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Gestational phthalate exposure and lung function during childhood: A prospective population-based study☆,☆☆

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ABSTRACT

The potential effect of gestational exposure to phthalates on the lung function levels during childhood is unclear. Therefore, we examined this association at different ages (from 4 to 11 years) and over the whole childhood. Specifically, we measured 9 phthalate metabolites (MEP, MiBP, MnBP, MCMHP, MBzP, MEHHP, MEOHP, MECPP, MEHP) in the urine of 641 gestating women from the INMA study (Spain) and the forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1) and FEV_1/FVC in their offspring at ages 4, 7, 9 and 11. We used linear regression and mixed linear regression with a random intercept for subject to assess the association between phthalates and lung function at each study visit and for the overall childhood, respectively. We also assessed the phthalate metabolites mixture effect on lung function using a Weighted Quantile Sum (WQS) regression. We observed that the phthalate metabolites gestational levels were consistently associated with lower FVC and FEV_1 at all ages, both when assessed individually and jointly as a mixture, although most associations were not statistically significant. Of note, a 10% increase in MiBP was related to lower FVC (−0.02 (−0.04, 0)) and FEV_1 z-scores (−0.02 (−0.04, −0.01) at age 4. Similar significant reductions in FVC were observed at ages 4 and 7 associated with an increase in MEP and MnBP, respectively, and for FEV_1 at age 4 associated with an increase in MBzP. WQS regression consistently identified MBzP as an important contributor to the phthalate mixture effect. We can conclude that the gestational exposure to phthalates was associated with children's lower FVC and FEV_1, especially in early childhood, and in a statistically significant manner for MEP, MiBP, MBzP and MnBP. Given the ubiquity of phthalate exposure and its established endocrine disrupting effects in children, our findings support current regulations that limit phthalate exposure.

Abbreviations: ATS, American Thoracic Society; BMI, Body Mass Index; DAG, Directed Acyclic Graph; DEHP, di (2-ethylhexyl) phthalate; ERS, European Respiratory Society; FVC, Forced Vital Capacity; FEV_1, Forced Expiratory Volume in 1 s; FEV_1/FVC, FEV_1 to FVC ratio; INMA, Infancia y Medio Ambiente; IMIM, Hospital del Mar Medical Research Institute; HMW, High-molecular weight; LOD, limit of detection; LMW, Low-molecular weight; MBzP, mono-benzyl phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHP, mono-(2-ethyl-hexyl) phthalate; MEP, mono-ethyl phthalate; MiBP, mono-iso-butyl phthalate; MCMHP, mono-2-carboxymethyl hexyl phthalate; MnBP, mono-n-butyl phthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; N, Number of (..); NIHP, Norwegian Institute of Public Health; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; SD, standard Deviation; WQS, Weighted Quantile Sum; Y, Years.

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1. Introduction

The chemical properties of the esters of the phthalic acid, commonly known as phthalates, have made them ubiquitous in plenty of modern man-made consumer products. High-molecular weight (HMW) phthalates increase the flexibility and transparency of polymers and they are primarily used as plasticizers (Nriagu, 2019). HMW phthalates may be found in a variety of products, ranging from toys to building materials, clothing, detergents, food packaging and medical devices. On the other hand, low-molecular weight (LMW) phthalates are extensively used to increase the spreadability and lubricity of lacquers and varnishes, cosmetic and personal-care products, as excipients in pharmaceuticals and solvents, and as aerosol delivery agents in consumer products (Schettler, 2006; Hubinger, 2010; Koniecki et al., 2011; Lin et al., 2018).

Additional to being omnipresent, phthalates in normal conditions leach, migrate, or gas out from the phthalate-containing products into, for example, the air, dust, or food (Wittassek et al., 2011; Braun et al., 2013) increasing human exposure to these synthetic substances. Exposure to phthalates starts as early as *in-utero* through maternal exposure, given that phthalates travel through the placenta barrier and reach the foetal bloodstream (Mose et al., 2007). Gestational exposures to phthalates are of concern given the inferior ability of unborn children to metabolize chemical products compared to adults (Weuve et al., 2006).

In this vulnerable exposure window, phthalate exposure has been associated with numerous reproductive health (Swan, 2008; Bornehag et al., 2015; Watkins et al., 2017) and developmental problems (Qian et al., 2019), but epidemiological evidence is unclear regarding respiratory outcomes in children. Research has consistently found that gestational phthalate exposure relates to increased asthma risk in childhood (Gascon et al., 2015; Ku et al., 2015; Vernet et al., 2017; Whyatt et al., 2014) but the evidence is scarce and unclear for childhood lung function. Gestational exposure to some specific phthalate metabolites, such as monocarboxyisooctyl phthalate (MCOP) and mono-ethyl phthalate (MEP), has been statistically associated with decreased lung function at ages 5 and 7 (Vernet et al., 2017; Berger et al., 2018, 2019; 2020) whereas other metabolites have shown similar inverse associations that were not statistically significant (Vernet et al., 2017; Berger et al., 2018, 2019). The possibility that gestational phthalates may affect children lung function is additionally supported by the reported associations in some cross-sectional studies between children's phthalate exposure and their lung function at the same time points (Just et al., 2012; Cakmak et al., 2014; Kim et al., 2018; Lin et al., 2018).

Potential explanations for the inconsistent longitudinal results are the small sample size of the studies (Vernet et al., 2017; Berger et al., 2018, 2019), the analysis restriction to a selected population (e.g. only boys in Vernet et al., 2017), the focus on single exposures, as only one study tested the effects of the overall phthalate mixture on lung function (Berger et al., 2020), and the different ages when these associations were tested. Of importance, a common limitation to previous longitudinal and cross-sectional studies is that lung function was assessed at only one time point during childhood thus preventing the possibility of assessing whether the observed effects persisted during childhood (Vernet et al., 2017; Berger et al., 2018, 2019).

