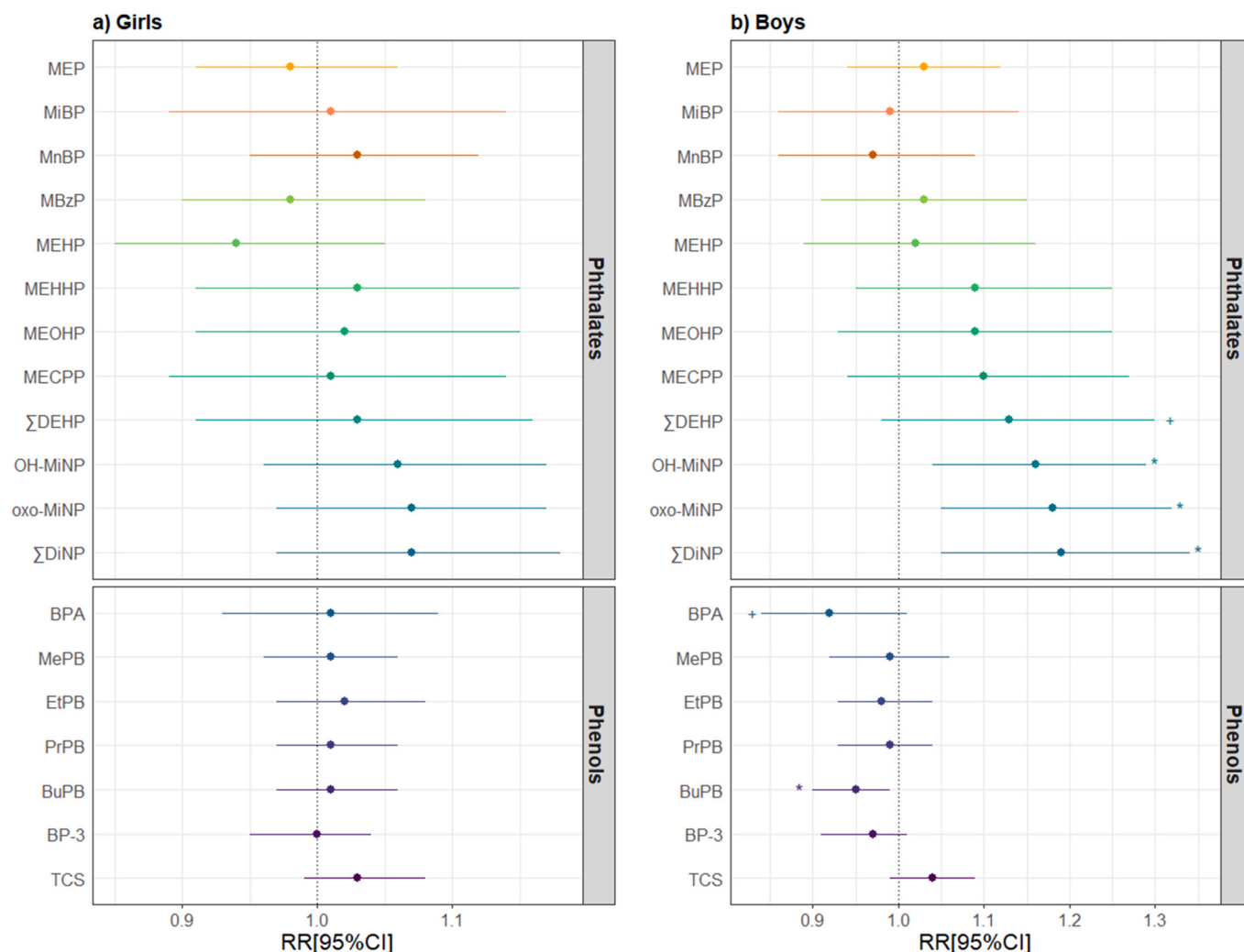


Fig. 2. Title: Single exposure models for the association of prenatal phthalate and phenol exposure with overall pubertal onset in girls and boys (7–12 years old). Legend:

Pubertal onset defined as being in stage 2+ based on the PDS (Carskadon and Acebo algorithm). All models are adjusted for mother's urinary creatinine (natural log-transformed), cohort (random effect), maternal education (up to primary; secondary; university) and pre-pregnancy BMI (continuous), and child age at puberty assessment. RR: Relative risk of puberty onset for a 2-fold increase in prenatal urinary phthalate metabolite or phenol; 95% CI: 95% Confidence interval. * $p < 0.05$, † $p < 0.10$. DiNP metabolites: 430 girls, 475 boys; MePB, EtPB: 465 girls, 515 boys; TCS: 465 girls, 527 boys.

