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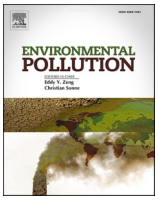

Prenatal exposure to synthetic phenols and phthalates and child respiratory health from 2 to 36 months of life☆

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ABSTRACT

Exposure to phthalates and synthetic phenols is ubiquitous. Some of them are suspected to impact child respiratory health, although evidence still remains insufficient. This study investigated the associations between prenatal exposure to phthalates and phenols, individually and as a mixture, and child respiratory health assessed by objective lung function measures since 2 months of age. Among 479 mother-child pairs from the SEPAGES cohort, 12 phenols, 13 phthalate and 2 non-phthalate plasticizer metabolites were measured in 2 pools including each 21 urine samples collected at the 2nd and 3rd pregnancy trimesters. Lung function was measured at 2 months using tidal breathing flow-volume loops and nitrogen multiple-breath washout, and at 3 years using oscillometry. Asthma, wheezing, bronchitis and bronchiolitis were assessed by repeated questionnaires. A cluster-based analysis was applied to identify exposure patterns to phenols and phthalates. Adjusted associations between clusters as well as each individual exposure biomarker and child respiratory health were estimated by regression models. We identified four prenatal exposure patterns: 1) low concentrations of all biomarkers (reference, n = 106), 2) low phenols–moderate phthalates (n = 162), 3) high concentrations of all biomarkers except bisphenol S (n = 109), 4) high parabens–moderate other phenols–low phthalates (n = 102). At 2 months, cluster 2 infants had lower functional residual capacity and tidal volume and higher ratio of time to peak tidal expiratory flow to expiratory time (t_{PTEF}/t_E) and cluster 3 had lower lung clearance index and higher t_{PTEF}/t_E. Clusters were not associated with respiratory health at 3 years but in the single-pollutant models, parabens were associated with increased area of the reactance curve, bronchitis (methyl, ethyl parabens) and bronchiolitis (propyl paraben). Our results suggested that prenatal exposure to mixtures of phthalates reduced lung volume in early life. Single exposure analyses suggested associations of parabens with impaired lung function and increased risk of respiratory diseases.

1. Introduction

Although lungs develop until early adulthood, the most important structural developments occur in early-life, including fetal life. Changes in lung development in this sensitive window of time can lead to lifelong respiratory morbidity (Stocks et al., 2013). This has raised the motivation to identify modifiable factors influencing early lung development, and has led to increased epidemiological evidence for impact of prenatal exposure to environmental factors on child lung development and respiratory diseases later in life (Johnson et al., 2021; Stocks et al., 2013).

Early-life exposure to endocrine disruptors, such as phthalates and synthetic phenols, is among the emergent possible risk factors of child

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Abbreviations

AOS	Airway Oscillometry
AX	Area under the reactance curve
BPA	Bisphenol A
BPS	Bisphenol S
BP3	Benzophenone-3
BUPA	Butyl paraben
DEHP	Di(2-ethylhexyl) phthalate
DINCH	Di(isononyl)cyclohexane-1,2-dicarboxylate
DiNP	Diisononyl phthalate
ETPA	Ethyl paraben
FRC	Functional residual capacity
ISAAC	International Study of Asthma and Allergies in Childhood
LCI	Lung clearance index
LOD	Limit of detection
LOQ	Limit of quantification
MBzP	Mono-benzyl phthalate
MEP	Monoethyl phthalate
MEPA	Methyl paraben
MiBP	Mono-isobutyl phthalate
MnBP	Mono-n-butyl phthalate
N_2MBW	Nitrogen multiple-breath washout
OH-MPHP	Mono-6-hydroxy-propylheptyl phthalate
R_7	Resistance at 7 Hz
$R_{7\text{-}19}$	Resistance between 7 and 19 Hz
TBFVL:	Tidal breathing flow-volume loops
TCS	Triclosan
t_{PTEF}/t_E	Ratio of time to peak tidal expiratory flow to expiratory time
V_t	Tidal volume
X_7	Reactance at 7 Hz

health, including respiratory health (Miller and Marty, 2010; Vernet et al., 2017). Exposure to synthetic phenols and phthalates is ubiquitous through food processing and packaging (bisphenol A or S, benzophenone-3, parabens, phthalates), personal care products (parabens, triclosan, benzophenones, some phthalates), pharmaceutical products (phthalates), and a large range of other consumer products (such as paints, varnishes, furniture and PVC plastics (phthalates)). Epidemiological studies have shown that phthalate metabolites and phenols are detected in almost all pregnant women in Europe and U.S (Dereumeaux et al., 2016; Haug et al., 2018; Mortensen et al., 2014; Philippat and Calafat, 2021; Rolland et al., 2020).

Several epidemiological studies investigated the associations between these compounds, mainly phthalates and bisphenol A and respiratory and allergic diseases in children (Casas and Gascon, 2020). However, the evidence remains insufficient because of limited result consistency. For example, although the majority of studies that addressed the role of prenatal bisphenol A exposure on respiratory health in children showed positive associations (Spanier et al., 2012), some identified positive associations in boys (Buckley et al., 2018; Vernet et al., 2017) or in girls (Zhou et al., 2017) only. One other study showed inverse associations (Donohue et al., 2013) while three did not observe any association (Agier et al., 2019; Berger et al., 2019; Gascon et al., 2015). Limited power and exposure misclassification may partly explain the discrepancy in results. Regarding the latter issue, most studies conducted so far relied on a single (Buckley et al., 2018; Donohue et al., 2013; Ku et al., 2015; Vernet et al., 2017; Whyatt et al., 2014; Zhou et al., 2017) or few urine samples (Agier et al., 2019; Berger et al., 2020, 2019; Gascon et al., 2015; Spanier et al., 2014, 2012). Because of the high within-subject variability of phenol and phthalate urinary concentrations in pregnant women (Casas et al., 2018; C. Vernet et al., 2018), a small number of urine samples imperfectly reflects the average exposure throughout the pregnancy. Depending on the chemicals, this exposure misclassification leads to moderate-to-high underestimation of exposure-health associations (Perrier et al., 2016; Céline Vernet et al., 2018). Regarding respiratory health assessment, few studies used objective measurements of lung function, and all were performed in children aged 5 years or older when spirometry becomes feasible (Agier et al., 2019; Berger et al., 2019; Spanier et al., 2014; Vernet et al., 2017). Finally, while in daily life populations are simultaneously exposed to a wide range of chemicals, most of the previous studies investigated the effect of each compound alone. Only one study addressed mixture effect (Berger et al., 2020). In the CHAMACOS cohort, a cluster-based analysis identified seven prenatal exposure patterns to phthalates and phenols. However, none was associated with child respiratory health (Berger et al., 2020). Further analyses are needed to explore the effect of chemical mixtures on respiratory health.

The goal of this study was to assess the associations between prenatal exposure to synthetic phenols and phthalates, considering mixture of chemicals, and child respiratory health assessed based on objective lung function measures as early as 2 months of age and up to 3 years.