To overcome previous limitations, in this study we aimed to estimate the association between gestational exposure to phthalates (considering them individually and as a mixture) and childhood lung function measured at different ages (from age 4 to 11) and over the whole period in 641 mother-child pairs from a population-based cohort study in Spain. Given that early lung function is a key determinant of adult lung function (Bui et al., 2018; Lange et al., 2015; Stern et al., 2007) and it is a strong predictor of chronic respiratory diseases later in life (Agusti and Faner, 2019; Bui et al., 2018) this study is crucial to understand the respiratory effects of such ubiquitous chemicals.

2. Material and methods

2.1. Design and study population

This study is based on longitudinal data collected within the Spanish INMA (INfancia y Medio Ambiente – Childhood and Environment) study, a network of population-based birth cohorts that recruited pregnant women in their first-trimester prenatal-care visits between 2003 and 2008 and followed them and their offspring prospectively (Guxens et al., 2012). For the present analysis we considered the Sabadell and Gipuzkoa INMA cohorts because they measured phthalates during pregnancy and lung function in children at several time points during childhood (at ages 4, 7, 9 and/or 11). From a total of 1415 mother-child pairs, in the present analysis we included those with gestational phthalate data that participated in at least two study visits with at least one acceptable lung function measurement at each visit (n = 641). Of note, not all the mother-child pairs were available for all the follow-up visits, and consequently the number of observations at each visit changed.

We obtained ethical clearance from all appropriate authorities as well as from the Ethical Committees at the recruitment centers. Additionally, we obtained written informed consent from all pregnant women, who also provided written consent for their child's participation.

2.2. Phthalates exposure assessment

Nine phthalate metabolites (mono-ethyl phthalate (MEP), mono-isobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), mono-2-carboxymethyl hexyl phthalate (MCMHP, only measured in the Gipuzkoa cohort), mono-benzyl phthalate (MBzP), (mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), and mono-(2-ethyl-hexyl) phthalate (MEHP)) were measured in urine samples of the participating women using slightly different methods by cohort as described below.

First, urine samples were collected both in Gipuzkoa and Sabadell participants using identical standardized procedures in the 1st and 3rd trimester of pregnancy (median (interquartile range, IQR) gestational weeks, respectively: 13.3 (1.6) weeks and 33.9 (2) weeks). Urine was aliquoted and stored in 10-mL polyethylene tubes at −20 °C until further analysis. A small number of mothers (n = 35.5% of the total) provided only one urine sample during their pregnancy (either in the 1st or 3rd trimester), and we assessed the effect of this in a sensitivity analysis. In the Sabadell cohort the Bioanalysis Research Group at Hospital del Mar Medical Research Institute (IMIM, Barcelona, Spain) quantified the phthalate metabolites in two urine samples independently and subsequently averaged them (Valvi et al., 2015). The limit of detection (LODs) values were 0.5 ng/mL for MEHHP, MEOHP, MBzP and MiBP, and 1 ng/ml for MEHP, MECPP, MEP and MnBP. In the Gipuzkoa cohort, the phthalates determination was done by the Environmental Exposure and Epidemiology department within the Norwegian Institute of Public Health (NIHP, Oslo, Norway). Two urine samples obtained in the 1st and 3rd gestational trimester were pooled and subsequently, the phthalate metabolites were quantified in the pooled sample (Sabaredzovic et al., 2015). The limit of detection (LOD) values were 0.2 ng/mL for MEHHP, MEOHP, MiBP, MEP, MnBP, MEHP, 0.07 ng/mL for MBzP, and 0.7 ng/mL for MECPP and MCMHP. In both laboratories, phthalate quantification was done by means of liquid chromatography coupled to mass spectrometry (Waters Corp., Milford, MA) and the arylsulfatase free beta-glucuronidase (from E. coli K 12) was used for the enzymatic hydrolyses of glucuronide conjugates in the urine samples.

In addition to the metabolites mentioned above obtained from the

laboratories, we calculated the molar sum of the di(2-ethylhexyl) phthalate (DEHP) metabolites (MEHP, MEHHP, MECPP and MEOHP) and named it ∑DEHP, as it represented an integrative measure of the overall exposure to the parent compound (DEHP) (Pearson's correlation coefficients among DEHP metabolites ranging from 0.74 to 0.98).

To ensure the comparability of phthalates measurements between laboratories, we conducted a blinded inter-laboratory comparison study using 37 urine samples of the Sabadell cohort. Phthalate metabolite concentrations between the two analytical centers showed moderate-to-high correlations (Spearman's rho ranging from 0.69 to 0.97) (Tamayo-Uria et al., 2019). In a final step, we adjusted the phthalate metabolites concentrations for creatinine levels (in micrograms (μg) per gram (g) of creatinine) to account for the inter and intra-individual urinary dilution due to hydration variability among and within the pregnant women. In both cohorts, creatinine urine levels were determined by the Jaffe method (kinetic with target measurement, compensated method) with a Beckman Coulter (Fullerton, Calif) reactive in AU5400 (IZASA, Barcelona, Spain). In the Sabadell cohort, we used the 2 creatinine readings from the 1st and 3rd trimester samples to adjust each of the independent phthalate metabolite quantifications whereas for the Gipuzkoa cohort we applied the creatinine measurement obtained in the pooled urine sample to the phthalate quantification. Previous to that we verified in a pilot study of 20 samples that estimating creatinine levels in a urine sample pooled from the samples collected in the 1st and 3rd trimester of pregnancy was highly correlated with the creatinine levels estimated by averaging the creatinine levels quantified in the two independent urine samples (Spearman correlation coefficient: Rho = 0.97).

