

Exposure to polycyclic aromatic hydrocarbons, vitamin D, and lung function in children with asthma

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Abstract

Background: It is unclear whether exposure to polycyclic aromatic hydrocarbons (PAH) affects lung function in children with asthma. Whether vitamin D insufficiency enhances any detrimental effects of PAH on lung function in asthmatic children is also unknown.

Methods: Cross-sectional study of 2459 children (6-17 years) who participated in the 2007-2012 National Health and Nutrition Examination Survey. Multivariable linear regression was used to analyze the relation between molar mass of urinary PAH metabolites (sum of all PAH (Σ molPAH), sum of PAH with 2 benzene rings (Σ mol2-PAH), or sum of PAH with 3 or 4 benzene rings (Σ mol3,4-PAH)) and lung function or exhaled fraction of nitric oxide (FeNO) in children with and without asthma. In this multivariable analysis, we tested whether vitamin D insufficiency (a serum 25(OH)D level <30 ng/mL) interacts with PAH exposure on lung function in children with asthma.

Results: Among children with asthma, higher Σ molPAH, and Σ mol2-PAH were significantly associated with decreased %predicted FEV₁ (by 3.5-3.8%). Urinary PAH were not associated with lung function in children without asthma or with FeNO. Given a significant interaction between vitamin D insufficiency and PAH metabolites on lung function in asthmatic children, we stratified the analysis by vitamin D status. In this analysis, urinary PAH metabolites were significantly associated with 2.0-5.6% reduced %predicted FEV₁ and %predicted FEV₁/FVC in children with asthma and vitamin D insufficiency, but not in those with asthma and vitamin D sufficiency.

Conclusions: Vitamin D insufficiency and PAH exposure may have synergistic detrimental effects on lung function in asthmatic children.

KEYWORDS

asthma, children, lung function, NHANES, polycyclic aromatic hydrocarbons

1 | INTRODUCTION

Asthma is one of the most common chronic diseases of childhood.¹ The prevalence of childhood asthma has substantially increased in industrialized countries over the last few decades, likely due to environmental changes.² Considerable evidence links exposure to air

pollutants (such as particulate matter, mixed traffic-related air pollution, and polycyclic aromatic hydrocarbons (PAH)) to asthma in children.^{3,4}

PAH, a group of hydrocarbons with two or more fused aromatic rings, are byproducts of incomplete combustion of tobacco, wood, coal, fossil fuels, and other organic substances. PAH can be readily

absorbed through the skin, lungs, and gastrointestinal tract.⁵ PAH metabolites in urine have been used as biomarkers of PAH exposure.⁶ Naphthalene, a PAH compound consisting of two aromatic rings, is the most volatile PAHs found in the air and can be a respiratory tract toxicant.⁷ PAH compounds consisting of 3–4 aromatic rings can exist in both gaseous and particulate phase.⁵

PAH exposure has been associated with asthma or asthma symptoms in children.^{8–10} Although several studies have also linked PAH exposure to reduced lung function measures (forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC)) among children and adults in the general population,^{11–13} little is known about PAH exposure and lung function or eosinophilic airway inflammation in children with asthma, who may be particularly susceptible to the detrimental effects of air pollutants. In the only study examining PAH exposure and lung function among children with and without asthma, exposure to the sum of PAH with 4, 5, or 6 rings for 3 months to 1 year was significantly associated with reduced post-bronchodilator FEV₁ among 162 children without asthma, but not among 135 children with asthma.¹⁴ Underlying mechanisms for the PAH-asthma association include increasing oxidative stress, stimulating inflammatory response, enhancing sensitization to aeroallergens, and epigenetic remodeling.^{4,15}

In addition to asthma, vitamin D insufficiency may modify the detrimental effects of PAH exposure on lung function, a marker of asthma severity. Indeed, vitamin D insufficiency has been previously associated with reduced lung function in the U.S. population,¹⁶ and we previously reported that proximity to a major highway (a broad marker of traffic-related air pollution) is more significantly and strongly associated with severe asthma exacerbations in children with asthma and vitamin D insufficiency (a circulating 25(OH)D level <30 ng/mL) than in those with asthma and sufficient vitamin D levels.¹⁷