2. Materials and methods

2.1. Study population

The study is based on the data from the SEPAGES cohort (2014–2017, Grenoble, France) (Lyon-Caen et al., 2019). Briefly, 484 pregnant women were included before the 19th gestational week and followed during pregnancy, and their child were subsequently followed-up. In the present study, the association analyses were conducted in 457 children with both prenatal urinary biomarkers of exposure to synthetic phenols and phthalates and either lung function test at 2 months or 3 years or history of respiratory diseases (Fig. 1).

The study was approved by the Comité de Protection des Personnes (CPP Sud-Est) and the Commission Nationale de l'Informatique et des Libertés (CNIL). Volunteers had signed a consent before their inclusion in this study.

2.2. Prenatal exposure to synthetic phenols and phthalate

At two time points of the pregnancy (2nd trimester: median of 18 gestational weeks, and 3rd trimester: median of 34 gestational weeks), women were asked to collect three urine samples per day, during a week. For each participant and each week of urine collection (2nd and 3rd trimester of pregnancy), equal volume pools of the repeated urine samples during the week were made (Philippat and Calafat, 2021; Vernet et al., 2019).

For each individual, an aliquot of these pools was sent on dry ice with a temperature sensor to the Norwegian Institute of Public Health (NIPH), where measurements of 12 synthetic phenols (5 bisphenols; triclosan; triclocarban; benzophenone-3; 4 parabens), 13 phthalate metabolites and 2 metabolites of a non-phthalate plasticizer (di(isononyl)cyclohexane-1,2-dicarboxylate, DINCH) concentrations were done (Supplementary Table S1). Phthalate and DINCH metabolites were analysed and quantified using high performance liquid chromatography coupled to mass spectrometry (HPLC-MS-MS) (Sabaredzovic et al., 2015), while for phenols we used ultra-high-performance liquid chromatography coupled to mass spectrometry (UPLC-MS-MS) (Sakhi et al., 2018). For both methods, procedural blanks, in-house quality controls and standard reference material (SRM 3673) from National Institute of Standards and Technology (NIST) were analysed along with the samples. The accuracy ranged from 70% to 126%, and the precision given as

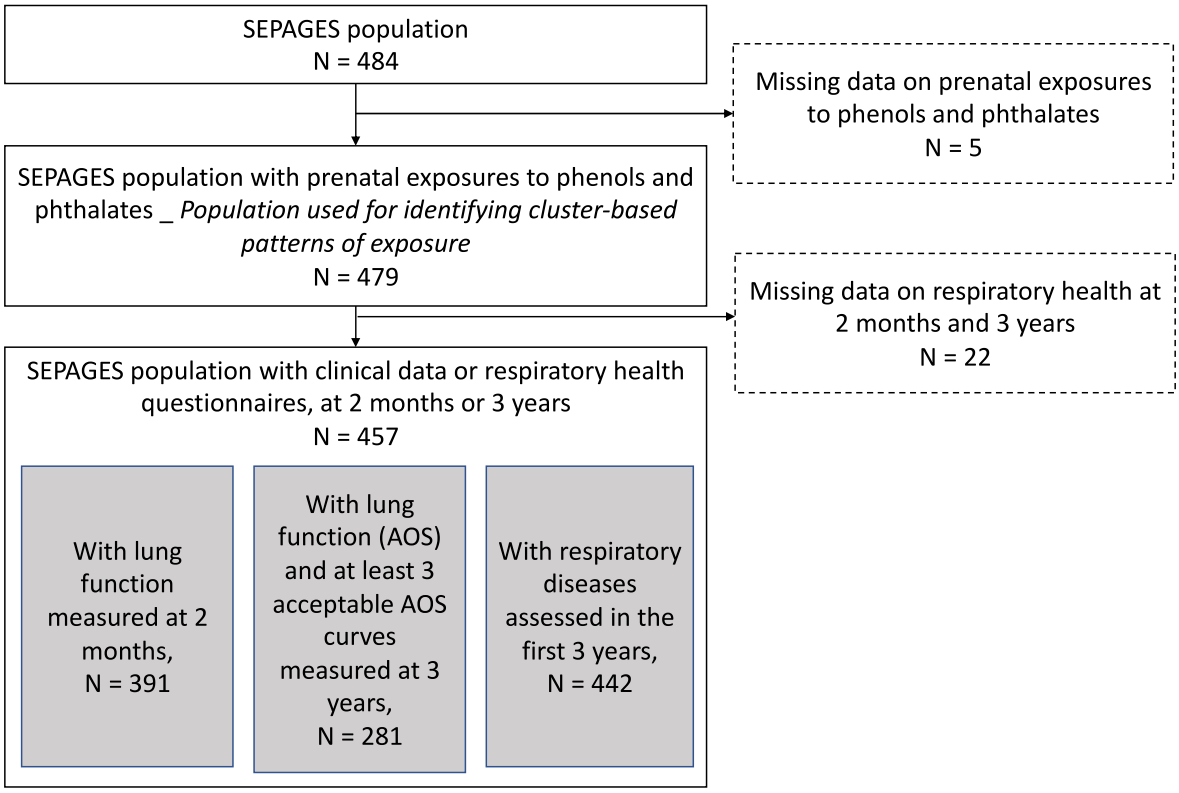

Fig. 1. Flowchart of the study population.
Abbreviations: AOS: Airway Oscillometry, SEPAGES: Suivi de l'Exposition à la Pollution Atmosphérique durant le Grossesse et Effet sur le Santé

relative standard deviation was below 26% for phenols, phthalates and DINCH metabolites. The free and conjugated forms of phenol biomarkers were preliminarily measured in samples from 50 women. Since these preliminary measurements did not suggest external contamination (Rolland et al., 2020), for the remaining participating women, we relied on the total form (free + conjugated).

Bisphenols AF, B, F and triclocarban were detected in less than 5% of the pooled samples and were not considered in our analysis. In addition, we created a molar sum of metabolites from the same parent compound (ΣDEHP (Di(2-ethylhexyl) phthalate), ΣDiNP (Diisononyl phthalate) and ΣDINCH) (Supplementary Table S1). In total, 16 exposures were included in the study with 8 synthetic phenols (methyl, ethyl, propyl, butyl parabens; bisphenol A and S; triclosan; benzophenone-3), 5 phthalate metabolites (MEP, MnBP, MiBP, MBzP, OH-MPHP), 3 molar sums (ΣDEHP, ΣDINCH, ΣDiNP).

Bisphenol S and butyl paraben, two biomarker concentrations with more than 30% of their values below the LOD (Limit of Detection), were binarized as detected, not detected. Others were singly imputed using the compound's probability distribution (Helsel, 2012).

Then, synthetic phenols and phthalates were standardized on analytical batch, transport time between participant's house and biobank and defrosting time during the pooling procedure using a 2-step standardization method based on regression residuals, when needed (i.e., when the conditions listed above were associated (p-val <0.2) with the studied biomarker (Guilbert et al., 2021; Mortamais et al., 2012)). The conditions for which each biomarker concentrations were standardized for are listed in Supplementary Table S2.