2.3. Lung function

Lung function was measured following identical methodology in Gipuzkoa and Sabadell cohorts. Trained pulmonary function technicians conducted forced spirometry at ages 4, 7 and 11 in Gipuzkoa and Sabadell, and at age 9 only in Sabadell, following the American Thoracic Society (ATS) and the European Respiratory Society (ERS) guidelines for lung function testing (Miller et al., 2005). We measured pre-bronchodilator forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) and we derived the FEV_1 to FVC ratio (FEV_1/FVC). A minimum of one technically acceptable maneuver per study visit was required for the main analysis and at least 2 acceptable and reproducible maneuvers per study visit were required for a sensitivity analysis (see below in Statistical Analysis). We expressed the lung function parameters as z-scores using the Global Lung Initiative equations (Quanjer et al., 2012), accounting for differences in ethnicity, age, sex and height.

2.4. Other participants' characteristics

Maternal information collected through questionnaire at the 1st and 3rd trimester of pregnancy included country of birth, ethnic origin (European and non-European), education level (primary, secondary and university education), socio-economic status (low, medium and high social class), smoking during pregnancy, alcohol consumption, history of allergic asthma and parity. At the 1st trimester study visit we measured maternal height and, jointly with reported pre-pregnancy weight, we calculated pre-pregnancy body mass index (BMI) (kg/m^2). We accessed clinical records to obtain the maternal age at delivery (years), gestational age (years), child's sex, and birth weight (grams) and height (cm.). At each study visit, the mothers reported children's ever asthma through interviewer-administered questionnaires based on the International Study of Asthma and Allergies in Childhood (ISAAC) phase-I questionnaire (Asher et al., 1998) and we recorded the exact age of the child and exposure to parental smoking (second-hand smoking both from the mother and from the father). Additionally, we measured children's height and weight, which were used to generate the age-and-sex standardized BMI (continuous variable) and BMI categories (underweight, normal weight, overweight, and obese) following the WHO guidelines for children aged 0 up to 5 (de Onis and Lobstein, 2010) and 5 to 19 (de Onis et al., 2007).

2.5. Statistical methods

The sample size was determined by the number of mother-child pairs that had gestational phthalate metabolites measured and lung function measurements at 2 or more timepoints. Using the GRANMO 7.12 software (Marrugat et al., 1998) we calculated that a sample of 641 mother-child pairs offered a statistical power of 98% to detect as statistically significant the association between gestational exposure to MBzP and FEV_1 previously observed in an age 7 population (Berger et al., 2019), accepting an alpha risk of 0.05 in a two-sided contrast.

Due to the small proportion of missing data (1.4% of the total observations) we used a complete case strategy and reported missing data in the footnotes of tables and figures. Due to the identical design and data collection methods (Guxens et al., 2012), we pooled data from Gipuzkoa and Sabadell cohorts and adjusted all analysis by cohort to control for observed differences in demographic variables (Supplemental Material, Table S1) and potential unmeasured confounders. We replaced the phthalate metabolites values < LOD with LOD/$\sqrt{2}$. Additionally, urinary metabolite concentrations were transformed using the natural log (ln) to achieve a normal distribution of their values.

To describe mothers' and children's characteristics, we used number and percentage, mean and standard deviation, or median and percentiles 25th-75th, depending on the variable distribution.

To assess the association between gestational exposure to phthalate metabolites (MEP, MiBP, MnBP, MBzP, ∑DEHP and MCMHP) and lung function (z-score FVC, FEV_1 and FEV_1/FVC), we first built multivariable linear regression models using one metabolite and one study visit (children ages 4, 7, 9 and 11) at a time. Second, we assessed the association between phthalates metabolites and lung function across all childhood including all repeated study visits in the same model using linear mixed regression and with the participant as a random intercept to acknowledge the existence of repeated measures from the same subject. In both analyses, we multiplied the estimates by the ln(1.10) in order to express the change in the outcome per 10% increase of phthalate metabolite concentrations in maternal urine. Statistical significance was set at 0.05, although the Bonferroni correction for multiple comparisons set the p-value threshold at 0.0017. Third, we used the Weighted Quantile Sum (WQS) regression to assess the association of the phthalate metabolites mixture over the children lung function at each study visit after verifying in the multivariable and mixed linear models the assumption of directional homogeneity (i.e., that all the individual metabolites were associated with the outcome in the same direction). Given our relatively small sample size we performed a WQS regression with repeated holdout validation in order to improve the stability of the WQS estimates (Tanner et al., 2019) and therefore the median, and the 2.5th and 97.5th centiles are reported as lower and upper limits from the sampling distribution. We computed the weights that estimate the contribution of each individual phthalate metabolite to the mixture index and used the inverse of the number of metabolites in the mixture (Carrico et al., 2015) to identify which metabolites had a significant weight.

In all previous three approaches, we used existing literature and a directed acyclic graph (DAG) (Supplemental Material, Fig. S1) to identify potential confounders. After bivariate analysis relating potential confounders with phthalates and lung function, we adjusted the final models for maternal age at delivery, maternal education, maternal social class and maternal ethnicity. We additionally included cohort (Gipuzkoa or Sabadell) as a covariate.

Secondary analyses included the use of the % of predicted FVC, FEV_1 and FEV_1/FVC instead of z-scores as outcome variables, and the stratification by child sex and parental smoking to test for potential effect modification. We set the p-value threshold for including an interaction

Table 1
Characteristics of the 641 mother-child pairs from the population-based INMA study.