We hypothesized that PAH exposure would have a stronger detrimental effect on lung function and eosinophilic airway inflammation (measured by fractional exhaled nitric oxide (FeNO)) in children with asthma than in those without asthma. We further hypothesized that any detrimental effects of PAH exposure on the lung function of children with asthma would be stronger in those with vitamin D insufficiency than in those with sufficient vitamin D levels. To test these hypothesis, we first examined the relation between urinary PAH metabolites and lung function or FeNO in a large sample of U.S. children with and without asthma. Among children with asthma, we then tested whether vitamin D insufficiency modifies the estimated effect of urinary PAH metabolites on lung function.

2 | METHODS

2.1 | Subject recruitment

In this analysis, we included children (6–17 years old) who participated in the 2007–2008, 2009–2010, and 2011–2012 NHANES cycles with urinary PAH measurements. NHANES is a cross-sectional nationwide survey designed to assess the health and nutritional status of the noninstitutionalized U.S. population. Participants are selected by using a stratified multistage probability sampling, and oversampling persons

60 years and older and ethnic minorities (African Americans and Hispanics), to represent the U. S. population of all ages and produce reliable statistics. The survey combines interviews and physical examinations that are administered by highly trained personnel. NHANES is approved by the Institutional Review Board of the National Center for Health Statistics of the U.S. Centers for Disease Control and Prevention (CDC). Informed consent is obtained from all participants. A proxy provided information for survey participants who were less than 16 years of age and for subjects who could not answer the questions by themselves.

2.2 | Study procedures

Current asthma was defined by a positive answer to both of the following questions: “Has a doctor or other health professional ever told you that you have asthma?” and “Do you still have asthma?” Participating children performed a pre-bronchodilator spirometry following American Thoracic Society recommendations.¹⁸ Participants were not eligible for spirometry if they were on supplemental oxygen or had painful ear infections, current chest pain, or a physical problem with forceful expiration, surgery (of the eye, chest, or the abdomen) in the previous 3 months, heart disease, history of a detached retina, hemoptysis, or history of a collapsed lung or tuberculosis exposure. The best FEV₁, FVC, and the FEV₁/FVC ratio were selected for analysis. Percent (%) predicted and z-score of FEV₁, FVC, and FEV₁/FVC were calculated using Global Lung Initiative equations that account for age, sex, race/ethnicity, and height.¹⁹ FeNO was measured using the Aerocrine NIOX MINO, a portable, hand-held NO analyzer (Aerocrine AB, Solna, Sweden). The NHANES protocol required two valid FeNO measurements that were reproducible. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared based on body measures. BMI z-scores were calculated based on the 2000 CDC growth charts.²⁰

2.3 | Laboratory procedures

Urinary PAH metabolites were examined in a randomly selected sample of subjects, comprising one-third of NHANES participants aged 6 years and older. Nine mono-hydroxylated metabolites of PAH (OH-PAH) in urine samples (including 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 3-hydroxyfluorene, 9-hydroxyfluorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, and 1-hydroxypyrene) were measured by isotope dilution capillary gas chromatography tandem mass spectrometry (GC-MS/MS, Agilent Technologies, Santa Clara, CA). Analytes with values under the lower limit of detected (LLOD) were assigned a constant value (lower limit of detection divided by square root of 2 (LLOD/sqrt(2))). Three variables were then created: the sum of individually calculated molar mass of all nine PAH metabolites ($\sum\text{molPAH}$), the sum of the naphthalene metabolites that have two benzene rings ($\sum\text{mol2-PAH}$), and the sum of PAH metabolites that have three (fluorene and phenanthrene), or four (pyrene) benzene rings ($\sum\text{mol3,4-PAH}$). Quartiles of $\sum\text{molPAH}$, $\sum\text{mol2-PAH}$, and $\sum\text{mol3,4-PAH}$ were categorized for the analysis of PAH

