Children's Urinary Phthalate Metabolites and Fractional Exhaled Nitric Oxide in an Urban Cohort

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Rationale: Phthalates are used widely in consumer products. Exposure to several phthalates has been associated with respiratory symptoms and decreased lung function. Associations between children's phthalate exposures and fractional exhaled nitric oxide (F_{ENO}), a biomarker of airway inflammation, have not been examined.

Objectives: We hypothesized that urinary concentrations of four phthalate metabolites would be positively associated with F_{ENO} and that these associations would be stronger among children with seroatopy or wheeze.

Methods: In an urban ongoing birth cohort, 244 children had phthalate metabolites determined in urine collected on the same day as F_{ENO} measurement. Repeated sampling gathered 313 observations between ages 4.9 and 9.1 years. Seroatopy was assessed by specific IgE. Wheeze in the past year was assessed by validated questionnaire. Regression models used generalized estimating equations.

Measurements and Main Results: Log-unit increases in urinary concentrations of metabolites of diethyl phthalate (DEP) and butylbenzyl phthalate (BBzP) were associated with a 6.6% (95% confidence interval [CI] 0.5–13.1%) and 8.7% (95% CI, 1.9–16.0%) increase in F_{ENO} , respectively, adjusting for other phthalate metabolites and

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Phthalates are used widely in consumer products, including plastics, vinyl flooring, and personal care products, such that exposure is ubiquitous. Exposure has been linked to adverse neurobehavioral and reproductive effects in children, and there is emerging evidence of associations between the phthalates commonly measured in indoor air and asthma, including associations with respiratory symptoms.

What This Study Adds to the Field

This study demonstrates an association between current phthalate exposure and fractional exhaled nitric oxide, a marker of airway inflammation. This association was observed among a well-characterized population-based study of inner-city children. The association was greater among children with recent wheeze, who may be most susceptible to changes in and consequences of airway inflammation, supporting a relevance of these exposures to asthma morbidity.

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potential covariates/confounders. There was no association between concentrations of metabolites of di(2-ethylhexyl) phthalate or di-*n*-butyl phthalate and F_{ENO} . There was no significant interaction by seroatopy. The BBzP metabolite association was significantly stronger among children who wheeze ($P = 0.016$).

Conclusions: Independent associations between exposures to DEP and BBzP and F_{ENO} in a cohort of inner-city children were observed. These results suggest that these two ubiquitous phthalates, previously shown to have substantial contributions from inhalation, are positively associated with airway inflammation in children.

Keywords: airway inflammation; asthma; diethyl phthalate; butylbenzyl phthalate; fractional exhaled nitric oxide

Phthalates are a group of high-production volume compounds added to plastics to confer flexibility (e.g., vinyl flooring) and used in personal care and other consumer products (1). Several phthalates can act as endocrine disruptors, and early life exposure has been associated with adverse neurobehavioral and reproductive effects in children (2–6). Numerous studies monitoring phthalate metabolites in urine have shown widespread exposure, including among inner-city populations, with higher concentrations observed among children than adults (7–12). Although phthalates are rapidly metabolized, the ubiquity of their metabolites in urine suggests that exposure occurs nearly constantly, with contributions from ingestion, inhalation, and dermal absorption (13). Personal air concentrations of two phthalates,

diethyl phthalate (DEP), which is more volatile than most other phthalates, and butylbenzyl phthalate (BBzP), which is found on respirable particles, were correlated with maternal urinary concentrations of the corresponding metabolites, suggesting inhalation as an important route of exposure (8, 11).

In a cross-sectional case-control analysis of Swedish children aged 3 to 8 years, di(2-ethylhexyl) phthalate (DEHP) and BBzP in bedroom dust were associated with physician-confirmed asthma and rhinitis, respectively (14). In a cross-sectional analysis of adults from the National Health and Nutrition Examination Survey (NHANES), urinary concentrations of monoethyl phthalate (MEP), the metabolite of DEP, and metabolites of di-*n*-butyl phthalate (DnBP) and di-isobutyl phthalate were associated with decreases in FEV₁ (15). Although these findings with asthma, rhinitis, and airflow obstruction suggest the potential for increased airway inflammation with phthalate exposure, it has not been demonstrated.