Maternal characteristics	*(n = 641)*
INMA cohort - n (%):	
Gipuzkoa	250 (39.0)
Sabadell	391 (61.0)
Age at delivery, years - mean ± SD	32.2 ± 3.7
Education level - n (%)	
Primary education	117 (18.4)
Secondary education	249 (39.1)
University	271 (42.5)
Social class - n (%)	
Semi-skilled/unskilled (low)	262 (40.9)
Skilled manual/non-manual (medium)	203 (31.7)
Professionals and managers (high)	176 (27.5)
Ethnic origin - n (%)	
European	615 (96.4)
Not European	23 (3.6)
Parity (viable pregnancies additional to this) - n (%)	
0	369 (57.7)
1	233 (36.5)
≥2	37 (5.8)
History of asthma - n (%)	
Yes	591 (92.3)
No	49 (7.7)
Smoking during pregnancy - n (%)	
Not smoking	539 (85.6)
Smoking during first trimester and quitting before week 32	8 (1.3)
Smoking during all pregnancy	83 (13.2)
Pre-pregnancy BMI categories – n (%)	
Underweight (BMI <18.5)	32 (5.0)
Normal (18.5 ≤ BMI<25)	453 (70.7)
Overweight (25 ≤ BMI<30)	113 (17.6)
Obese (BMI≥30)	43 (6.7)
Child characteristics (fixed variables)	*(n = 641)*
Sex - n (%)	
Male	331 (51.6)
Female	310 (48.4)
Preterm birth (<37 weeks), yes - n (%)	17 (2.7)
Low birth weight (<2500 g), yes - n (%)	28 (4.4)
Number of weeks breastfeeding - n (%)	
0	105 (17.1)
>0 - 16	270 (44.0)
>16 - 26	210 (34.3)
>26	28 (4.6)

Abbreviations: n represents the number of participants; SD standard deviation; BMI body mass index.

term for sex and parental smoking in the final model at 0.1. As sensitivity analyses, we repeated the analysis (i) restricting it to participants with at least 2 acceptable and reproducible maneuvers per study visit in order to reduce potential outcome misclassification; (ii) restricting to participants with 2 urine spot samples in order to reduce potential exposure misclassification; (iii) excluding children with a history of asthma, to remove those participants that due to their condition may have a lower lung function and affect the studied association; (iv) additionally adjusting for maternal smoking during pregnancy to remove any potential residual confounding, as phthalates have been previously identified in cigarettes (Xu et al., 2019); and (v) adjusting for BMI of the children at each follow-up visit to indirectly assess its potential mediation role. We performed the analyses using STATA 14.0 (StataCorp LP, Tx USA) and R version 3.6.0 (gWQS R Package).

3. Results

The analysis included 641 gestating mother-child pairs for which phthalate metabolites were quantified in maternal urine and spirometry was performed in their offspring at ages 4 (n = 465), 7 (n = 560), 9 (n = 272) and/or age 11 (n = 443). Mothers mean ± standard deviation age at the time of childbirth was 32.2 ± 3.7 years, 81.6% of them had secondary or university education and only 14% smoked during pregnancy (Table 1). Compared to those mothers not included in this analysis (n = 774), the included mothers were slightly older at the time of childbirth, in a higher frequency from European origin, and reported higher education and smoking during pregnancy in a higher frequency (Supplemental Material, Table S2).

Children were 51.6% male, 82.9% were breastfed (Table 1) and among them the median breastfeeding duration was 14.9 weeks. At all age visits, around 40% of the participants fell in the categories of overweight or obesity, and lung function values (FVC and FEV_1) were normal (Table 2). No differences were observed between included and not-included children (Supplemental Material, Table S2).

Seven phthalate metabolites were detected in 100% of the samples and the two remaining metabolites (MBzP and MEHP) were detected in 99.8% of them. MEP urinary phthalate median concentrations were the highest with a concentration of 257.8 μg/g of creatinine. The rest of the phthalate metabolites presented median concentrations that were smaller; from 7.9 μg/g of creatinine for MEHP to 32.8 μg/g of creatinine for MECPP (Table 3).

Overall, phthalate metabolites were associated with lower FVC and FEV_1 but most of the associations did not reach statistical significance

Table 2
Time-varying characteristics of the 641 children included in the present analysis of the population-based INMA study.

Study population characteristics	Age 4 (n = 465)	Age 7 (n = 560)	Age 9 (n = 272)	Age 11 (n = 443)
Age, years - median (IQR)	4.5 (0.1)	7.3 (1.1)	9.0 (0.9)	10.9 (0.6)
Height, cm - mean ± SD	106.2 ± 4.4	124.3 ± 6.4	134.5 ± 6.9	145.0 ± 7.2
Weight, kg - median (IQR)	17.9 (3.2)	25.6 (7.4)	32.2 (11.3)	38.8 (13)
BMI, z-score categories* - n (%)				
Underweight	2 (0.4)	1 (0.2)	1 (0.4)	3 (0.7)
Normal	313 (67.5)	346 (62.8)	159 (58.5)	267 (60.3)
At risk of overweight	111 (23.9)			
Overweight	29 (6.3)	116 (21.1)	62 (22.8)	107 (24.2)
Obese	9 (1.9)	88 (16.0)	50 (18.4)	66 (14.9)
FVC (liters) - mean ± SD	1.0 ± 0.2	1.7 ± 0.3	2.1 ± 0.4	2.5 ± 0.4
FVC (% of predicted) - mean ± SD	93.3 ± 17.5	106.3 ± 12.1	102.4 ± 12.7	100.2 ± 11.9
FVC (z-score) - mean ± SD	−0.5 ± 1.3	0.5 ± 1.0	0.2 ± 1.1	0.0 ± 1.0
FEV_1 (liters) - mean ± SD	0.9 ± 0.2	1.5 ± 0.3	1.8 ± 0.3	2.2 ± 0.4
FEV_1 (% of predicted), - mean ± SD	92.1 ± 16.4	102.0 ± 11.7	100.5 ± 12.3	97.6 ± 11.8
FEV_1 (z-score) – mean ± SD	−0.6 ± 1.2	0.2 ± 1.0	0.0 ± 1.1	−0.2 ± 1.0
FEV_1/FVC (liters) – mean ± SD	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
FEV_1/FVC (% of predicted) - mean ± SD	98.7 ± 7.5	95.6 ± 7.0	97.6 ± 7.0	97.0 ± 7.3
FEV_1/FVC (z-score) - mean ± SD	−0.1 ± 1.0	−0.6 ± 0.9	−0.3 ± 1.0	−0.4 ± 1.0