metabolites and current asthma or lung function. Urinary creatinine was measured by the Roche/Hitachi Modular P Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN) to account for variation in dilution in spot urinary samples. Total serum 25(OH)D level was measured by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Serum total 25(OH)D (in SI units of nmol/L) was defined as the sum of 25(OH)D₃ and 25(OH)D₂, and then converted to conventional units (ng/mL), using the suggested conversion factors: 1 nmol/L = 0.40066 ng/mL.²¹ Vitamin D insufficiency was defined as a serum 25(OH)D level lower than 30 ng/mL. Further details of the methods, protocols, and definitions used in NHANES can be found at <http://www.cdc.gov/nchs/nhanes.htm>.

Because humans are often exposed to mixtures of the PAH compounds, sum of the individual PAH metabolites by chemical structures was calculated to estimate overall exposure.

2.4 | Statistical analysis

Primary sampling units and strata for the complex NHANES survey design were applied for data analysis. Subsample weights, stratification, and clusters provided in the NHANES dataset were incorporated into the analysis to obtain proper estimates and their standard errors. Wald chi-square tests and t-tests were used for bivariate analyses of binary and continuous variables, respectively. Logistic regression was used for the analysis of urinary PAH metabolites and current asthma. Linear regression was used to examine urinary PAH metabolites and %predicted lung function or FeNO, separately in children with and without current asthma. All multivariable models were adjusted for age, gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other), health insurance coverage, BMI z-score, serum cotinine (a marker of second-hand smoke exposure, SHS), urinary creatinine levels, and use of oral or inhaled steroids in the past 2 days. Family history of asthma was also included in the model for the analysis of PAH exposure and current asthma. We then tested for a multiplicative interaction between urinary PAH metabolites and vitamin D insufficiency on lung function in children with asthma. Since this interaction was statistically significant, we repeated the multivariable analysis after stratification by vitamin D insufficiency. All statistical analyses were conducted using the SAS SURVEY procedure and SAS 9.3 software (SAS Institute Inc., Cary, NC).

3 | RESULTS

A total of 2459 children who participated in NHANES (2007-2008, 2010-2011, and 2011-2012) and who had measures of urinary PAH, and data on asthma and lung function, were included in this analysis (Table 1). The overall prevalence of current asthma among study participants was 11.8%. Compared to children without asthma, those with asthma were more likely to be non-Hispanic black, and to have: health insurance, a higher BMI z-score, vitamin D insufficiency, family history of asthma, a higher FeNO, used oral, or inhaled steroid in the past 2 days, and lower %predicted FEV₁ and lower %predicted FEV₁/FVC. Compared with children without asthma, those with asthma had slightly

higher levels of Σ molPAH, Σ mol2-PAH, or Σ mol3,4-PAHs, but these differences were not statistically significant. With the exception of BMI z-score and urinary creatinine level, there were no significant differences in the main characteristics of children who were ($n = 2,459$) and were not ($n = 6,346$) included in the current analysis (E-Table S1).

The results of the multivariable analysis of urinary PAH metabolites (by quartiles) and current asthma are shown in Figure 1. After adjusting for age, gender, race/ethnicity, health insurance coverage, family history of asthma, serum cotinine, and urinary creatinine, children in the highest quartile of urinary Σ mol3,4-PAH had 2.84 times higher odds of current asthma than those in the lowest quartiles of urinary Σ mol3,4-PAH (95% confidence interval [CI] for the odds ratio [OR] for 4th quartile vs 1st quartile = 1.20 to 6.70, $P = 0.02$). There was no significant association between either urinary Σ molPAH or urinary Σ mol2-PAH and current asthma.