For continuous exposures, we computed the mean of naturally log transformed concentrations measured in urine sample pools collected in the 1st and 2nd collection week. For categorical exposures, we used a binary variable (not detected at any trimester vs. detected at least one trimester).

2.3. Respiratory health assessment

At the 2-month visit (median: 6.9 weeks), infants performed a lung function test during natural sleep, in supine position and with the head midline as recommended by the ATS/ERS (American Thorax Society/European Respiratory Society) guidelines (Bates et al., 2000), see also detail in Supplemental Material. Tidal breathing flow-volume loops (TBFVL) were recorded and three measurements of nitrogen multiple-breath washout (N_2MBW) were performed. The 2 TBFVL parameters retained in the present analysis, tidal volume (V_T) and the ratio of the time to peak tidal expiratory flow (t_{PTEF}) to expiratory time (t_E), was estimated on the first 30 to 50 regular breaths. t_{PTEF}/t_E ratio is expected to decrease with airflow obstruction. The N_2MBW technique measures lung volumes and ventilation heterogeneity. Using the Exhalyzer© device and Spiroware© software (Ecomedics), infants inhaled pure oxygen (O_2) and the concentration of exhaled N_2 was monitored. The estimated parameters were: 1) functional residual capacity (FRC), and; 2) lung clearance index (LCI), defined as the number of respirations needed to achieve a concentration of N_2 below 2.5% (Supplementary Table S3). LCI increases with peripheral ventilation inhomogeneity due to airway obstruction. Because the use of pure oxygen may induce a transient decrease in tidal volume that can affect FRC and LCI measures (Gustafsson et al., 2017), FRC and LCI values were corrected for the degree of hypoventilation using a 2-step standardization method based on regression residuals (Mortamais et al., 2012). A total of 865 valid N_2MBW tests were retained, with a median [Q1; Q3] of 3 [2; 3] tests per child. For each child, both LCI and FRC corrected values were averaged.

At the age of 3 years (median: 3.1 yrs), the impedance of the respiratory system was assessed based on oscillometry using a commercial device (TremoFlo, Thorasys Systems Montreal, Quebec, Canada) complying with current European standards (Beydon et al., 2007; King et al., 2020). Pressure waves with frequencies varying from 7 to 41 Hz are applied during tidal breaths and lung impedance is calculated from the changes in flow and pressure. Three to five acceptable measurements

(excluding leakage, swallowing, glottis closure, vocalization or obstruction of the mouthpiece by the tongue) were obtained and averaged. The parameters included in this study are raw values of resistance and reactance at a frequency of 7 Hz (R_7 and X_7), the area of the reactance curve (AX) and the frequency-dependence of resistance, defined by the resistance difference between 7 and 19 Hz ($R_{7\text{-}19}$). R_7 is a parameter that reflects large airway resistance, while AX and $R_{7\text{-}19}$ better characterize the peripheral airways. $R_{7\text{-}19}$ also evaluates the heterogeneous obstruction of the distal airway. Increased R_7, $R_{7\text{-}19}$ and AX, and decreased X_7 are indicators of a reduced lung function (Supplementary Table S3). Among the 320 children who performed oscillometry measurements, 281 children produced technically acceptable and reproducible measurements, i.e. at least 3 measurements with a coefficient of variation <15% for R_7. For each child, the mean value of the valid measurements was calculated for each parameter and used for the analyses.

Parents of the children replied to a respiratory questionnaire at 2 months, 1 year, 2 years and 3 years, based on the ISAAC (International Study questionnaire of Asthma and Allergies in Childhood) questionnaire and administrated by an interviewer. Ever wheezing, bronchitis over the first 3-years of life and bronchiolitis over the 2 years of life were defined by a positive response to the questions: "In the last 12 months (or since birth for the 2 month-questionnaire), has your child had wheezing in the chest (respectively bronchitis, bronchiolitis)?". Doctor diagnosed asthma was defined by a positive answer to: "In the last 12 months (or since birth for the 2 month-questionnaire), has your child had asthma" and to: "Was the asthma diagnosis confirmed by a medical doctor?" in at least one questionnaire.

2.4. Statistical analyses

2.4.1. Identification of prenatal exposure patterns through cluster analysis

We applied a cluster-based statistical model, the Variable Selection for Model-Based Clustering of Mixed-Type Data method (package VarSelLCM, R) to identify prenatal exposure patterns based on biomarker concentrations. This (unsupervised) model uses mixture clustering model to create clusters of individuals independently of health outcomes (Marbac and Sedki, 2017). Models with a different number of clusters were successively conducted and compared in terms of statistical performance (including stability, discriminative power, distribution within classes and posterior probability), ease of interpretation (*a priori* meaningful clusters) and clusters size to select the most appropriate model. Cluster were presented in a radar chart, showing the percentage of children with a prenatal biomarker's concentration above the whole population median concentration for continuous compounds and detected concentration for categorical compounds. "Low", "moderate" and "high" exposure concentrations were defined as <49%, between 49% and 59% and >59% of women with concentration level above the whole population median concentration, respectively.

2.4.2. Association studies between prenatal exposures and respiratory health

For comparison with previous studies relying on uni-pollutant models, linear (for lung function parameters measured at 2 months and 3 years) and logistic regression models (for binary respiratory diseases/symptoms) adjusted on potential confounders were performed to assess the associations first between cluster-based prenatal exposure patterns to synthetic phenols and phthalates and respiratory health and secondly between prenatal exposure to each chemical separately (Exposome Wide Association Study (ExWAS) approach) and respiratory health.

Based on the literature, we built a DAG (Directed Acyclic Graph, DAgitty program) from which we selected the adjustment factors included in the main models: maternal body mass index (BMI) before pregnancy (continuous), maternal age (continuous), highest parental educational level (maximum number of study years after the high-school degree between the parents in two categories: more or less than 5 years), parental history of rhinitis, parity, season of urine sampling, passive smoking (in utero (maternal active smoking), and until the assessment of child respiratory function), breastfeeding at 2 months (yes / no), child's sex, weight and height (continuous) at the clinical visit. To avoid losing individuals due to missing data in confounders, multiple imputations (m = 20 datasets) were done with Multivariate Imputation by Chained Equations (package mice, R).

We investigated a potential modifying effect of the child's sex in the association between prenatal exposure patterns and respiratory health by integrating an interaction term between exposure patterns and child's sex in the regression models. Analysis stratified for child sex were run if the p-value for interaction was ≤ 0.20. A sensitivity analysis to address the robustness of the results to the uncertainty in the cluster assignment was conducted by excluding mother-child pairs whose highest posterior probability in cluster membership was below 0.70 (n = 61). A second sensitivity analysis was performed to assess the effect of the extreme values of the outcome on the associations between respiratory health data and clusters. For each outcome, 1% of their maximum values and 1% of their minimum values were removed from the analyses.