The measurement of fractional exhaled nitric oxide (F_{ENO}), a biomarker of airway inflammation, offers a method to test for associations between phthalate exposure and subclinical changes in airway inflammation (16). Prospective studies in children reported associations of higher F_{ENO} with subsequent exacerbations in children with asymptomatic asthma after withdrawal of inhaled corticosteroids and increased risk of new-onset asthma among children without asthma (17–19). F_{ENO} is responsive to environmental pollutants that contribute to respiratory health problems. Exposure to inhalant air pollutants, such as respirable particulate matter (PM_{2.5}), black carbon, and formaldehyde, has been associated positively with F_{ENO} (20–23). We are unaware of any population-based studies examining the association between phthalates and F_{ENO}. Airway inflammation and F_{ENO} are increased in both serotopic children and children with asthma; thus, these children may be more responsive to exposures that affect airway inflammation.

Based on the prior human observational studies, we hypothesized that higher urinary concentrations of four phthalate metabolites—MEP, mono-*n*-butyl phthalate (MnBP; the metabolite of DnBP), monobenzyl phthalate (MBzP; the major metabolite of BBzP), and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP; a metabolite of DEHP)—would be associated with higher concurrent F_{ENO} in children. We also hypothesized that children with serotopy and wheeze, as surrogate measures of allergy and the hyperresponsive airways of asthma, would be more susceptible to airway inflammation triggered by these environmental exposures.

METHODS

Participants were selected based on available samples from the Columbia Center for Children's Environmental Health study, a longitudinal birth cohort that enrolled 727 nonsmoking Dominican and African American pregnant women free of hypertension, diabetes, and known HIV living in Northern Manhattan and the South Bronx (24). Although expectant mothers were recruited from neighborhoods that also had high asthma prevalence (25), women were not recruited based on reported history of asthma or allergy. Children (aged 4.9–9.1 yr) included in these analyses provided at least one spot urine sample during an office visit when F_{ENO} was measured between 2006 and 2010. This study was approved by the institutional review boards of Columbia University and the Centers for Disease Control and Prevention (CDC).

Phthalate metabolites in urine collected between midmorning and early evening were measured at the CDC as described (8, 10). We used urinary concentrations of MEHHP as the proxy for exposure to DEHP (*see* online supplement for justification). Correction factors of 0.66 and 0.72 were applied to the MEP and MBzP concentrations, respectively, to adjust for previous overestimations of the analytic standards (*see* online supplement for further details) (12).

F_{ENO} Collection

F_{ENO} was collected with a modified offline device along with ambient NO as described previously and in the online supplement (26–28). F_{ENO} data were excluded if the child reported having a cold at the time of the measurement.

Seroatopy Measurement

Seroatopy was defined as specific IgE to dust mite, cockroach, or mouse allergens (≥ 0.35 IU/ml) by ImmunoCap (Phadia, Uppsala, Sweden) in serum collected within 1 year of the F_{ENO} measure (29).

International Study of Allergy and Asthma in Children Wheeze by Questionnaire

Mothers were asked whether their child had wheezed in the past 12 months at age 5, 6, 7, and 9 years using the International Study of Allergy and Asthma in Children (ISAAC) questionnaire (30). Children with a report of wheeze on the questionnaire administered on the day of F_{ENO} collection or at the next study visit (within 1 year) were classified as having wheezed.