Since all analyses of urinary PAH metabolites (by quartiles) and lung function measures showed significant linear trends, continuous measures of urinary PAH metabolites were used in the analysis of lung function. Table 2 presents the results of the multivariable analyses of urinary PAH metabolites and lung function or FeNO, stratified by current asthma. Among children with asthma, each molar mass increase in Σ molPAH was associated with 3.80% decrements in % predicted FEV₁ (95%CI = -6.68 to -0.88) and each molar mass increase in Σ mol2-PAH were associated with 3.47% decreased % predicted FEV₁ (95%CI = -6.07 to -0.88). There was no significant association between urinary PAH and lung function among children without asthma, or with FeNO in children with or without asthma.

Next, we tested for interactions between urinary PAH metabolites (as continuous) and vitamin D insufficiency (as binary) on lung function (%predicted FEV₁, %predicted FVC, and %predicted FEV₁/FVC) in children with asthma. Because we indeed found significant interactions between vitamin D insufficiency and urinary PAH metabolites in children with asthma (eg, Σ molPAH, Σ mol2-PAH, and Σ mol3,4-PAH) on %predicted FEV₁/FVC, $P < 0.01$ in all instances, we repeated the multivariable analysis after stratification by vitamin D insufficiency. In this analysis, there was a significant inverse association between urinary PAH metabolites (Σ molPAH, Σ mol2-PAH, and Σ mol3,4-PAH) and both %predicted FEV₁ and %predicted FEV₁/FVC in children with asthma and vitamin D insufficiency, but not in children with asthma and sufficient vitamin D levels (Table 3). Similar results were obtained in an analysis using z-scores for spirometry variables (E-Table S2).

4 | DISCUSSION

In a large study of U.S. children, high levels of urinary Σ mol3,4-PAH were associated with current asthma. Among children with asthma, urinary Σ molPAH and Σ mol2-PAH metabolites were associated with lower FEV₁. After stratification by vitamin D status, urinary Σ molPAH, Σ mol2-PAH, and Σ mol3,4-PAH were associated with lower FEV₁ and FEV₁/FVC in children with asthma and vitamin D insufficiency, but not in those with asthma and sufficient vitamin D levels. Thus, our findings suggest potential synergistic detrimental

TABLE 1 Characteristics of participating children (6–17 years) with and without asthma, NHANES 2007–2012

Characteristics	No current asthma (n = 2,170)	With current asthma (n = 289)
Age (years)	11.5 ± 0.1	12.1 ± 0.3
Male gender	1106 (50.9)	141 (49.8)
Race/ethnicity		
Non-Hispanic white	642 (56.7)	86 (54.6)**
Non-Hispanic Black	485 (13.2)	103 (22.6)
Mexican American/other Hispanic	865 (23.0)	70 (13.9)
Other	178 (7.1)	30 (8.9)
Had health insurance coverage	1418 (89.6)	198 (95.1)**
Annual household income <\$20 000	437 (14.5)	81 (21.0)
Body mass index (BMI) z-score	0.65 ± 0.03	0.90 ± 0.09**
Serum cotinine level (ng/mL)	4.5 ± 0.9	6.9 ± 2.7
Serum vitamin D level (ng/mL)	27.5 ± 0.5	26.2 ± 1.0
Vitamin D insufficiency (<30 ng/mL)	1395 (62.7)	205 (71.4)*
Family history of asthma	561 (24.4)	175 (59.0)**
Fractional exhaled nitric oxide (ppb)	15.4 ± 0.5	23.2 ± 2.0**
Use oral or inhaled steroid in past 2 days	13 (0.7)	56 (18.4)**
%predicted FEV ₁	104.2 ± 0.4	99.2 ± 1.0**
%predicted FVC	106.2 ± 0.5	105.2 ± 1.2
%predicted FEV ₁ /FVC	97.7 ± 0.2	93.7 ± 0.6**
ΣPAHs (ng/L) ^a	9861 ± 470	13322 ± 2880
Σ2-PAHs (ng/L) ^b	8474 ± 441	11717 ± 2837
Σ3,4-PAHs (ng/L) ^c	1371 ± 54	1512 ± 121
Urinary creatinine (mg/dL)	118.0 ± 2.3	132.6 ± 7.7