For all analyses, associations with p-values less than 0.05 were considered statistically significant and those with p-values between 0.05 and 0.1 were considered as suggestive associations. Because the main association analysis was based on the cluster-based exposure patterns and because the ExWAS analysis was considered as a support for interpreting the results from the main analysis, we did not correct for multiple hypotheses testing.

All statistical analyses were conducted with the version 4.0.5 of R.

3. Results

3.1. Population characteristics

Women included in the analysis (mean (sd) age at conception = 32.5 (3.9) years) were highly educated (70% with Master's or higher degree) (Table 1). Among children (46% girls, mean (sd) birth weight = 3.3 kg (0.4)), 85% were breastfed for at least 2 months and 14% were exposed to tobacco smoke in early-life (in-utero exposure and/or passive smoking in the first 2 months). Regarding respiratory health in the first 3 years of life, 14%, 38% and 21% had ever had asthma, bronchiolitis and bronchitis, respectively.

Correlation between lung function parameters varied from −0.23 between LCI and t_{PTEF}/t_E to 0.59 between V_T and LCI at 2 months and from −0.72 between AX and X_7 to 0.75 between R_7 and AX at 3 years (Supplementary Table S3).

3.2. Identification of prenatal exposure patterns using cluster analysis

The pairwise correlations between biomarkers did not show strong correlations (all Spearman's coefficients of correlation were below 0.6) (Supplementary Fig. S1). Among phthalate metabolites, the highest median concentration was observed for MEP and among synthetic phenols it was observed for methyl paraben (Supplementary Table S4). The cluster analysis identified 4 clusters characterized by specific prenatal exposure patterns: 1) low concentration of phthalate metabolites and phenols (N = 106), 2) low concentration of phenols and moderate concentration of phthalate metabolites (N = 162), 3) high concentration of all biomarkers except bisphenol S (N = 109), 4) high concentration of parabens, moderate concentration of other phenols and low concentration of phthalate metabolites (N = 102) (Fig. 2). The overall mean posterior probability was high (mean (min-max) 0.90 (0.41–1.00)) and was similar across the 4 clusters (mean for cluster 1, 2, 3 and 4 were 0.90, 0.88, 0.93 and 0.91, respectively).

Table 1
Characteristics of included mother-child pairs from SEPAGES cohort population (N = 457).

Characteristics	N (%)	Mean (SD)
Maternal characteristics		
Maternal BMI before pregnancy (kg/m^2)	453	22.4 (3.8)
Maternal age at conception (years)	457	32.5 (3.9)
Parity		
Nulliparous	207 (45.3)	
Multiparous	250 (54.7)	
Season of sampling		
January–March	131 (28.8)	
April–June	134 (29.5)	
July–September	105 (23.1)	
October–December	85 (18.7)	
Child characteristics		
Child sex		
Boys	246 (53.8)	
Girls	211 (46.2)	
Gestational age at delivery (days)	457	278.3 (10.1)
Child weight at birth (kg)	457	3.3 (0.4)
Parents' highest educational level		
< master degree	136 (29.8)	
≥ master degree	321 (70.2)	
Breastfeeding duration		
<2 months	67 (14.9)	
≥2 months	383 (85.1)	
Parental history of rhinitis		
No	157 (36.7)	
Yes	271 (63.3)	
Data at the 2-month visit		
Child height at 2 months (cm)	430	56.4 (2.2)
Child weight at 2 months (kg)	431	4.8 (0.6)
Tobacco smoking exposure up to 2 months		
No	388 (85.5)	
Yes	66 (14.5)	
LCI	350	7.6 (1.1)
FRC (ml)	350	104.9 (18.1)
V_T (ml)	325	34.2 (7.1)
t_{PTEF}/t_E (%)	325	35.2 (9.9)
Data at the 3-year visit		
Child height at 3 years (cm)	306	95.5 (3.7)
Child weight at 3 years (kg)	306	14.7 (1.6)
Tobacco smoking exposure up to 3 years		
No	327 (71.9)	
Yes	128 (28.1)	
R_7 (cmH2O.s/L)	306	11.9 (2.3)
$R_{7\text{-}19}$ (cmH2O.s/L)	306	1.3 (1.0)
X_7 (cmH2O.s/L)	306	−4.2 (1.2)
AX (cmH2O/L)	306	73.6 (35.3)
Child asthma ever		
No	363 (85.8)	
Yes	60 (14.2)	
Child wheeze ever		
No	280 (66.0)	
Yes	144 (34.0)	
Child bronchiolitis up to the 2nd year of life		
No	262 (61.9)	
Yes	161 (38.1)	
Child bronchitis ever		
No	332 (78.5)	
Yes	91 (21.5)	

Abbreviations: AX: area under the reactance curve, BMI: Body Mass Index, FRC: Functional Residual Capacity, LCI: Lung Clearance Index, R_7: Resistance at 7 Hz, $R_{7\text{-}19}$: Resistance between 7 and 19 Hz, t_{PTEF}/t_E: ratio of time to peak tidal expiratory flow to expiratory time, V_T: tidal volume, X_7: Reactance at 7 Hz.

3.3. Associations between prenatal exposure patterns and child respiratory health: the mixture effects

Compared to children belonging to cluster 1 (low phenols - low phthalates), children belonging to cluster 2 (low phenols - moderate phthalates) had lower FRC (β = −5.72 [−10.50; −0.95]), lower V_T (β = −2.67 [−4.56; −0.78]), higher t_{PTEF}/t_E (β = 3.41 [0.34; 6.48]) and a suggestive association was observed with a lower LCI (β = −0.25 [−0.54; 0.04]) at 2 months, while they did not differ for oscillometry parameters and symptoms assessed at 3 years (Fig. 3 with re-scaled lung function parameters, i.e. divided by their standard deviation, Supplementary Table S5 with raw lung-function parameters). Children belonging to cluster 3 (high concentrations of all biomarkers except bisphenol S) had lower LCI (β = −0.35 [−0.68; −0.02]) and higher t_{PTEF}/t_E (β = 4.18 [0.71; 7.64]) at 2 months, while no difference was observed for oscillometry parameters and symptoms at 3 years. Children belonging to cluster 4 (high paraben-moderate phenols and low phthalates) did not differ from those belonging to cluster 1 for any of the respiratory health parameters (all p-values above 0.12).

3.4. Associations between each exposure biomarker and child respiratory health: the ExWAS approach (Fig. 4 and Supplementary Tables S6, S7, S8)

In the single-pollutant models, among phthalate metabolites, increased urinary concentration of MiBP was associated with lower LCI (β = −0.27, 95% CI: (−0.46; −0.09) for an increase by 1 unit in ln (MiBP)) and lower V_T (β = −1.67 mL, 95% CI: (−2.85; −0.48)). It also tended to be associated with lower FRC (β = −2.83 mL, 95% CI: (−5.87; 0.21)) and higher t_{PTEF}/t_E (β = 1.71%, 95% CI: (−0.22; 3.65)) at 2 months. MnBP was associated with lower LCI (β = −0.27, 95% CI: (−0.47; −0.07)) and there was trend for lower FRC (β = −3.04 mL, 95% CI: (−6.34; 0.27)). MBzP was associated with lower FRC (β = −3.69 mL, 95% CI: (−6.34; −1.04)). No association with any oscillometry parameters and respiratory function at 3 years was observed for these phthalate metabolites. ΣDINCH was associated with higher t_{PTEF}/t_E at 2 months (β = 1.64%, 95% CI: (0.24; 3.03)) and higher risk of wheeze over the first 3 years of life (OR = 1.32, 95% CI: (1.01; 1.72)). The other phthalate metabolites were not associated with respiratory health parameters.