not associated with pubertal onset in the BMI-stratified analysis, and MEHP (one of the DEHP metabolite) was associated with delayed rather than earlier adrenarche in boys. By contrast, a cohort study in Mexico associated maternal urinary concentrations of DEHP metabolites in the 3rd trimester of pregnancy with reduced odds of adrenarche and gonadarche in males (aged 8–14 years) (Ferguson et al., 2014; Watkins et al., 2017b). The association observed between MnBP and delayed gonadarche is opposite to the association previously observed in normal weight boys in the INMA cohorts (Freire et al., 2022); however, it is partially consistent with findings in the Mexican cohort of associations between prenatal MBP and delayed pubarche (Cathey et al., 2020) and lower odds of adrenarche and gonadarche in boys (Ferguson et al., 2014), although prenatal MnBP was not associated with pubertal development in males in the CHAMACOS cohort (Harley et al., 2019). Furthermore, the association of MiBP with earlier adrenarche was not observed in our previous analysis (Freire et al., 2022) and contrasts with the finding in Mexican boys of no association between prenatal MiBP and any pubertal outcome (Ferguson et al., 2014). Results suggesting puberty advancement (*i.e.*, DEHP, DiNP, MiBP) are not consistent with *in vitro* evidence of complete inhibition of the AR by DEHP, DiNP, and DBP (Engel et al., 2017). They are also in disagreement with studies in

mammals of an association between *in utero* exposure during sexual differentiation to DEHP and DBP, among other phthalates, with the disruption of androgen-signaling and androgen-dependent reproductive development, such as the suppression of fetal testosterone production in male rats (Howdeshell et al., 2007, 2008a, 2008b).

Results of single exposure models indicate that the apparent accelerating effect of DiNP on puberty may be stronger in boys with higher BMI, and this was not evidenced in our previous study (Freire et al., 2022). In this line, Berger et al. (2018) found that the association between advanced pubertal timing and *in utero* exposure to DEHP, MCOP, and MBzP was stronger in overweight/obese boys (aged 9–13 years). A possible explanation for this finding is that boys with an excess of adiposity may have a greater predisposition for earlier puberty due to the interaction between leptin and kisspeptin and the peripheral action of adipose tissue (Reinehr and Roth, 2019). However, the tendency towards earlier puberty onset is less clear in boys than in girls with obesity (Reinehr and Roth, 2019). Another hypothesis is that prenatal exposure to DiNP may lead to both childhood obesity and earlier puberty. Nonetheless, prenatal DiNP exposure was not associated with risk of obesity in boys from the CHAMACOS cohort (Harley et al., 2017) and the present data do not suggest mediation because associations appear