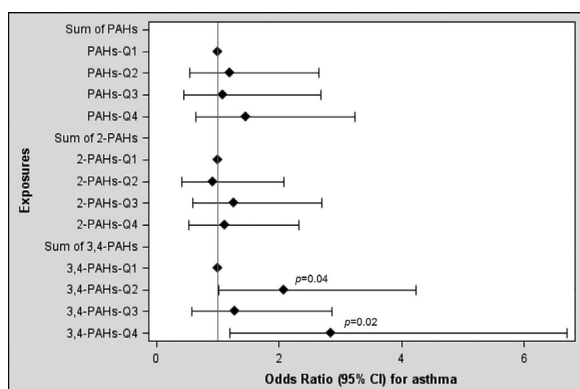
Results are shown as mean ± standard error (SE) for continuous variables, and as N (%) for binary variables. PAHs = polycyclic aromatic hydrocarbons.

aΣPAHs = sum of all urinary PAH metabolites.

bΣ2-PAHs = sum of urinary PAHs with 2 benzene rings.

cΣ3,4-PAHs = sum of urinary PAHs with 3 or 4 benzene rings.

* $P < 0.05$ or ** $P < 0.01$ for comparison between children with and without current asthma.

**FIGURE 1** Urinary PAHs by quartiles (Q) and current asthma in children, NHANES 2007–2012. All models adjusting for age, gender, race/ethnicity, health insurance coverage, family history of asthma, serum cotinine, and urinary creatinine level. PAH levels were calculated as molecular weight/L.

effects of PAH exposure and vitamin D insufficiency on lung function in children with asthma.

PAH that have 3 or 4-rings (phenanthrene and pyrene) are dominant components of total PAH emission from diesel vehicles²² and coal combustion.²³ Our results for urinary Σmol3,4-PAH and childhood asthma are consistent with those of prior studies showing that urinary PAH metabolites such as phenanthrene are associated with asthma or wheeze in U.S. children.^{9–11,24} Prenatal exposure to airborne PAH has been associated with increased benzo[a]pyrene-DNA adducts, reduced expression of interferon-γ, and changes in DNA methylation in cord blood that have been ultimately linked to childhood asthma.^{8,25,26} In addition, a combination of prenatal exposure to PAH and exposure to second-hand smoke or particulate matter has been linked to respiratory symptoms and probable asthma in young children,^{27,28} and pyrene exposure during pregnancy has been associated with childhood asthma.²⁹ The association between Σmol3,4-PAH and childhood asthma was seen in the 2nd and 4th quartiles of Σmol3,4-PAH (but not 3rd quartile), suggesting a non-linear association.

TABLE 2 Urinary PAH and lung function (%predicted) and FeNO in children, by asthma status

Outcomes	No current asthma (n = 2170)	Current asthma (n = 289)
	β (95% confidence interval)	
$\Sigma_{\text{mol}}\text{PAHs}^{\text{a}}$		
%predicted FEV ₁	0.94 (−0.31, 2.18)	−3.80 (−6.68, −0.88)*
%predicted FVC	1.19 (−0.01, 2.36)	−2.13 (−5.12, 0.87)
%predicted FEV ₁ /FVC	−0.10 (−0.84, 0.64)	−2.43 (−5.34, 0.49)
FeNO (ppb)	−0.57 (−2.01, 0.87)	−0.71 (−5.23, 3.80)
$\Sigma_{\text{mol}2}\text{-PAHs}^{\text{b}}$		
%predicted FEV ₁	0.67 (−0.50, 1.84)	−3.47 (−6.07, −0.88)*
%predicted FVC	0.90 (−0.19, 2.00)	−2.20 (−4.79, 0.39)
%predicted FEV ₁ /FVC	−0.09 (−0.80, 0.62)	−1.14 (−3.23, 0.96)
FeNO (ppb)	−0.44 (−1.81, 0.93)	−0.15 (−4.10, 3.79)
$\Sigma_{\text{mol}3,4}\text{-PAHs}^{\text{c}}$		
%predicted FEV ₁	0.59 (−1.33, 2.51)	−3.83 (−8.74, 1.09)
%predicted FVC	0.42 (−1.37, 2.21)	−2.93 (−7.28, 1.42)
%predicted FEV ₁ /FVC	0.30 (−0.81, 1.42)	−1.27 (−4.33, 1.79)
FeNO (ppb)	−1.87 (−3.73, 0.01)	2.12 (−3.98, 8.21)