Among synthetic phenols, all parabens tended to be positively associated with increased AX (p-values ranged from 0.05 for ethyl paraben to 0.12 for methyl paraben). Ethyl and methyl parabens were also associated with a higher risk of bronchitis (OR = 1.18, 95% CI: (0.98; 1.41) and 1.19, 95% CI: (1.01; 1.40) for ethyl and methyl paraben, respectively) while propyl paraben was associated with increased risk of bronchiolitis at 3 years (OR: 1.09, 95% CI: (1.00; 1.18)). Several associations were observed with bisphenol S. Infants with detected prenatal bisphenol S concentrations had higher risk of asthma (OR = 1.89, 95% CI: (1.05; 3.38)) and bronchiolitis (OR = 1.56, 95% CI: (1.01; 2.38)) at 3 years and higher FRC (β = 4.71 mL, 95% CI: (1.24; 8.18)) and V_T (β = 1.43 mL, 95% CI: (0.04; 2.82)) at 2 months. This compound also tended to be negatively associated with $R_{7\text{-}19}$ (β = −0.22 cmH2O.s/L, 95% CI: (−0.44; 0.01)) at 3 years. We did not observe association for the other synthetic phenols.

3.5. Sensitivity analyses

A single interaction test between the prenatal exposure patterns and child's sex was observed at p value < 0.20 (for cluster 3 and bronchiolitis, p = 0.14), not providing strong evidence for a modification of association by sex. The sensitivity analyses excluding women whose highest posterior probability in cluster membership was below 0.7 (Supplementary Table S9) or children with extreme outcome values (Supplementary Table S10) showed similar results to the main analysis.

4. Discussion

This study is one of the first to address the associations between prenatal exposure to synthetic phenols, phthalates and one non-phthalate plasticizer, considered as a mixture (through exposure patterns assessed by cluster analysis), and objective lung function parameters measured as early as 2 months of age. The main results indicated

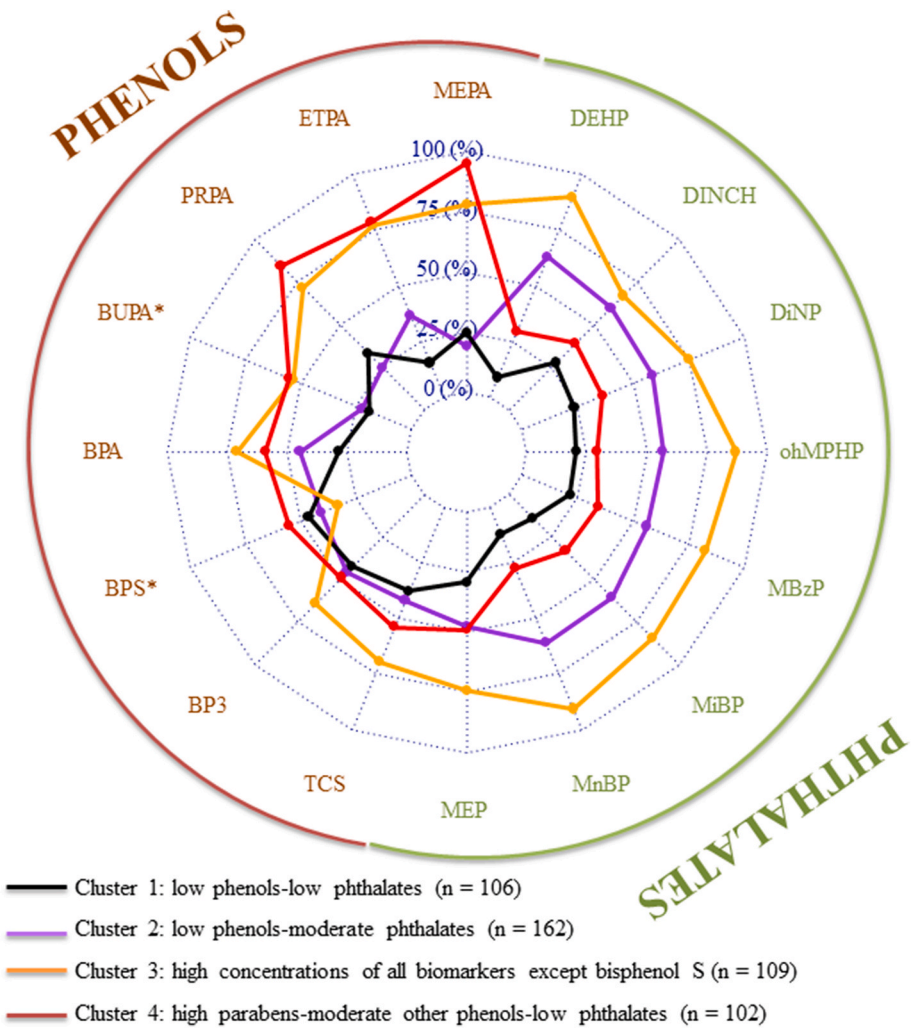

Fig. 2. Radar chart displaying the prenatal biomarker of exposure patterns identified by cluster analysis.

The clustering analysis was performed on ln-transformed concentrations. For each cluster, the radar chart shows the percentage of children with a prenatal biomarker's concentration above the whole population median concentration for continuous compounds and detected concentration for categorical compounds. "Low", "moderate" and "high" exposure concentrations were defined as <49%, between 49% and 59% and >59% of women with concentration level above the whole population median concentration, respectively.

There were 4 clusters: 1) low concentration of all exposures (black, N = 106), 2) low concentration of phenols and moderate concentration of phthalates (purple, N = 162), 3) high concentration of all exposures except bisphenol S (orange, N = 109), 4) high concentration of parabens, moderate concentration of phenols and low concentration of phthalates (red, N = 102).

Abbreviations: BPA: Bisphenol A, BPS: Bisphenol S, BUPA: Butyl paraben, BP3: Benzophenone-3, DEHP: Di(2-ethylhexyl) phthalate, DINCH: Di(isononyl) cyclohexane-1,2-dicarboxylate, DiNP: Diisononyl phthalate, ETPA: Ethyl paraben, MEPA: Methyl paraben, MBzP: Mono-benzyl phthalate, MEP: Mono-ethyl phthalate, MiBP: Mono-isobutyl phthalate, MnBP: Mono-n-butyl phthalate, OH-MPHP: Mono-6-hydroxy-propylheptyl phthalate, PRPA: Propyl paraben, TCS: Triclosan

that, compared to children with low exposure to the mixture, those with moderate concentrations of phthalate metabolites and low concentration of synthetic phenols had lower FRC and V_T, indicating lower lung volumes. However, the exposure pattern characterized by a high concentration of all of the biomarkers except bisphenol S was associated with higher t_{PTEF}/t_E and lower LCI at 2 months which was unexpected since t_{PTEF}/t_E should be decreased and LCI increased with airway obstruction. The ExWAS results, somewhat converged with those of the cluster analysis, and also showed suggestive associations between parabens and lung health at 3 years.