Modeled Probability of Wheeze

Because reports on single questionnaires may not characterize the episodic and variable timing of wheeze in childhood, a larger set of repeated questionnaires also was used to model the probability of wheezing in the last 3 months as a continuous measure at each F_{ENO} collection for all ($n = 313$) observations. Four childhood wheeze phenotypes (patterns) were identified using latent class growth analysis, and the posterior probabilities of phenotype membership as well as the probabilities of wheezing in each phenotype were estimated for each child in the study at the age of F_{ENO} measurement (31).

Statistical Analysis

Individual children had one to three observations with phthalate and F_{ENO} collections; therefore, repeated measures were modeled using generalized estimating equations (GEE) with robust standard errors. Models included specific gravity to adjust for urinary dilution (32), as well as potential confounders: age, sex, race/ethnicity, time of day of F_{ENO} collection, and ambient NO. Phthalate metabolite, F_{ENO}, and ambient NO variables were log transformed due to the right skew and high variance at the upper range of their distributions. Analyses were conducted using R version 2.13.1 (33–35).

RESULTS

The 244 participants included 125 girls and 119 boys (Table 1). Of the 244 children enrolled who provided at least one measure for both phthalates and F_{ENO}, 65 had two measures, and two children had three, resulting in a total of 313 paired observations of phthalate metabolites and F_{ENO}. The demographics of the children included in the analyses were similar to those who were enrolled, but excluded from these analyses, except a slightly higher frequency of first-born children (*see* Table E1 in the online supplement). Exposures to the phthalates examined were widespread, as suggested by the detection of the metabolites of all four phthalates in 100% of urine samples (Table E2). The concentrations of the four phthalate metabolites within each sample were positively correlated with a range of pairwise Spearman correlations from $\rho = 0.21$ to 0.68 (Table E3).

To characterize predictors of children's phthalate metabolite urinary concentrations, GEE models with age, sex, and race/ethnicity as predictors were fit for each of the four log-transformed metabolite concentrations, also including specific gravity as a covariate. The urinary concentrations of three of the metabolites were higher in girls than in boys: MEP (41% higher; 95% confidence

TABLE 1. DEMOGRAPHICS OF PARTICIPANTS AND DISTRIBUTION OF URINARY METABOLITES STRATIFIED BY WHEEZE AND SEROATOPY (N = 244)

	Overall (n = 244)	Seroatopy*		Wheeze†	
		No (n = 153)	Yes (n = 64)	No (n = 177)	Yes (n = 54)
Age, mean (SD), yr	6.5 (1.3)	6.3 (1.2)	6.8 (1.3)‡	6.5 (1.3)	6.1 (1.1)‡
Male, n (%)	119/244 (49)	70/153 (46)	36/64 (56)	84/177 (47)	29/54 (54)
African American race, n (%)§	80/244 (33)	49/153 (32)	19/64 (30)	57/177 (32)	15/54 (28)
Dominican ethnicity, n (%)§	164/244 (67)	104/153 (68)	45/64 (70)	120/177 (68)	39/54 (72)
Mother has no high school degree, n (%)	81/244 (33)	52/153 (34)	20/64 (31)	58/177 (33)	19/54 (35)
First born, no older siblings, n (%)	127/244 (52)	70/153 (46)	42/64 (66)‡	92/177 (52)	27/54 (50)
Inhaled/oral corticosteroid use, n (%)	14/244 (6)	8/153 (5)	6/64 (9)	3/177 (2)	10/54 (19)‡
Material hardship, n (%)	96/244 (39)	59/153 (39)	29/64 (45)	66/177 (37)	28/54 (52)
Urinary metabolites, unadjusted geometric means in ng/ml (95% CI)					
MEP¶	111 (96–129)	109 (91–131)	122 (90–165)	116 (97–139)	109 (78–154)
MnBP	41 (36–46)	38 (32–45)	48 (38–60)	40 (35–47)	39 (29–53)
MBzP¶	24 (20–28)	22 (18–27)	30 (22–41)	22 (18–27)	24 (16–34)
MEHHP	42 (36–49)	39 (32–47)	46 (36–58)	41 (34–48)	45 (32–62)

Definition of abbreviations: CI = confidence interval; F_{ENO} = fractional exhaled nitric oxide; ISAAC = International Study of Allergy and Asthma in Children; MBzP = monobenzyl phthalate; MEHHP = mono(2-ethyl-5-hydroxyhexyl) phthalate; MEP = monoethyl phthalate; MnBP = mono-*n*-butyl phthalate.