All models adjusting for health insurance coverage, BMI z-score, use of oral or inhaled steroids in the past 2 days, serum cotinine, and urinary creatinine level. Models for FeNO in additionally adjusting for age, gender, and race/ethnicity.

a $\Sigma_{\text{mol}}\text{PAHs}$ = sum of all urinary PAH metabolites.

b $\Sigma_{\text{mol}2}\text{-PAHs}$ = sum of urinary PAHs with 2 benzene rings.

c $\Sigma_{\text{mol}3,4}\text{-PAHs}$ = sum of urinary PAHs with 3 or 4 benzene rings.

* $P < 0.05$.

In our study, urinary PAH metabolites were inversely associated with pre-bronchodilator FEV₁ and FEV₁/FVC among children with asthma and vitamin D insufficiency. In a prior study in California, the sum of PAH with 4, 5, or 6 rings was associated with decreased

post-bronchodilator lung function in children without asthma, but not in children with asthma.¹⁴ In further contrast to our results, PAH exposure was not associated with pre-bronchodilator lung function in children with or without asthma.¹⁴ However, vitamin D status, use of oral or

TABLE 3 Urinary PAH and lung function (%predicted) in asthmatic children, by vitamin D status

Outcomes	Vitamin D ≥ 30 ng/mL (n = 52)	Vitamin D < 30 ng/mL (n = 205)
	β (95% confidence interval)	
$\Sigma_{\text{mol}}\text{PAHs}^{\text{a}}$		
%predicted FEV ₁	0.63 (−3.71, 4.97)	−5.13 (−8.60, −1.66)**
%predicted FVC	−1.44 (−6.25, 3.37)	−2.70 (−6.65, 1.25)
%predicted FEV ₁ /FVC	1.96 (−0.80, 4.72)	−2.33 (−4.26, −0.41)*
$\Sigma_{\text{mol}2}\text{-PAHs}^{\text{b}}$		
%predicted FEV ₁	0.60 (−2.77, 3.97)	−4.87 (−8.20, −1.54)**
%predicted FVC	−1.34 (−4.77, 2.09)	−2.72 (−6.47, 1.03)
%predicted FEV ₁ /FVC	1.86 (−0.70, 4.42)	−2.04 (−3.88, −0.19)*
$\Sigma_{\text{mol}3,4}\text{-PAHs}^{\text{c}}$		
%predicted FEV ₁	−1.82 (−11.05, 7.41)	−5.57 (−11.12, −0.02)*
%predicted FVC	−0.89 (−11.23, 9.45)	−1.95 (−7.42, 3.52)
%predicted FEV ₁ /FVC	−1.05 (−4.61, 2.50)	−3.94 (−6.39, −1.49)*

All models adjusting for health insurance coverage, BMI z-score, use of oral or inhaled steroids in the past 2 days, serum cotinine, and urinary creatinine level.

a $\Sigma_{\text{mol}}\text{PAHs}$ = sum of all urinary PAH metabolites.

b $\Sigma_{\text{mol}2}\text{-PAHs}$ = sum of urinary PAHs with 2 benzene rings.

c $\Sigma_{\text{mol}3,4}\text{-PAHs}$ = sum of urinary PAHs with 3 or 4 benzene rings.