To the best of our knowledge, only two previous studies assessed mixture effects of a large number (>10) of biomarkers (Adgent et al., 2020; Berger et al., 2020). On 319 mother-child pairs from the CHAMACOS study, Berger et al. identified 7 clusters of prenatal exposure (*vs.* 4 in our analysis) and none was associated with asthma or FEV_1 (Forced Expiratory Volume in 1 s) measured at age 7 years (Berger et al., 2020). However, sample size in each cluster was relatively low (between 26 and 68 mother child-pairs depending on the clusters), potentially limiting power of their analysis. Using a different statistical approach, the weighted quantile sum regression, Adgent et al. identified, in 1481 mother-child pairs, a phthalate index associated with increased risk of asthma and wheeze at age 4–6 years in boys only, with MEP weighted highest in the association (Adgent et al., 2020). Our findings do not converge with these results, with no indication of association between cluster-based exposure patterns characterized by moderate or high phthalate biomarkers and asthma and wheezing and no indication of sex-specific effect in these associations. Comparison of our results to those from previous studies is not straightforward because this is the first study assessing lung function as early as at 2 months of age, while the previous studies assessed lung function by spirometry in children 5 years or older (Abellan et al., 2022; Agier et al., 2019; Berger et al., 2020, 2019; 2018; Spanier et al., 2014; Vernet et al., 2017). Differences in exposure timing (3rd trimester *vs.* a 2nd and 3rd trimesters) in number of urinary samples considered (single *vs.* up-to 42 samples) and in biomarkers jointly considered through clusters (phthalates *vs.* phenols and phthalates) might explain results discrepancies. Differences in exposure levels between cohorts could lead to different results as well. Overall, concentrations measured in the SEPAGES cohort were lower or of similar range (e.g., bisphenol A, ΣDEHP, MnBP, MiBP) than those of previous studies (Supplementary Fig. S2).

4.1. Phthalates and child respiratory health

Both our cluster and ExWAS analysis suggested associations between phthalates and lower lung volumes at 2 months. Indeed, our results showed associations between the exposure pattern characterized by a mixture of moderate phthalate metabolite concentrations and low synthetic phenol concentrations and lower FRC and V_T, both indicating lower lung volumes at two months. The lack of association with lung function parameters at 3 years suggests that effects of prenatal exposure to endocrine disruptors on child respiratory health might be easier to evidence soon after birth, possibly because lung function at 3 years is also influenced by postnatal exposures. Children prenatally exposed to both high phthalates and phenols (cluster 3) also showed a trend for

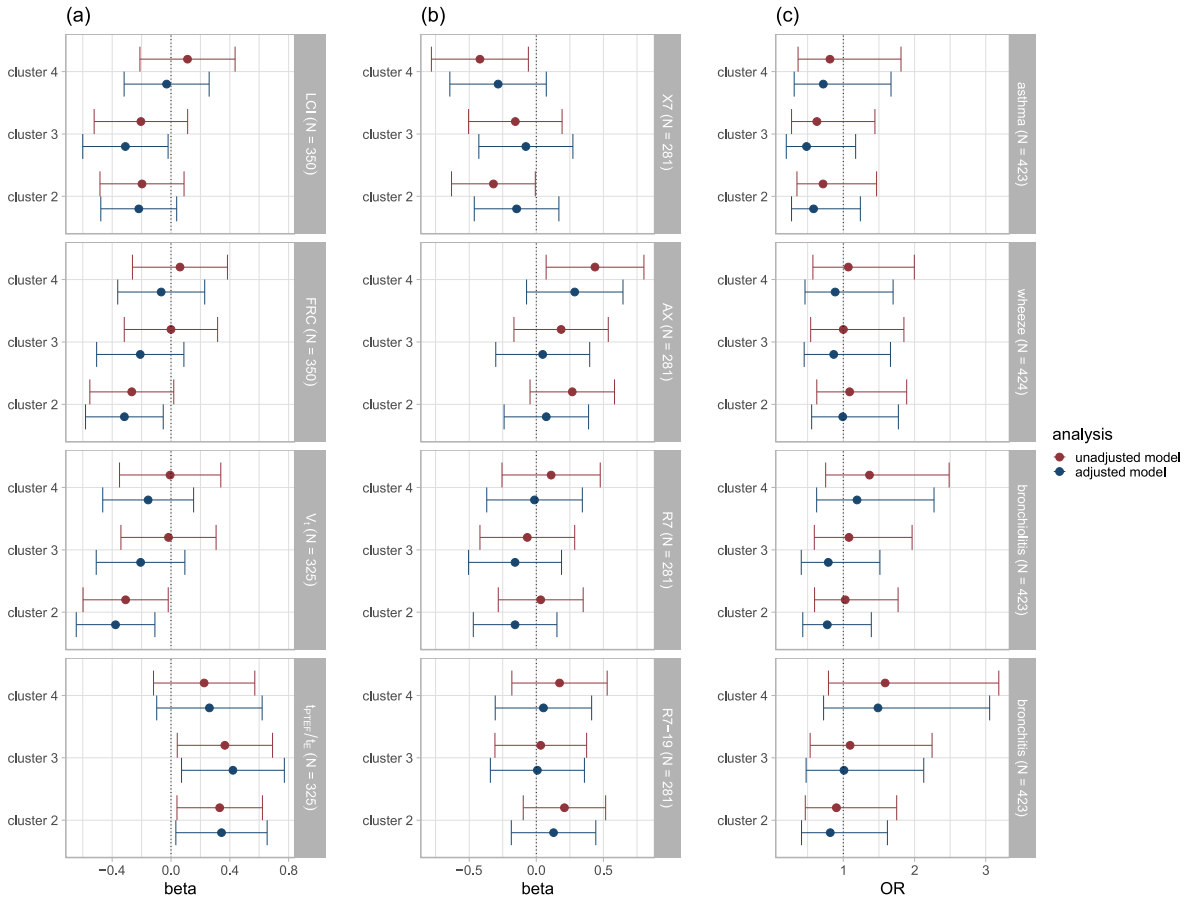

Fig. 3. Unadjusted and adjusted-associations between prenatal exposure patterns to phenols and phthalate metabolites and (a) child lung function at 2 months, (b) child lung function at 3 years, (c) child respiratory outcomes in the first 3 years of life.
Cluster 2 "low phenols-moderate phthalates" (n = 162), cluster 3 "high phenols-high phthalates (except bisphenol S)" (n = 109) and cluster 4 "high parabens-moderate phenols-low phthalates" (n = 102) were compared to cluster 1 "low phenols-low phthalates" (reference group, n = 106). Each continuous outcome (figure (a) and (b)) was re-scaled (divided by its standard deviation) to allow direct comparison of the beta estimates. See Supplemental Material, Table S5 for associations with raw lung function parameters. Beta values and their 95%CI for figure (a) and (b) were estimated by linear regression models, and OR and their 95% CI for figure (c) were estimated by logistic regression models.
Abbreviations: AX: area under the reactance curve, FRC: Functional Residual Capacity, LCI: Lung Clearance Index, R_7: Resistance at 7 Hz, $R_{7\text{-}19}$: Resistance between 7 and 19 Hz, t_{PTEF}/t_E: ratio of time to peak tidal expiratory flow to expiratory time, V_t: tidal volume, X_7: Reactance at 7 Hz