Potentially time-varying variables (e.g., wheeze and seroatopy) are reported for the children at the youngest age measured among the children, with metabolites and F_{ENO} measured at multiple ages.

* Specific IgE to dust mite, cockroach, or mouse allergens (≥ 0.35 IU/ml) in serum collected within 1 year of the F_{ENO} measure.

† Children with a report of wheeze on the ISAAC questionnaire after F_{ENO} collection were classified as having wheezed.

‡ $P < 0.05$ difference in demographic variables between children with and without seroatopy or with and without wheeze.

§ African American race and Dominican Republic ethnicity were reported for the mother.

|| Mother reported at closest questionnaire that she and her family could not afford needed food, rent, clothing, or medical care or that gas/electricity was suspended because of bill nonpayment in the previous 6 months.

¶ Corrected for updated Centers for Disease Control and Prevention standards as of January 2012 as described in the online supplement.

interval [CI], 8.0–83%; $P = 0.011$), MnBP (32% higher; 95% CI, 9.0–60%; $P = 0.005$), and MEHHP (45% higher; 95% CI, 16–81%; $P = 0.001$). There was no significant difference by age or race/ethnicity for the urinary concentrations of any of the metabolites.

Phthalate Metabolites and F_{ENO}

F_{ENO} measures ranged from 2.3 to 71.6 ppb, with a median of 7.9 ppb and an interquartile range from 5.7 to 12.3 ppb, and were approximately log-normally distributed. In a GEE model of F_{ENO} with age, sex, race/ethnicity, time of day, and ambient NO concentration as predictors, only ambient NO concentration was significantly positively associated with F_{ENO}. In four separate adjusted models, F_{ENO} was associated with urinary concentrations of MEP, MnBP, and MBzP, but not MEHHP, after controlling for specific gravity, age, sex, race/ethnicity, time of day of F_{ENO} collection, and ambient NO concentration (Table 2). The effect size for MnBP was slightly larger than for MEP and MBzP, although all three were very similar and were consistent with linearity in adjusted models using quintiles of urinary concentrations (Figure 1). For example, there was a 6.8% increase in F_{ENO} (95% CI, 1.1–12.9%; $P = 0.019$) for each log-unit increase in MBzP concentrations, adjusting for covariates. Similarly, a log-unit increase in MEP concentration was associated with a 6.5% increase in F_{ENO} (95% CI, 1.0–12.4%; $P = 0.021$) adjusted for covariates, whereas a log-unit change in MnBP concentration was associated with an 8.5% increase in F_{ENO} (95% CI, 0.2–17.6%; $P = 0.045$) adjusting for covariates. Effect estimates of the metabolites on F_{ENO} were altered less than 10% in adjusted analyses including potential confounding variables individually: oral/inhaled corticosteroid use, older siblings, number of people living in the home, type of heating, having a smoker in the home, and indicators of socioeconomic status, maternal education, and material hardship (data not shown) (36). There was no significant interaction by child sex (data not shown).

Seroatopy measured within 1 year was available for 88% of observations (n = 274 of 313) from 217 children with 31% of measures classified as seroatopic at their F_{ENO} observation (n = 86 of 274). Adjusting for seroatopy did not substantially alter the estimates for the individual phthalate metabolites (Table 2). In sensitivity analyses, similar results were seen when restricting to seroatopy measured on the day of F_{ENO} collection (n = 160; Table E4).