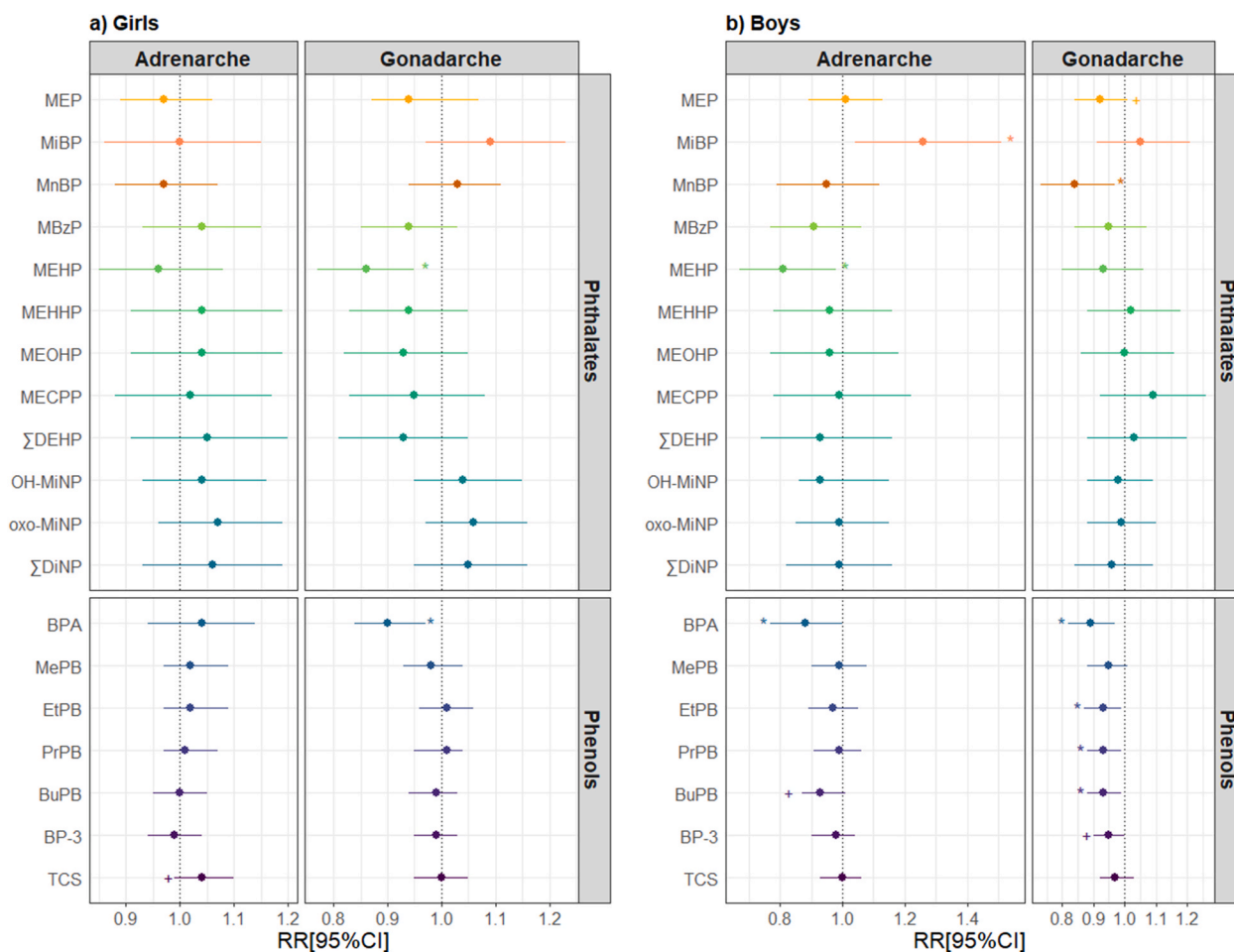


Fig. 3. Title: Single exposure models for the association of prenatal phthalate and phenol exposure with adrenarche and gonadarche in girls and boys.

Legend:

Adrenarche and gonadarche defined as being in stage 2+ based on the PDS (Shirtcliff et al. algorithm). All models are adjusted for mother's urinary creatinine, cohort (random effect), maternal education, and pre-pregnancy BMI, and child age. RR: Relative risk for a 2-fold increase in prenatal urinary phthalate metabolite or phenol; 95% CI: 95% Confidence interval. * $p < 0.05$, † $p < 0.10$. DiNP metabolites: 430 girls, 475 boys; MePB, EtPB: 465 girls, 515 boys; TCS: 465 girls, 527 boys.

independent of child BMI.

In general, the lack of association found in the present and previous study of INMA cohorts (Freire et al., 2022) between phthalate biomarkers and pubertal development in girls contrasts with previous reports associating prenatal exposure to DEHP and other phthalates with earlier puberty in girls aged between 8 and 16 years (Cathey et al., 2020; Harley et al., 2019; Hart et al., 2014; Watkins et al., 2014, 2017a). However, the association of MEHP with delayed gonadarche is partially consistent with the study by Berger et al. (2018), which associated maternal ΣDEHP (mean of 1st and 3rd trimesters of gestation) with later thelarche and menarche in daughters, and with the Mexican study by Watkins et al. (2017a), which associated urinary MEHP across pregnancy with later breast development in offspring. In the present study, prenatal MEHP exposure was associated with delayed gonadarche in girls with underweight/normal weight alone. Likewise, the CHAMACOS study associated prenatal ΣDEHP concentrations with later puberty in normal-weight girls alone (Berger et al., 2018). One possible explanation is that the delay in sexual maturation might be counteracted by obesity, which might produce an opposite effect. However, Berger et al. (2018) also found that MCOP was associated with later pubarche and menarche, mostly in normal-weight girls, whereas a weak association was observed in the present study between prenatal DiNP exposure and higher probability of having started puberty in all girls, which was attenuated after adjusting for their BMI. In addition, the chemical mixture was not associated with pubertal timing in the present girls, as

also found for the phthalate mixture in our previous investigation (Freire et al., 2022). To our knowledge, the combined effect of *in utero* phthalate exposure on pubertal timing has not previously been examined, although Berman et al. (2021) associated the summed concentration of high-molecular-weight phthalate metabolites in maternal serum with delayed menarche of daughters in an Australian cohort.