lower lung volume parameters at 2 months, although associations were not statistically significant. Results do not indicate stronger association with cluster 3 as compared to cluster 2 as we could have expected. We do not have an explanation for this observation, but lower sample size can be part of it (33% less individuals in cluster 3 than in cluster 2). In addition, the fact that clusters were defined on exposure patterns and not on similarities in biological effects of the different compounds may limit our ability to identify the additive and dose-response effects of multiple compounds involved in same biological pathways.

In the ExWAS approach, in accordance with the mixture-effect analysis, increased prenatal exposure to MnBP, MiBP and MBzP was associated with lower lung volume parameters (FRC and V_T) at 2 months, but not with lung function parameters at 3 years, nor with respiratory diseases/symptoms. Prenatal exposure to MBzP was associated with wheezing and asthma in several previous studies, including in 0.5–7 years old children in INMA cohort (Gascon et al., 2015), in 8 year old boys in the Taiwan Maternal Infant Cohort Study (Ku et al., 2015), in 9 year-old children from the REPRO_PL cohort in Poland (Podlecka et al., 2020), in 5–11 year old children in CCCEH US cohort (Whyatt et al., 2014), although other studies did not evidence any association (Adgent et al., 2020; Agier et al., 2019; Buckley et al., 2018; Vernet et al., 2017).

Unexpectedly, both the mixture and single-pollutant analyses also suggested "protective" effects of phthalates on two other lung function parameters measured at 2 months, the LCI and t_{PTEF}/t_E, both markers of airflow obstruction. LCI is recognized as more reliable and sensitive to airway obstruction than t_{PTEF}/t_E, the latter being possibly influenced by central control of ventilation. Taking into account the respiratory rate in the analysis (a determinant of these two lung function parameters) did not change the results (data not shown). We do not have any clear explanation, but potential missing confounders (e.g., nutrition) might partly explain these unexpected results.

4.2. Phenols and child respiratory health

In our study population, prenatal exposure to the parabens tended to be associated with health parameters assessed at 3 years (e.g., AX, increased risk of bronchitis or bronchiolitis), while no association was observed in neonates. The positive association with AX at 3 years, was also suggested in the cluster analysis (p-value of 0.12 for the association with cluster 4 characterized by high paraben concentrations). Although there are limits in extrapolating findings from experimental studies in murine models to humans, it is interesting to note that the lower association in the cluster analysis compared to the single exposure analysis for parabens, is in agreement with a study considering an experimental mouse model (Junge et al., 2022). Indeed, Junge and co-authors

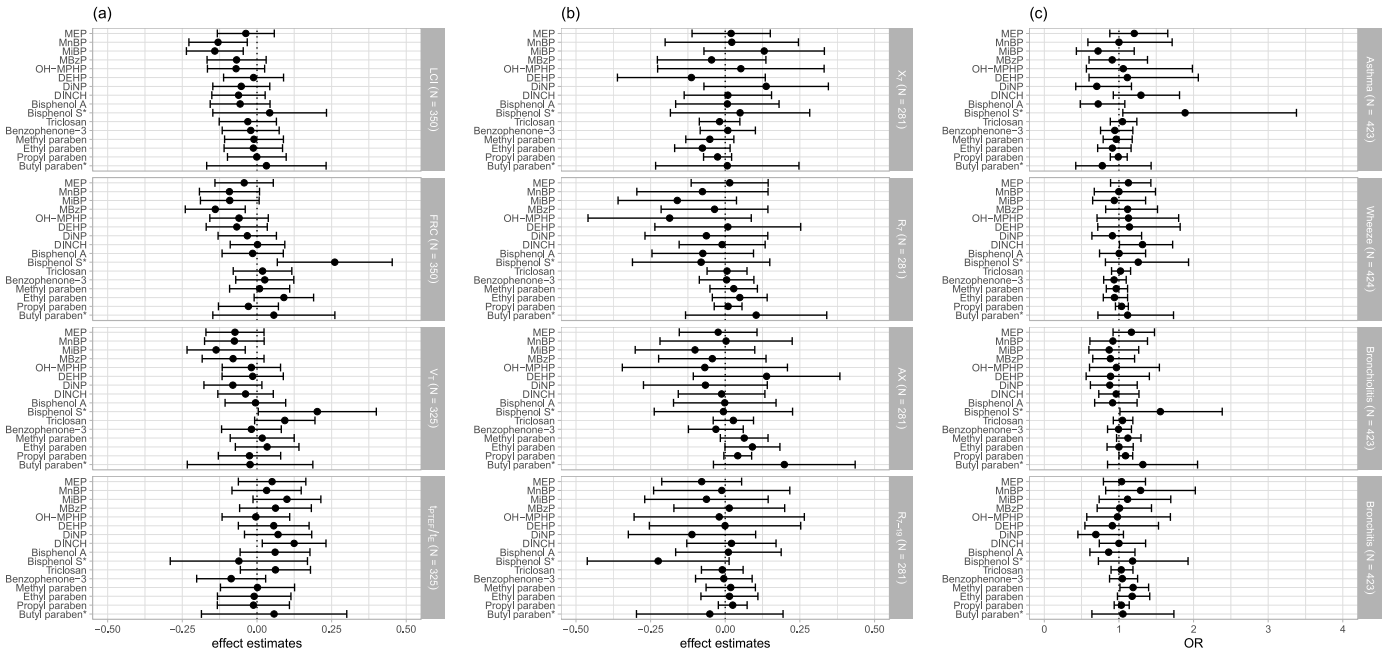

Fig. 4. Adjusted associations between each prenatal exposure biomarker and (a) child lung function at 2 months, (b) child lung function at 3 years, (c) child respiratory outcomes in the first 3 years of life.

Beta values and their 95%CI were estimated by linear regression models and OR and their 95%CI were estimated by logistic regression models. Each continuous outcome was re-scaled (divided by its standard deviation) to allow direct comparison of the beta estimates. See Supplemental Material, Table S6, S7, S8 for associations with raw lung function parameters.

*Represents categorical exposures coding with 2 categories: 1) not detected, 2) detected at least one trimester.