In an adjusted multipollutant model of F_{ENO} including concentrations of all four metabolites (MEP, MnBP, MBzP, and MEHHP) that was also adjusted for seroatopy, MEP and MBzP

TABLE 2. ADJUSTED PERCENT DIFFERENCE IN FRACTIONAL CONCENTRATION OF NITRIC OXIDE FOR A ONE LOG-UNIT HIGHER PHTHALATE METABOLITE URINARY CONCENTRATION FROM GENERALIZED ESTIMATING EQUATION REGRESSION MODELS

Metabolite	% Difference	95% CI	P Value
Single metabolites, four separate models (n = 313)			
MEP	6.5	(1.0, 12.4)	0.021
MnBP	8.5	(0.2, 17.6)	0.045
MBzP	6.8	(1.1, 12.9)	0.019
MEHHP	2.7	(−3.3, 9.1)	0.38
Single metabolites, separate models adjusted for seroatopy (n = 274)			
MEP	6.8	(1.0, 12.9)	0.020
MnBP	8.4	(−0.1, 17.5)	0.053
MBzP	8.3	(2.3, 14.8)	0.007
MEHHP	2.1	(−4.3, 9.0)	0.53
All four metabolites in one model adjusted for seroatopy (n = 274)			
MEP	6.6	(0.5, 13.1)	0.034
MnBP	−1.5	(−11.1, 9.1)	0.77
MBzP	8.7	(1.9, 16.0)	0.011
MEHHP	0.0	(−6.4, 6.9)	0.99

Definition of abbreviations: CI = confidence interval; MBzP = monobenzyl phthalate; MEHHP = mono(2-ethyl-5-hydroxyhexyl) phthalate; MEP = monoethyl phthalate; MnBP = mono-*n*-butyl phthalate.

All models adjusted for specific gravity, sex, race/ethnicity, age, time of day of F_{ENO} collection, and ambient NO concentration.

Figure 1. The association between urinary concentrations of three phthalate metabolites: monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), and monobenzyl phthalate (MBzP), and fractional exhaled nitric oxide (FeNO) from separate models ($n = 313$ observations from 244 children). Adjusted estimates for each quintile are shown as horizontal segments. The continuous linear association is shown as a wider gray line. Both quintile and continuous model estimates are adjusted and shown at mean levels of specific gravity, age, sex, race/ethnicity, time of day of FeNO collection, and ambient NO concentration.

remained statistically significant independent predictors of FeNO with similar effect size estimates to those in the single metabolite models, as shown in Table 2. Although MEHHP concentrations remained nonsignificant, as in the single metabolite models, the single-metabolite β estimate for MnBP decreased from 0.080 in the seroatopy-adjusted model to a β of -0.016 in the multipollutant model and was no longer statistically significantly associated with FeNO after adjusting for the other phthalate metabolite concentrations ($P = 0.77$). Therefore, the MnBP association with FeNO seen in the single phthalate model may be due to the high correlation between MnBP and MBzP (Spearman

correlation = 0.68). In the adjusted four-pollutant model, a log-unit higher concentration of MEP and MBzP was associated with a 6.6% increase (95% CI, 0.5–13.1%; $P = 0.034$) and an 8.7% increase (95% CI, 1.9–16.0%; $P = 0.011$) in FeNO , respectively, after adjusting for all four metabolite concentrations, seroatopy, specific gravity, age, sex, race/ethnicity, time of day of FeNO collection, and ambient NO.

Interaction between Phthalate Metabolites and Seroatopy or Wheeze

There was no direct association between the urinary concentrations of any of the four metabolites with incident seroatopy or reported wheeze (*see online supplement*). As seen in Figure 2, FeNO was higher among children with seroatopy (geometric mean, 11.6 ppb; $n = 86$) than those without seroatopy (geometric mean, 7.5 ppb; $n = 188$; $P < 0.001$). Although the slopes of the MEP or MBzP and FeNO associations were slightly larger among those with seroatopy than those without seroatopy (Figure 2), testing interaction terms in the GEE models showed that the differences in the slopes were not statistically significant ($P = 0.51$ and $P = 0.60$).