The inverse association observed between BPA and pubertal development indicates a delaying effect of *in utero* BPA exposure in both girls and boys, particularly in those with low-normal BMI, specifically for exposure in the 3rd trimester in boys and in the 1st trimester in girls. As noted above, the modifying effect of BMI suggests that pubertal delay may be counteracted by child obesity. Berger et al. (2018) reported a similar result for girls (aged 9–13 years), associating maternal urinary BPA with later menarche and pubarche in normal-weight girls but with earlier gonadarche and pubarche in overweight/obese boys. However, Ferguson et al. (2014) found no association between maternal urinary BPA at the 3rd trimester and pubertal timing in males (aged 8–14 years). The present findings are plausible, given that BPA antagonizes androgen receptor (AR) signaling *in vitro* (Molina-Molina et al., 2013; Wang et al., 2017) and *in utero* exposure results in reduced testosterone concentrations, impaired testicular development and spermatogenesis, and reduced sperm counts in male mice (Hart, 2020). However, BPA also acts as a potent estrogen (Alonso-Magdalena et al., 2012), and premature puberty was induced in female rodents by prenatal and neonatal exposure to high BPA doses (Alonso-Magdalena et al., 2012; Losa-Ward

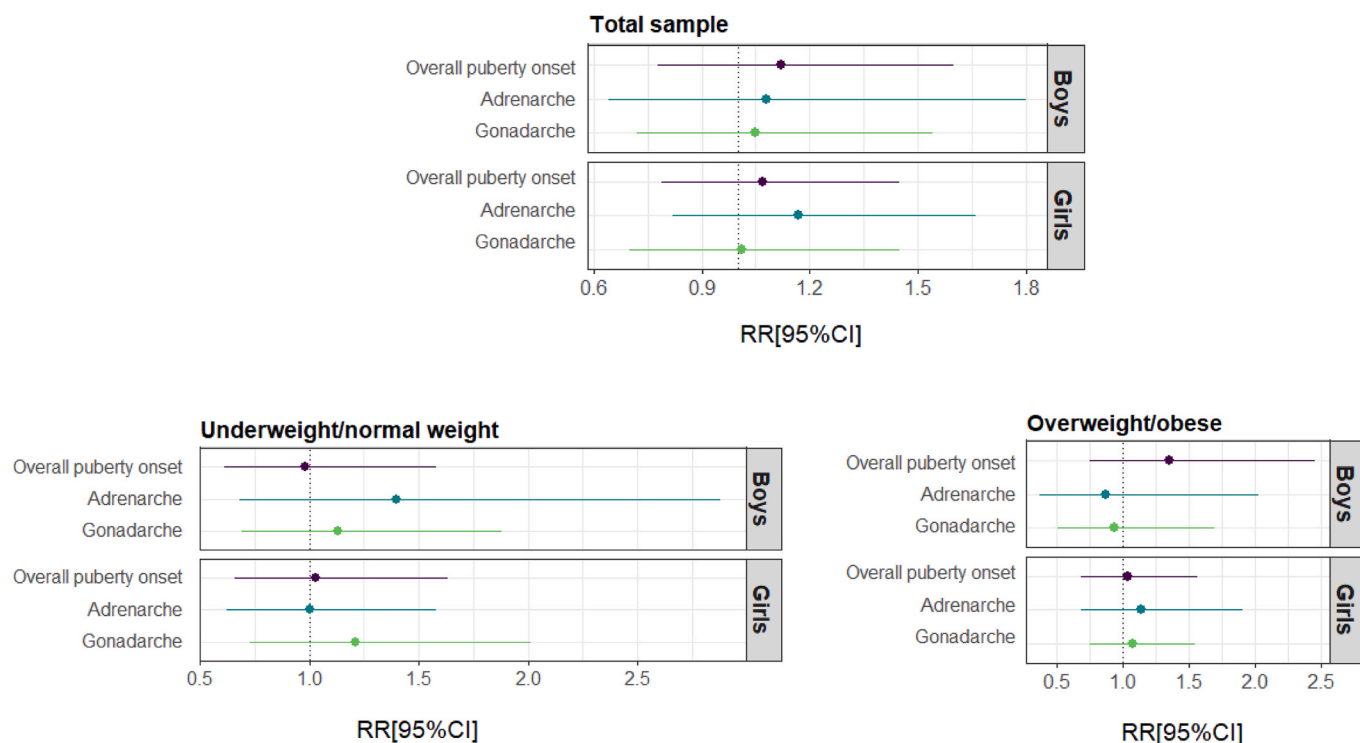


Fig. 4. Title: Chemical mixture effect on pubertal development using quantile g-computation.

Legend:

Mixture components: MEP, MiBP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, BuPB, PrPB, BPA, BP-3. All models are adjusted for mother's urinary creatinine, cohort, maternal education and pre-pregnancy BMI, and child age. RR: Relative risk of pubertal onset for a quartile increase in the mixture concentration derived by G-computation regression; 95% CI: 95% confidence interval.

et al., 2012; Wang et al., 2023). In addition, BPA was associated with delayed gonadarche but not adrenarche in girls, suggesting that it may impact the adrenal gland of boys but not girls, although this remains merely speculative.

As observed for BPA, parabens and BP-3 were associated with delayed gonadarche in boys, mostly in those with underweight/normal weight, despite the capacity of these chemicals to display weak estrogenic activities and bind with the estrogen receptors (Boberg et al., 2010; Okubo et al., 2001; Schlumpf et al., 2001). The only available human study relating pubertal development to prenatal exposure to these chemicals found no association between exposure biomarkers during pregnancy and gonadarche or pubarche timing in boys in the CHAMACOS cohort, although it described an association between peripubertal PrPB and earlier gonadarche (Harley et al., 2019). In another study, peripubertal BP-3 was associated with delayed genital and pubic hair development in boys aged 10–12 years, in partial agreement with the present findings, and analysis of the mixture effect of several phthalates and UV filters during childhood identified BP-3 as one of the chemicals contributing most to delayed male genital