Abbreviations: n represents the number of participants; IQR Inter-quartile Range; SD Standard deviation; FVC Forced Vital Capacity; FEV_1 Forced Expiratory Volume in 1 s; and FEV_1/FVC, FEV_1 to FVC ratio. * The WHO BMI z-score categories are detailed in the methods section.

Table 3
Levels of phthalate metabolites in maternal urine in 641 pregnant mothers from the population-based INMA study.

		n	>LOD	Crude (μg/L)	Creatinine adjusted (μg/g creatinine)	Ln-transformed creatinine-adjusted (μg/g creatinine)
				Median (p25-p75)	Median (p25-p75)	Geometric mean (95% CI)
Mono-ethyl phthalate	**MEP**	640	99.8%	218.4 (93.5, 532.2)	257.8 (111.3, 621.6)	5.4 (5.3, 5.5)
Mono-iso-butyl phthalate	**MiBP**	641	100%	26.4 (16.7, 42.3)	30.0 (20.3, 44.5)	3.4 (3.3, 3.4)
Mono-n-butyl phthalate	**MnBP**	635	99.1%	20.3 (10.6, 34.3)	22.3 (13.8, 38)	3.1 (3, 3.1)
Mono benzyl phthalate	**MBzP**	635	99.1%	7.4 (3.8, 14)	14.1 (5.5, 25)	2.3 (2.2, 2.4)
Mono-2-carboxymethyl hexyl phthalate[a]	**MCMHP**[a]	250	100%	16.9 (11.2, 23.8)	20.5 (14.5, 27.2)	3 (2.9, 3.1)
Mono-2-ethyl-5-hydroxyhexyl phthalate	**MEHHP**	641	100%	18.7 (10.6, 32.6)	21.1 (12.8, 35.2)	3 (2.9, 3.1)
Mono-2-ethyl-5-oxohexyl phthalate	**MEOHP**	641	100%	13.8 (7.6, 24.2)	15.4 (9.5, 25.4)	2.7 (2.6, 2.7)
Mono-2-ethyl 5-carboxypentyl phthalate	**MECPP**	640	99.8%	28.8 (18.1, 45.6)	32.8 (22.1, 50.1)	3.5 (3.4, 3.5)
Mono-2-ethylhexyl phthalate	**MEHP**	636	99.2%	6.4 (3.6, 11.5) (μmol/L)	7.9 (4.3, 13.1) (μmol/L g creatinine)	1.8 (1.8, 1.9) (μmol/L g creatinine)
Molar sum of di(2-ethylhexyl) phthalate metabolites[b]	**∑DEHP**[b]	636	99.2%	0.2 (0.1, 0.4)	0.3 (0.2, 0.4)	0.5 (0.4, 0.7)

Abbreviations: n represents the number of samples in which the metabolite was above the limit of detection, LOD limit of detection; (p25- p75), 25th percentile and 75th percentile; 95% CI, 95% confidence interval.
[a] Phthalate metabolite only available for the Gipuzkoa cohort.
[b] ∑DEHP corresponds to the molar sum of the following DEHP metabolites: MEHP, MEHHP, MECPP and MEOHP.

(Table 4). Among the observed associations, a 10% increase in MEP was associated with a reduction of the FVC z-scores at age 9 of −0.013 (−0.026, −0.001; p-value = 0.033). This inverse association was also observed at ages 4, 7 and 11, although it did not reach statistical significance (Table 4). Similarly, a 10% increase in MiBP was statistically related to lower FVC and FEV_1 z-score coefficients at age 4. For MnBP, only the associations with FVC z-score at age 7 and in the mixed model (using all the lung function measures available) were statistically significant. In the case of MBzP, we observed statistical significance for the FEV_1 z-score coefficient at age 4 and in the mixed model. ∑DEHP and MCMHP were associated with non-statistically significant lower FVC and FEV_1 z-scores. No clear pattern of associations was observed between any of the phthalate metabolites and the FEV_1/FVC ratio. When correcting for multiple testing, none of the phthalate metabolite-lung function parameter associations mentioned above remained statistically significant.

In the mixture approach (WQR model), a one-unit increase in the WQS index (i.e., the phthalate metabolites mixture) was consistently associated with lower FEV_1 and FVC (but not FEV_1/FVC ratio) z-scores at the different study visits (from ages 4 to 11), although the associations were not statistically significant (Table 5). Among the different metabolites, MBzP consistently presented a WQS estimated weight >20% (i.e., higher than equal weighting) and therefore was considered one of the metabolites driving the mixture effect over FVC and FEV_1 at most ages (Supplemental Material, Fig. S2).

Most secondary and sensitivity analyses produced results very similar to the main analysis, although some associations lost precision due to the reduction in sample size (Supplemental Material, Table S3, Table S6, Table S7, Table S8, Table S9, and S10). After sex stratification, the associations were stronger in boys than in girls (Supplemental Material, Table S4), although there was no evidence of statistical interaction with sex (p-value for interaction >0.1 in all models). Similarly, after stratifying by parental smoking exposure (Supplemental Material Table S5), the associations remained among children non-exposed to smoking, whereas generally null associations were observed among the exposed to smoking. In general, there was no evidence of statistical interaction for most metabolites except for the interactions between parental smoking and MnBP observed in the FVC and FEV_1 models at the 4-year study visit, for the FVC model at the 7-year study visit, and between parental smoking and MBzP observed in the FVC model at the 4-year study visit.