We also assessed the interaction between phthalate concentrations and wheeze using data from the ISAAC questionnaire responses to test whether children who wheeze are more susceptible to airway inflammation associated with phthalate exposure. Overall, 23% of children had a report of wheezing in the past 12 months at their FeNO observation or their next questionnaire collected within 12 months (available for $n = 284$ observations). There was no significant interaction between concentrations of MEP, MnBP, or MEHHP and report of wheeze in the past year in the association with FeNO ($P = 0.96$, 0.17, and 0.52, respectively). There was a significant positive interaction of the MBzP and FeNO association by ISAAC wheeze (Figure 3; $P = 0.016$, $n = 284$). In sensitivity analyses, this interaction was stronger and remained significant when restricted to ISAAC wheeze questionnaires asked on the same day as FeNO collection ($P = 0.011$, $n = 187$).

As a further exploration of the interaction between phthalates and wheeze, a latent class growth analysis (LCGA) was used to model the probability of wheezing in the past 3 months. This LCGA included 7,048 questionnaires on report of wheeze in the past 3 months from 15 time points between 3 months and 9 years of age in a larger subset ($n = 689$) of the children in the Columbia Center for Children's Environmental Health study (31). The resulting predicted probability of wheezing was available for all ($n = 313$) observations and ranged from 0.01 to 0.66, although they were highly skewed, with a median of 0.04, a 75th percentile of 0.08, and a 95th percentile of 0.44. There was no evidence of interaction between concentrations of MEP, MnBP, or MEHHP and LCGA predicted probability of wheeze on FeNO . However, as in the analysis using a dichotomous ISAAC wheeze question, the interaction between MBzP urinary concentrations and the LCGA probability of wheezing on FeNO was positive and highly significant ($P = 0.006$). The association between concentrations of MBzP and FeNO was of a larger magnitude among those with a higher probability of wheezing (Figure 4).

DISCUSSION

In this observational study of children, urinary concentrations of three phthalate metabolites (MEP, MnBP, and MBzP) were associated positively with FeNO measured on the same day in separate adjusted models. This is the first population-based study to show an association between phthalate exposure and

Figure 2. The association between (A) monoethyl phthalate (MEP) or (B) monobenzyl phthalate (MBzP) urinary concentrations and fractional exhaled nitric oxide ($F_{E_{NO}}$) stratified by seroatopy of the child ($n = 188$ and $n = 86$ observations from 217 children). Box plots show the distribution of $F_{E_{NO}}$ concentrations is higher among children with atopy than children without atopy ($P < 0.001$). The interaction terms testing for a difference in slopes were not statistically significant ($P = 0.51$ and $P = 0.60$), shown at the mean specific gravity, age, sex, race/ethnicity, time of day of $F_{E_{NO}}$ collection, and ambient NO concentration. Main effects for both metabolites remained statistically significant after adjusting for seroatopy without interaction.

this measure of airway inflammation. All three associations remained largely unchanged after adjusting for seroatopy. When all four metabolites (MEP, MnBP, MBzP, MEHHP) were included in the same adjusted model, MEP and MBzP remained independent predictors of higher $F_{E_{NO}}$, whereas concentrations of MnBP, which are highly correlated with concentrations of MBzP, were no longer associated with $F_{E_{NO}}$. These results suggest that urinary biomarkers of exposure to two phthalates, believed to have substantial inhalational exposure, are associated with a measure of subclinical airway inflammation in children.