development (Zhou et al., 2023). In girls, neither the present study nor the study by Harley et al. (2019) found any significant association between BP-3 and pubertal timing, except for the present finding of a slight increase in the probability of adrenarche in relation to exposure in the 3rd trimester. In the present study, BuPB was the only paraben associated with delayed puberty in all boys, in line with a report that gestational BuPB exposure delays testicular descent and preputial separation in male rats (Maske et al., 2020). Our data also suggest that prenatal TCS exposure may slightly increase the probability of puberty onset in normal-weight boys and girls, more strongly in the 1st trimester in all girls, while Harley et al. (2019) associated prenatal TCS with earlier puberty in girls (earlier menarche) but not in boys. We have found no other human study on the link between prenatal TCS exposure and pubertal timing. *In vivo* studies in mammals suggest that TCS exerts estrogenic or anti-androgenic

effects (Wang and Tian, 2015), and postnatal exposure to high TCS doses was found to advance vaginal opening in female rats (Stoker et al., 2010). However, *in vitro* studies show that TCS may act as either agonist or antagonist of estrogen and androgen receptors (Wang and Tian, 2015). Despite accumulated evidence on the toxicological effects of parabens, BP-3, and TCS, further research is needed to explore their developmental effects on the reproductive system and the underlying mechanisms involved.

As noted above, the effect of prenatal phthalate/phenol exposure on puberty appears to depend on the developmental window in a sex-dimorphic manner, with pubertal development more likely affected by exposures in the 1st trimester of pregnancy in girls and exposures in the 3rd trimester in boys. A rodent study identified early and middle gestation as the critical time window for maternal BPA exposure-induced early onset of puberty in female offspring (Wang et al., 2023), in line with the present results. Other rodent studies have also demonstrated that the critical masculinization programming window of exposure to EDCs is probably in early and middle gestation (Hart, 2020), corresponding to 8–14 weeks of pregnancy in humans (Welsh et al., 2014). However, the present data suggest that the exposure of boys to EDCs in later rather than earlier pregnancy alters the development or activation of endocrine axes, particularly the gonadal axis, which warrants further investigation.

The mixture analysis did not suggest a combined effect of phthalates and phenols on advancing or delaying puberty in boys or girls, in line with the previous INMA study on phthalates (Freire et al., 2022). The mechanisms of action of phthalates, synthetic phenols, and many other EDCs are complex and may involve anti-androgenic and estrogenic action and other mechanisms, complicating interpretation of their mixture effects. The present results highlight the need for further investigation of interactions among these chemicals and their overall impact on pubertal development.