4. Discussion

To the best of our knowledge, this is the first study to estimate the effect of the gestational exposure to phthalates on the children's lung function at multiple times during childhood (from 4 to 11 years), combining individual and mixture-exposure approaches. We observed that (1) all phthalate metabolites seemed associated with reduced lung function parameters during childhood (both when assessed individually and in the mixture), although most of these associations were not statistically significant; (2) MiBP and MBzP exhibited statistically significant associations; (3) the statistically significant associations were observed generally at younger ages; and (4) MnBP seemed to have a consistently statistically significant effect in boys than in girls. These results were robust to diverse secondary and sensitivity analyses.

Our results are in agreement with previous research that has reported either inverse associations, but not statistically significant, between gestational exposure to phthalates and childhood lung function (Vernet et al., 2017; Agier et al., 2019) or null associations (Berger et al., 2018, 2019; 2020). Of relevance, we found that some phthalate metabolites were significantly associated with lower lung function levels at early ages but not later, a pattern that cannot be compared with previous literature due to the lack of studies with repeated lung function measures. Some of our findings for specific metabolites and specific ages can be compared, though. First, in our study MiBP was statistically

Table 4
Adjusted* associations of ln-transformed creatinine-adjusted gestational phthalates with standardized (z-score) lung function parameters (FVC, FEV_1 and FEV_1/FVC) at different age periods (age 4, 7, 9 and 11, linear regression), and for the entire childhood (mixed linear regression). Coefficient estimates express the change in the outcome per 10% increase of exposure.

	n	z-score FVC		z-score FEV_1		z-score FEV_1/FVC	
		Coefficient (95% CI)	p-	Coefficient (95% CI)	p	Coefficient (95% CI)	p
ln MEP							
Age 4	461	−0.007 (−0.018, 0.003)	0.181	−0.007 (−0.018, 0.003)	0.159	0.001 (−0.007, 0.01)	0.737
Age 7	554	−0.007 (−0.014, 0)	0.056	−0.005 (−0.012, 0.002)	0.185	0.003 (−0.004, 0.01)	0.411
Age 9	266	−0.013 (−0.026, −0.001)	0.033	−0.01 (−0.022, 0.002)	0.116	0.005 (−0.007, 0.017)	0.402
Age 11	439	−0.007 (−0.016, 0.002)	0.11	−0.002 (−0.011, 0.007)	0.684	0.007 (−0.001, 0.016)	0.092
Entire childhood (4–11 years)	1724	−0.006 (−0.012, 0)	0.063	−0.004 (−0.01, 0.002)	0.209	0.003 (−0.003, 0.008)	0.352
ln MiBP							
Age 4	461	−0.02 (−0.037, −0.003)	0.024	−0.024 (−0.041, −0.008)	0.004	−0.005 (−0.018, 0.008)	0.433
Age 7	554	−0.003 (−0.014, 0.008)	0.564	−0.001 (−0.012, 0.01)	0.901	0.003 (−0.008, 0.014)	0.563
Age 9	266	−0.006 (−0.025, 0.013)	0.522	−0.002 (−0.021, 0.017)	0.831	0.008 (−0.01, 0.027)	0.362
Age 11	439	0 (−0.013, 0.013)	0.992	0.003 (−0.01, 0.017)	0.622	0.004 (−0.009, 0.017)	0.541
Entire childhood (4–11 years)	1720	−0.006 (−0.016, 0.004)	0.227	−0.005 (−0.015, 0.005)	0.349	0.002 (−0.006, 0.011)	0.597
ln MnBP							
Age 4	461	−0.013 (−0.03, 0.004)	0.141	−0.016 (−0.032, 0.001)	0.061	−0.004 (−0.017, 0.009)	0.574
Age 7	554	−0.012 (−0.022, −0.001)	0.039	−0.006 (−0.017, 0.005)	0.276	0.007 (−0.004, 0.018)	0.198
Age 9	266	−0.013 (−0.029, 0.004)	0.126	−0.007 (−0.024, 0.009)	0.394	0.012 (−0.004, 0.027)	0.137
Age 11	439	−0.004 (−0.017, 0.009)	0.515	−0.005 (−0.017, 0.008)	0.482	−0.001 (−0.013, 0.012)	0.887
Entire childhood (4–11 years)	1718	−0.009 (−0.019, 0)	0.048	−0.007 (−0.016, 0.003)	0.154	0.003 (−0.005, 0.012)	0.403
ln MBzP							
Age 4	461	−0.012 (−0.027, 0.002)	0.098	−0.018 (−0.032, −0.004)	0.014	−0.004 (−0.016, 0.007)	0.465
Age 7	554	−0.009 (−0.019, 0)	0.06	−0.007 (−0.017, 0.002)	0.142	0.002 (−0.007, 0.012)	0.618
Age 9	266	−0.015 (−0.03, 0)	0.055	−0.012 (−0.027, 0.003)	0.13	0.008 (−0.006, 0.022)	0.271
Age 11	439	−0.001 (−0.012, 0.011)	0.898	−0.005 (−0.016, 0.007)	0.414	−0.008 (−0.019, 0.004)	0.18
Entire childhood (4–11 years)	1715	−0.008 (−0.017, 0)	0.051	−0.009 (−0.017, 0)	0.043	0 (−0.008, 0.007)	0.943
ln ∑DEHP							
Age 4	461	−0.004 (−0.022, 0.014)	0.668	−0.006 (−0.023, 0.011)	0.512	−0.007 (−0.021, 0.006)	0.283
Age 7	553	−0.005 (−0.017, 0.007)	0.443	−0.005 (−0.017, 0.007)	0.419	−0.003 (−0.015, 0.009)	0.584
Age 9	266	−0.006 (−0.026, 0.015)	0.588	0.003 (−0.017, 0.024)	0.745	0.015 (−0.004, 0.034)	0.12
Age 11	439	−0.005 (−0.02, 0.01)	0.503	−0.003 (−0.018, 0.012)	0.673	0.002 (−0.013, 0.016)	0.83
Entire childhood (4–11 years)	1718	−0.002 (−0.012, 0.008)	0.725	−0.001 (−0.011, 0.01)	0.907	−0.001 (−0.01, 0.008)	0.841
ln MCMHP							
Age 4	172	−0.024 (−0.054, 0.007)	0.125	−0.026 (−0.056, 0.004)	0.085	−0.004 (−0.027, 0.019)	0.76
Age 7	239	−0.013 (−0.032, 0.006)	0.19	−0.01 (−0.029, 0.009)	0.299	0.001 (−0.019, 0.021)	0.927
Age 9	–	–	–	–	–	–	–
Age 11	205	−0.005 (−0.029, 0.019)	0.694	−0.003 (−0.027, 0.021)	0.786	0.005 (−0.021, 0.031)	0.707
Entire childhood (4–11 years)	616	−0.011 (−0.029, 0.007)	0.228	−0.01 (−0.027, 0.008)	0.280	0.001 (−0.015, 0.017)	0.922