This observational study of children is the first to report a positive association between biomarkers of exposure to phthalates and $F_{E_{NO}}$. The methodology for measuring $F_{E_{NO}}$ allows for noninvasive collection and measurement of NO produced by resident airway cells in response to inflammatory cytokines and mediators (37). Although its diagnostic use in children remains debated (37, 38), $F_{E_{NO}}$ has been established as a biomarker of airway inflammation in response to air pollutants, with more than a decade of epidemiology studies (20, 22, 23, 39, 40). In population-based studies, the majority of subjects with asthma have mild disease with infrequent exacerbations, making temporal associations between environmental exposures and exacerbations difficult to

establish. Elevated $F_{E_{NO}}$ reflects eosinophilic airway inflammation in response to known asthma triggers and has been associated both with increased symptoms among subjects with asthma and with the development of asthma among children without asthma (20, 41). As such, it offers an objective biomarker to detect subclinical variations in airway inflammation reflecting increased risk for exacerbation. Our findings of associations with phthalates and $F_{E_{NO}}$ contrast with those from two small controlled chamber studies of adults exposed to polyvinyl chloride surfaces in which there was no increase in post- versus prechallenge $F_{E_{NO}}$ (42, 43).

We hypothesized that children with report of wheeze in the last 12 months would be more susceptible to airway inflammation triggered by phthalate exposures than the nonwheezing children in our cohort. We observed a positive interaction between MBzP urinary concentrations and wheeze. There was also a positive interaction between urinary concentrations of MBzP and the probability of wheezing predicted from a latent class growth analysis of questionnaire data, suggesting that the MBzP and $F_{E_{NO}}$ association is stronger among a subset of children who wheeze. Although wheeze itself is episodic, the LCGA-based prediction was based on the pattern of wheezing at up to 15 time points between 3 months and 9 years of age and may offer a more stable indication of children with airway disease than a single

Figure 3. The association between monobenzyl phthalate (MBzP) urinary concentrations and fractional exhaled nitric oxide (F_{ENO}) varies by whether the mother reported on the International Study of Allergy and Asthma in Children questionnaire, administered on the day of F_{ENO} collection or at the next study visit (within 1 year), that the child had wheezing or whistling in the chest in the past 12 months ($n = 284$ observations from 231 children). The interaction term between MBzP and the report of wheeze was positive and significant ($P = 0.016$) in the adjusted model shown at the mean levels of specific gravity, age, sex, race/ethnicity, time of day of F_{ENO} collection, and ambient NO concentration.

questionnaire response. Additional advantages of the LCGA-based wheeze prediction are that it could be computed for all observations and that the output on the continuous probability scale may be more informative than a dichotomous classification. Direct comparison of our two wheezing measures is difficult, because the LCGA-derived probability of wheeze over 3 months was based on a different question than the ISAAC wheeze, and uncertainty estimates from the original LCGA model were not propagated into the LCGA-based probability of wheeze. The consistency of these observations using two different measures of wheeze lends support to these findings. Although corticosteroids may affect the strength of the correlation between F_{ENO} and wheeze (44), the positive interaction remained after adjusting for or excluding children with reported use of corticosteroids (data not shown).

Urinary concentrations of MBzP recently have been shown to be associated with a nonspecific marker of systemic inflammation in a large population-based analysis. MBzP concentrations were associated with increased serum C-reactive protein in a dose-dependent fashion among 8,346 NHANES participants (45). An interquartile range higher urinary MBzP concentration was associated with an average of 6.0% (95% CI, 1.7–10.8%) higher C-reactive protein, and this association held in analyses restricted to younger NHANES participants 6 to 12 or 13 to 19 years old. However, the mechanism through which phthalates are associated with systemic or airway inflammation remains unclear. Some phthalate

metabolites, especially MEHP and MBzP, activate the ubiquitously expressed nuclear peroxisome proliferator-activated receptors (PPAR) α and γ (46), which are ligand-activated transcription factors important in a variety of physiological processes, including airway inflammation and airway remodeling. For example, it has been shown that the expression of peroxisome proliferator-activated receptor γ is higher in the bronchial submucosa, airway epithelium, and smooth muscle of subjects with asthma than control subjects and is associated with decrements in lung function (47).