Abbreviations: AX: area under the reactance curve, DEHP: Di(2-ethylhexyl) phthalate, DINCH: Di(isononyl)cyclohexane-1, 2-dicarboxylate, DiNP: Diisononyl phthalate, FRC: functional residual capacity, LCI: lung clearance index, MBzP: Mono-benzyl phthalate, MEP: Monoethyl phthalate, MiBP: Mono-isobutyl phthalate, MnBP: Mono-n-butyl phthalate, OH-MPHP: Mono-6-hydroxy-propylheptyl phthalate, R_7: resistance at 7 Hz, $R_{7\text{-}19}$: resistance between 7 and 19 Hz, $t_{PTEF/tE}$: ratio of time to peak tidal expiratory flow to expiratory time, V_T: tidal volume, X_7: reactance at 7 Hz

identified an asthma-promoting effect of prenatal exposure to ethyl paraben in the female offspring but no effect of exposure to a mixture of ethyl paraben and butyl paraben, indicating that co-exposure can counterbalance the biological effect of each paraben. Several studies previously looked at the associations between exposure to parabens and child respiratory health assessed using questionnaires and FEV_1 measurements. Two reported decreased FEV_1 with increased exposure to ethyl paraben, however, one found this association with prenatal exposure (Vernet et al., 2017) while for the other study, the association was only observed with postnatal exposure (Agier et al., 2019). This paraben was not associated with asthma in children in a recent study (Junge et al., 2022) or was not assessed in the three other studies (Berger et al., 2020, 2018; Lee-Sarwar et al., 2018). Two studies also reported an association between propyl paraben and asthma but the sign of the association diverged (Berger et al., 2020; Vernet et al., 2017). Regarding methyl paraben, Vernet et al. reported a reduced rates of bronchiolitis/bronchitis in association with prenatal exposure to methyl paraben in boys while an increased risk for bronchitis was observed in our study. Although most of the previous epidemiological studies reported associations with at least one paraben, the respiratory health parameter affected, the sign of the associations and the timing of exposure (pre *vs.* post natal) often differed, preventing us to draw strong conclusions (Berger et al., 2020, 2018; Junge et al., 2022; Lee-Sarwar et al., 2018). Nevertheless, studies indicating that the antimicrobial properties of parabens could affect gut and respiratory tract microbiome diversity, which could in turn increase the risk of developing asthma and allergic diseases, provide support for a possible effect of parabens on respiratory health.

The other synthetic phenol for which we detected associations was bisphenol S. Bisphenol S was associated with increased risk of asthma and bronchiolitis symptoms at 3 years, while the associations observed between this compound and the other parameters tended to be protective (decreased $R_{7\text{-}19}$ at 3 years suggesting decreased peripheral airways resistance, increased FRC and V_T at 2 months suggesting higher lung volume). The only study looking at the associations between prenatal exposure to bisphenol S and child respiratory health assessed the risk of wheezing, asthma and FEV_1 and reported a protective association with wheezing risk among 1 to 11 year-old children (Abellan et al., 2022). As in our study, the rate of detection for this compound was relatively low (49% > LOD). Further analyses on recent populations, for which frequency of detection is likely to be higher for this compound that has replaced bisphenol A in some products, are needed. Authors also reported sex specific association for bisphenol A that was associated with increased risk of wheezing and asthma in girls (Abellan et al., 2022). However, the lack of statistical interaction between sex and bisphenol A in our study did not support a sex-specific effect.

4.3. Strengths and limitations

We relied on objective measures of lung function in children, as early as 2-months of age and at 3 years, using validated techniques (TBFVL, muliple-breath, Airwaves oscillometry) that remain poorly used in population-based cohort studies. In addition, this study is one of the first to estimate the combined effect of multiple exposures on child respiratory health through the identification of exposure patterns by cluster analysis. Using unsupervised cluster-based approach, meaning that exposures were combined given co-exposures in the study population, independently of the respiratory health and without any *a priori* knowledge on their effects on child health, the consistency of associations between exposure profiles and various respiratory health can be addressed. Moreover, this approach has the advantage to drastically reduce the number of tests, which is desired to diminish the false

discovery rate.

Our study also has limitations to consider. The sample size of our study, with 281–442 mother-child pairs according to the health outcome considered, is among the smallest compared to previous studies that were based on 171 (Ku et al., 2015) to more than 1033 mother-child pairs (Agier et al., 2019). This has a direct impact in our ability to detect statistically significant associations. Nevertheless, the statistical power is not only driven by the sample size, and has to be discussed in light of additional parameters, including the accuracy in the exposure assessment. It has been demonstrated that, for a given sample size, increasing the number of samples collected per participant to assess biomarkers with strong temporal variability resulted in increased accuracy of exposure assessment and in improved power (Perrier et al., 2016; Vernet et al., 2019). While 1 urine sample was used in almost all previous publications, except for few studies that used 2 or 3 samples (Berger et al., 2020, 2019; Spanier et al., 2014, 2012), our study relied on 2 pools of 21 urine samples each collected during pregnancy.

Regarding the respiratory health assessment, although LCI and FRC values were *a posteriori* statistically corrected to the degree of hypoventilation due to the use of pure oxygen in the N_2MBW technique (because it is not allowed to use SF6 in France), we acknowledge that a measurement error in these parameters due to hypoventilation might have decreased our ability to detect associations. Because 2 different techniques of lung function measurement were used at 2 months and 3 years of age, the effect of prenatal exposure on lung function growth could not be assessed. The mixtures of exposures were defined by the biomarker concentrations and do not account for toxicological knowledges, the latter being too limited to allow us to *a priori* define cluster of exposure. Mothers included in the SEPAGES cohort are highly educated, which can be seen as a limit in terms of generalization of our results but it is also a strength by analyzing a homogenous population, thus limiting risk for confounding bias.

5. Conclusion

In conclusion, besides some unexpected and null associations, our study was suggestive of associations between prenatal exposure to several phthalates and low lung volumes at 2 months and between parabens and markers of chest stiffness and bronchitis at 3 years.

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Author contribution statement

Ophélie Coiffier: software (lead), writing – original draft (equal); format analysis (lead), visualization (lead); Sarah Lyon-Caen: investigation (equal); review and editing (equal); Anne Boudier: Resources (equal); review and editing (equal); Joane Quentin: investigation (equal); review and editing (equal); Yoann Gioria: investigation (equal); review and editing (equal); Isabelle Pin: ressources (equal); review and editing (equal); Sam Bayat: ressources (equal); review and editing (equal); Cathrine Thomsen: investigation (equal); review and editing (equal); Amrit K. Sakhi: investigation (equal); review and editing (equal); Azemira Sabaredzovic: investigation (equal); review and editing (equal); Rémy Slama: ressources (equal); review and editing (equal); Claire Philippat: conceptualization (equal), writing – original draft (equal), supervision (equal), project administration (equal); Valérie Siroux: conceptualization (equal) writing – original draft (equal); funding acquisition (lead), supervision (equal), project administration (equal).

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121794.

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