Abbreviations: FVC, Forced vital capacity; FEV_1, Forced expiratory volume the first 1 s; and FEV_1/FVC, FEV1 to FVC ratio; 95% CI, 95% confidence interval. n represents the number of children that were included in the regression model of each study visit, whereas for the "entire childhood (4–11 years)" n represents the number of children · study visits included in the mixed models.
**Model adjusted for maternal age, education, social class, ethnic origin and cohort. Mixed model additionally included participant as random intercept.
Some variables had missing values: 1 in maternal age, 4 in education, and 3 in ethnic origin.

Table 5
Adjusted* associations of gestational phthalate metabolites mixture with standardized (z-score) lung function parameters (FVC, FEV_1 and FEV_1/FVC) at different age periods (age 4, 7, 9, and 11, Weighted Quantile Sum (WQS) linear regression).

Study visit	n	WQS Index β Coefficients and 2.5th and 97.5th percentiles*		
		z-score FVC	z-score FEV_1	z-score FEV_1/FVC
Age 4	458	−0.12 (−0.29, 0.04)	−0.19 (−0.36, 0.03)	−0.02 (−0.18, 0.13)
Age 7	550	−0.11 (−0.24, 0.02)	−0.07 (−0.2, 0.05)	0.00 (−0.13, 0.12)
Age 9	265	−0.15 (−0.31, 0.01)	−0.12 (−0.29, 0.04)	0.04 (−0.1, 0.19)
Age 11	436	−0.06 (−0.2, 0.08)	−0.01 (−0.15, 0.17)	−0.04 (−0.17, 0.09)

Abbreviations: FVC, Forced vital capacity; FEV_1, Forced expiratory volume the first 1 s; and FEV_1/FVC, FEV_1 to FVC ratio. n represents the number of children that were included in the regression model of each study visit. * Model adjusted for maternal age, education, social class, ethnic origin and cohort.
Some variables had missing values: 1 in maternal age, 4 in education, and 3 in ethnic origin.

associated with FVC and FEV_1 at age 4, while in later ages we observed a similar association but not statistically significant. This is aligned with the inverse (but non-statistically significant) association at age 5 in the EDEN study (Vernet et al., 2017), and the null associations at age 7 in the HELIX and CHAMACOS studies (Agier et al., 2019; Berger et al., 2018). Second, we observed a significant association between MBzP and lower FEV_1 at age 4 and across childhood (mixed model) in agreement with the results reported in the EDEN study at age 5 (Vernet et al., 2017) and the CHAMACOS study at age 7 (although $p > 0.05$) (Berger et al., 2019). Of note, MBzP, the metabolite identified in our study as the main driver of the mixture effect over lung function, was recognized as the second strongest contributing metabolite in the only previous study (CHAMACOS) that used a mixture approach (Berger et al., 2020). Finally, for the remaining metabolites (MEP, MnBP, ∑DEHP and MCMHP) for which we found consistent inverse associations but no clear pattern of statistical significance, similar associations had been reported in the EDEN study at age 4 (Vernet et al., 2017) and in the CHAMACOS study at age 7 ($p > 0.05$) (Berger et al., 2018). The results obtained in our and in

previous studies suggest that, among all the assessed metabolites, MiBP and MBzP stand out as potential key players in the observed lower levels of FVC and FEV_1 during childhood.

The joint interpretation of our and previous studies results needs to critically consider some methodological issues such as sample size and metabolite concentrations. Small sample size can be behind the fact that, although most studies consistently identified inverse associations between gestational exposure to phthalates and lung function parameters, most of the observed associations were not statistically significant. Differences in the concentrations of specific metabolites can explain some differences across studies. In particular, in our study (INMA) the levels of MiBP and MEP concentrations were one order of magnitude larger than in the CHAMACOS and EDEN studies (MiBP: 3.4 in CHAMACOS vs 41.2 μg/L in INMA; MEP: 99 in EDEN vs 402.3 μg/L in INMA), suggesting differential phthalate exposure sources among the cohorts. This could explain why statistically significant associations with these metabolites were identified only in our study. Another aspect to take into account is that our mean spirometry values were smaller (FEV_1: 1.5 (0.3) liters) than those reported by Berger et al. at age 7 (Berger et al., 2019) (FEV_1: 1.8 (0.5) liters). Additional unmeasured study-specific factors could play a role and explain why they found marginally statistically significant associations between gestational levels of MBzP similar to those quantified in our population (8.9 ng/mL vs 7.4 μg/L in INMA) and FEV_1. Aligned with this, differences in the prevalence of parental smoking exposure among the different cohorts could partially explain the differences observed. In our study, despite the generalized lack of statistical evidence for interaction, results suggest that the exposure to parental smoking could have masked the association between gestational levels of MiBP, MnBP and MBzP and lung function parameters among second-hand smoke exposed children, as we were only able to observe these associations among non-exposed children.