Diurnal variations both in measures of urinary phthalates and F_{ENO} have been reported, allowing for the potential for confounding of an association between them. Among adults and children enrolled in NHANES, MEP was higher during midday collections, whereas MBzP was nonsignificantly higher during the evenings (7). However, a panel study of adults found that 76% of the variance in urinary MEP was explained by between-person differences (48). There are conflicting findings in the literature about whether F_{ENO} has circadian variation (49), but at least one study in children reported higher F_{ENO} measured in the morning as compared with evening (50). We recorded the time of day of F_{ENO} , but not urine collection. Nonetheless, F_{ENO} and urine were collected during a single office visit that typically lasted less than 2 hours. Therefore, the robustness of the findings after adjustment for time of day of F_{ENO} collection reduces the likelihood that these observations were due to confounding by the time of collection.

Figure 4. The association between monobenzyl phthalate (MBzP) urinary concentrations and fractional exhaled nitric oxide (F_{ENO}) is stronger among children predicted to wheeze ($n = 313$ observations from 244 children). The area of points is proportional to the probability of wheezing predicted from a latent class growth analysis (continuous variable ranging from 0.01 to 0.66). The three lines display the MBzP and F_{ENO} association for participants at the median, 75th percentile, and the 95th percentile of the probability of wheeze shown at the mean specific gravity, age, sex, race/ethnicity, time of day of F_{ENO} collection, and ambient NO concentration from a multivariable generalized estimating equation model. The interaction term between MBzP and the probability of wheezing was positive and highly significant in the model ($P = 0.006$).

One strength of the cross-sectional study design is that it allows for the observation of effects that may be short-lived in response to exposures that vary over time. Studies of exposure to air pollutants, such as elemental carbon (22), have demonstrated increased FE_{NO} hours after exposure, suggesting the importance of assessing the impact of pollutant exposures on FE_{NO} in short time windows. Associations between FE_{NO} and phthalate metabolites were evaluated cross-sectionally. However, it will be important in future studies to determine the relevance of chronic exposure to DEP and BBzP and elevated FE_{NO} to airway disease.

This study had several limitations. In an observational study, urinary concentrations of particular phthalate metabolites also may indicate exposure to other chemicals that share sources or chemical properties with the parent phthalates. For example, the mothers of the children in this analysis who reported the use of perfume or an index of uses of other personal care products during pregnancy had higher urinary concentrations of MEP, the main metabolite of DEP, than other pregnant women within the same cohort (51). These participants also can be expected to have higher exposure to other chemicals in those products that could confound observed associations, such as artificial fragrances as seen in sampling of indoor air (52). The substantially higher urinary concentrations of MEP in girls than boys warrants further investigation, as the burden of asthma and respiratory disease differentially increases for girls in adolescence, when exposure to DEP might be expected to remain high (53). Furthermore, with phthalates, exposures occur to mixtures of correlated compounds. For example, in this study MnBP and MBzP were highly correlated ($\rho = 0.68$), which may be due to shared sources of exposure and the fact that MnBP is a minor product of BBzP (6%) (54). MBzP has been shown previously to be a more reproducible biomarker than MnBP in spot urine samples collected over 6 months from children in New York City (9); its concentration may serve as a better marker of the mixture of related compounds than does the concentration of MnBP.

Conclusions

We report cross-sectional associations between children's urinary concentrations of three phthalate metabolites and FE_{NO} , a marker of airway inflammation. Concentrations of both MEP and MBzP remained associated with FE_{NO} in a four-pollutant model. The association of concentrations of MBzP and FE_{NO} was significantly stronger among children in this study with as compared with those without reported wheeze. The children with wheeze are presumed to have hyperreactive airways more susceptible to environmental exposures. These findings suggest a role for a ubiquitous exposure on airway inflammation in a susceptible population. Future studies can prospectively follow these children to observe whether associations persist through childhood and the long-term consequences to respiratory health of increased airway inflammation in children.

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