The study has certain limitations. Phthalates and synthetic phenols

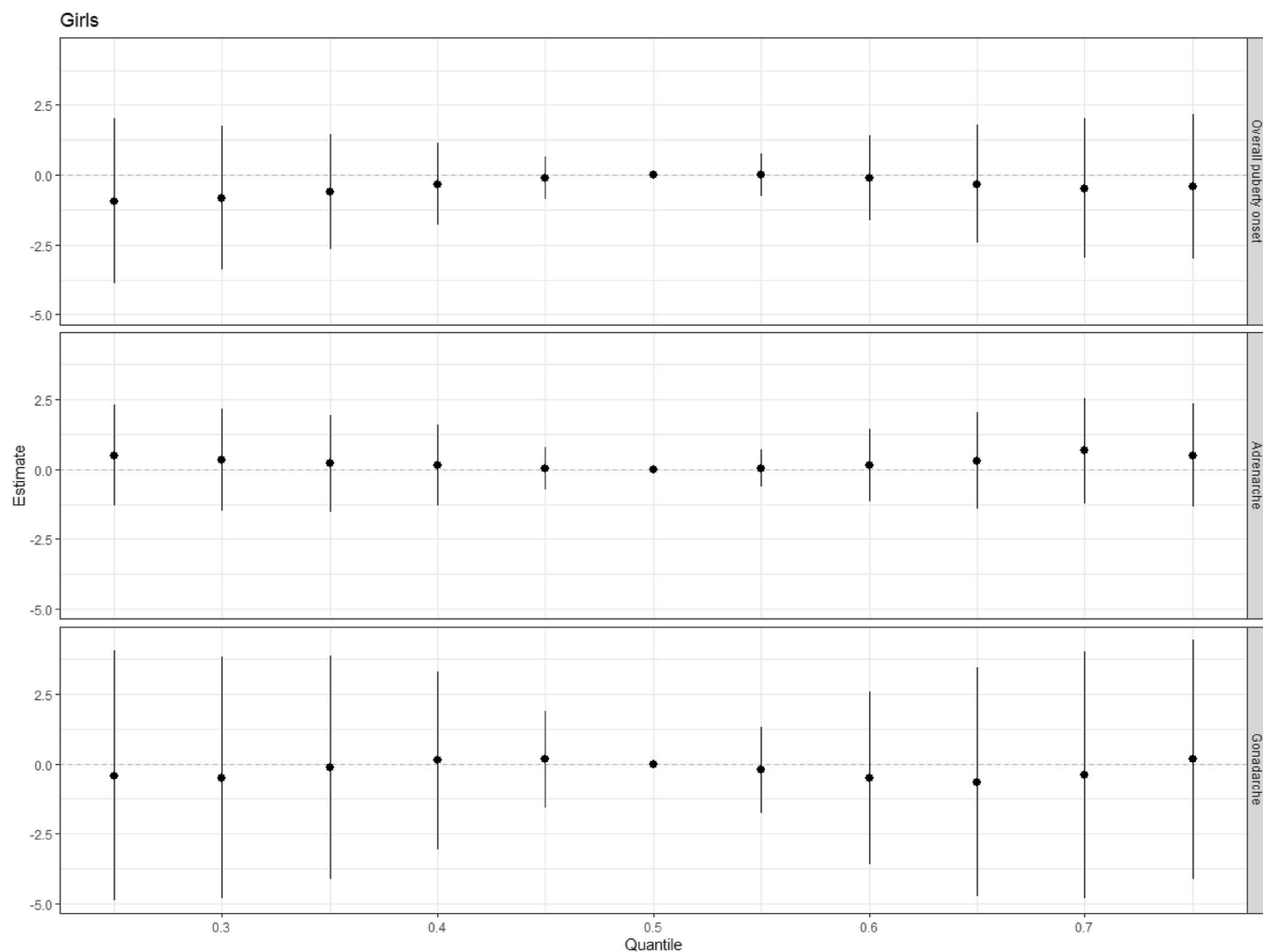


Fig. 5. Title: Chemical mixture effect on pubertal development in girls and boys using BKMR (posterior mean and 95% credible interval). N = 579 girls, 644 boys.