Evidence from experimental research supports the plausibility of the potential association between phthalate metabolites and reduced FVC and FEV_1 levels via excess of oxidative stress (Ferguson et al., 2015; Gläser et al., 2012; Rasmussen et al., 2009; Sweeney et al., 2019), delayed foetal lung maturation and decreased alveolarization in rats (Chen et al., 2010), and interference with sex hormones (Qian et al., 2020). Of interest, in these animal models some of these mechanisms reverted with time, potentially supporting our general findings of associations at ages 4 or 7 but not later (Chen et al., 2010) (Schittny, 2017).

Despite the lack of precision in most of the observed potential associations ($p > 0.05$) our results have implications for public health and future research. We found significant associations for metabolites that are highly ubiquitous (present in personal care products (Parlett et al., 2013) or used as plasticizers). Currently some of these (or their parent compounds) are banned from consumer products in the European Union under REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). Therefore, and despite the small magnitude of the observed associations and the inconsistency in statistical significance, our findings support these restrictions and suggest they are extended to additional phthalates and to those countries without restrictions in all consumer products. For future research, our results encourage the inclusion of repeated outcome measurements during childhood when assessing the health effects of gestational exposures. Also, our results encourage future studies to combine the single-exposure with the multi-exposure approach, for a better understanding of the health effects of the phthalates and the identification of the main contributors to the exposure.

This study has several limitations. First, sample size at each study visit was relatively small which, as previously mentioned, may be behind the mostly non-significant but consistent associations. Second, the measure of lung function in younger children may be subject to measurement error given the difficulties of the spirometry maneuver. This could have biased the observed associations to the null, although our results were consistent in sensitivity analyses following stricter validity and reproducibility criteria. Third, the use of only two individual urine samples in pregnant women may be insufficient to characterize their personal exposure to phthalates due to their between-trimester variability, potentially resulting in exposure misclassification; although phthalates have less temporal variability than other non-persistent pollutants such as phenols (Casas et al., 2018). Fourth, the mother-child pairs included in the study differed in terms of some sociodemographic factors with those mothers not included, which could have resulted in some selection bias. However, we adjusted for demographic factors and for cohort, which indirectly accounts for unmeasured confounding. Fifth, we could not formally test the role of urban vs. rural environment on the observed associations because virtually no children (<1% of the sample) resided in rural settings, although excluding them from the analysis had no impact on the results (data not shown). Additionally, the absence of phthalate concentration levels measured at the time of the spirometry tests prevent us to evaluate the effect that exposure to phthalates during childhood may have had in the relationship we are assessing here (gestation exposure to phthalates and lung function during childhood).

One of the main strengths of this study is the availability of up to 4 lung function measurements, covering most of childhood for respiratory health, which had never been done before and allowed us testing whether any effects of the gestational exposure to phthalates on lung function are specific to some age periods, persist or remit, at the same time that increased the statistical power when assessing the potential effect over the entire childhood through mixed models. Second, the combination of individual phthalate metabolites and mixture-approach allows comparing our results with previous literature and testing the joint effect of those metabolites. Finally, our results can be directly compared with and extrapolated to a larger population, as the average phthalate metabolite concentrations in our population (except for those mentioned earlier in the discussion) were generally in the range with those reported in other European pregnancy cohorts during similar periods (Haug et al., 2018; Vernet et al., 2017).

5. Conclusion

In conclusion, gestational phthalates exposure seemed associated with children's lower FVC and FEV_1, especially in early childhood, and in statistically significant manner for MEP, MiBP, MBzP and MnBP. Given the ubiquity of phthalate exposure and its established endocrine disrupting effects in children, our findings support current regulations that limit phthalate exposure.

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Disclosure of interests

JGA's institution has received consulting and lecture fees from AstraZeneca (not related to this study); JGA has received lecture fees from Esteve and Chiesi (not related to this study).

All other authors declare they have nothing to disclose.

Human participant protection

This study has been reviewed and approved by the accredited committees of the following institutions: Ethics Committee of the Parc de Salut Mar, and Hospital de Zumarraga in Gipuzkoa. The protocol has also been approved by all appropriate ethic committees and written informed consent was obtained by all participants and their offspring prior to commencing the epidemiological study.

Author's contribution

JS devised the INMA project and the main conceptual ideas. JS, RS-B and JI planned and carried out the data and measurements collection. MBB, MC and JG-A helped shape the research, analysis and manuscript. MBB, A-EC and IC carried out and technically assisted in the statistical analysis. MBB, A-EC, AA, AL, NM, RS-B, JI, MV, JS, MC and JG-A contributed to the interpretation of the results. MBB took the lead in writing the manuscript and all authors provided critical feedback and approved the final version of the manuscript.

Author's statement

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Judith Garcia-Aymerich reports a relationship with AstraZeneca Pharmaceuticals LP that includes: consulting or advisory and speaking and lecture fees. Judith Garcia-Aymerich reports a relationship with Esteve Quimica SA that includes: speaking and lecture fees. Judith Garcia-Aymerich reports a relationship with Chiesi Pharmaceuticals Inc that includes: speaking and lecture fees.

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.2022.119833.

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