Legend:

Mixture components: MEP, MiBP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, BuPB, PrPB, BPA, BP-3. All models are adjusted for mother's urinary creatinine, cohort, maternal education and pre-pregnancy BMI, and child age.

are non-persistent chemicals, and maternal urine measurements at one or two time points may not accurately reflect exposure throughout pregnancy. In fact, only poor to fair between-trimester reliability was found for urinary measurements of BPA, phthalate metabolites, parabens, BP-3, and TCS in pregnant women from the INMA study, particularly for BPA (intraclass correlation coefficient = 0.01 (Sarzo et al., 2022) and 0.24 (Casas et al., 2018)), indicating dietary or other lifestyle changes. As already noted, the integration of DiNP metabolites differed between INMA and EDEN/MoBa, further limiting the interpretation of DiNP results. Developmental epidemiological studies are needed to comprehensively characterize *in utero* exposure and consider particular windows of susceptibility. A further potential study limitation is the utilization of the PDS for puberty assessment rather than Tanner stages, considered the gold standard. Phthalate metabolite and phenol concentrations were comparable between the present and previous cohort studies, and difference in outcome measures may at least partially explain inconsistencies in results. PDS does not provide a complete estimation of Tanner stages and does not include direct questions on pubic hair, but it has been validated in previous studies (e.g., in the INMA-Valencia subcohort) and has demonstrated moderate to good agreement with physician-assessed Tanner stages (Beneito et al., 2017; Bond et al., 2006; Koopman-Verhoeff et al., 2020). It has also been reported that both self-reported and parent-reported PDS have strong internal consistency and test-retest reliability and can adequately

differentiate between pre-, mid-, and post-pubertal stages (Koopman-Verhoeff et al., 2020). At any rate, possible errors in the PDS assessment of pubertal stage are likely to be non-differential. Finally, the lack of information on the maternal diet quality during pregnancy or on post-natal exposure to phthalates, phenols, and other EDCs may be responsible for some degree of residual confounding.

Study strengths include the relatively large sample size, the inclusion of children from different geographical locations in Europe, the evaluation of trimester-specific associations, and the assessment of the mixture effect of 17 phthalate metabolites and synthetic phenols. In addition, utilization of the PDS permitted the assessment of secondary sexual characteristics that are not captured by Tanner stages, including growth spurt, voice deepening, facial and body hair growth, and skin changes, providing an overall score for pubertal development and additional outcomes related to the maturation of adrenal and gonadal axes. Moreover, most research on the link between EDCs and pubertal timing has focused on childhood exposure, whereas the longitudinal design of this study allowed the impact of *in utero* exposure to be evaluated.

5. Conclusions

This study examined the association of prenatal exposure to phthalates and synthetic phenols with pubertal development in boys and girls

aged 7–12 years, considering the effect of these compounds both individually and as a mixture. Maternal urinary concentrations of certain phthalate metabolites (DEHP, DiNP) and synthetic phenols, including BPA, were associated with altered pubertal development in boys, specifically earlier puberty for phthalates and delayed puberty for phenols, and this effect was modified by their weight status and the pregnancy exposure window. Among girls, *in utero* BPA exposure may also delay the pubertal development while other phenols such as TCS may accelerate puberty, and BMI and trimester-specific associations were also observed. However, effects of the chemical mixture on either boys or girls remain uncertain. Given the limited and conflicting data on the effects of prenatal exposure to these environmental chemicals on puberty and the widespread exposure of the general population, further research is warranted to elucidate their role on changes in pubertal timing.

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CRediT authorship contribution statement

Carmen Freire: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Francesca Castiello:** Methodology, Formal analysis. **Izaro Babarro:** Writing – review & editing, Resources, Data curation. **Augusto Anguita-Ruiz:** Resources, Methodology, Formal analysis. **Maribel Casas:** Writing – review & editing, Supervision, Investigation, Funding acquisition. **Martine Vrijheid:** Project administration, Investigation, Funding acquisition. **Blanca Sarzo:** Formal analysis, Software, Writing – review & editing. **Andrea Beneito:** Methodology, Data curation. **Manik Kadawathagedara:** Writing – review & editing, Data curation. **Claire Philippat:** Writing – review & editing, Supervision, Data curation. **Cathrine Thomsen:** Resources, Formal analysis. **Amrit Kaur Sakhi:** Validation, Formal analysis, Data curation. **Maria-Jose Lopez-Espinosa:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

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