* $P < 0.05$, ** $P < 0.01$.

inhaled steroids, or exposure to second-hand smoke exposure were not assessed in that prior study, which also had a small sample size.¹⁴

Our finding of potential joint effects of vitamin D insufficiency and urinary PAH metabolites on lung function in asthmatic children is consistent with and expand those of our previous study in a Puerto Rican cohort, in whom residential proximity to a major highway (a surrogate marker of traffic-related air pollution) and vitamin D insufficiency conferred a higher risk of severe asthma exacerbations than having only one or none of these two risk factors.¹⁷ Our results suggest that airway inflammation and oxidative stress induced by air pollutants is worsened by vitamin D insufficiency, as vitamin D has antioxidant effects.³⁰ In further support of this hypothesis, a longitudinal study showed that vitamin D deficiency enhanced the detrimental effects of smoking on lung function decline, indirectly suggesting that vitamin D may mitigate the damaging effects of smoking on lung function.³¹ Alternatively, vitamin D insufficiency and PAH exposure may impair response to inhaled corticosteroids in children with asthma, ultimately leading to abnormal lung function.³⁰ In experimental studies, particulate matter (which includes PAH as components) has been shown to promote T-helper 17 cell (Th17) responses in human myeloid dendritic cells, while vitamin D treatment attenuated expression of Th17 related cytokines and maintained IL-10 synthesis in dendritic cells treated with particulate matter.³² Although we found no significant association between urinary PAH metabolites and FeNO (a marker of eosinophilic airway inflammation) in children with or without asthma, previous studies have shown that traffic exhaust contributes to allergic sensitization (not measured in the current study) and respiratory symptoms in children,³³ and that urinary phenanthrene and fluorene are linked to allergic sensitization to mouse in inner-city children.³⁴ Diesel exhaust particles that comprise PAHs have been shown to not only increase the effects of antigen exposure in those sensitized but also to promote antigen sensitization after allergen exposure.³⁵ Moreover, higher serum PAH have been positively correlated with serum total IgE, IL-4 and IL-5 in children with asthma.³⁶

Our study has several strengths, including data on a large and ethnically diverse cohort of children, performance of standardized procedures by uniformly trained personnel, and the ability to account for several potential confounders. We also recognize several limitations to our findings. First, a temporal relationship between PAH exposure and asthma or lung function cannot be determined due to the cross-sectional study design. For example, children exposed to PAH through air pollution may reduce outdoor activities and thus have lower vitamin D levels. Second, while aggregating PAH metabolites allowed us to measure PAH from all routes of exposure, it also limited our ability to study specific exposures from inhalation or single PAH or separating the PAH sources (eg, cigarette smoking or air pollution). Third, single urinary samples reflect recent exposures, and thus do not account for seasonal variability or long-term exposure to PAH. Lastly, the study did not account for several potential confounders, including outdoor activity, dietary factors, or exposure to other air pollutants.

In summary, exposure to PAH was associated with worse lung function in U.S. children with asthma, particularly in those with vitamin

D insufficiency. Ongoing clinical trials should help determine whether vitamin D supplementation ameliorates the detrimental effects of air pollution on lung function and disease severity among children with asthma.

CONFLICTS OF INTEREST

Dr. Celedon has received research material from Merck, GSK and Pharmavite, to provide medications free of cost to participants in NIH-funded studies, unrelated to the current work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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E-Table 1- Characteristics of children (6-17 years) who were and were not included in the current analysis, NHANES 2007-2012

Characteristics	Included n=2,459	Not included n=6,346
Age (years)	11.6 ± 0.1	11.6 ± 0.1
Male gender	1254 (50.8)	3271 (50.7)
Race/ethnicity		
Non-Hispanic white	728 (56.5)	1889 (57.4)
Non-Hispanic Black	588 (14.3)	1535 (14.2)
Mexican American/other Hispanic	935 (21.9)	2333 (20.4)
Other	208 (7.3)	589 (7.9)
Had health insurance coverage	1616 (90.2)	4196 (91.0)
Annual household income < \$20,000	518 (15.3)	1338 (15.1)
BMI z-score	0.68 ± 0.03	0.60 ± 0.02 [†]
Serum cotinine level (ng/ml)	4.8 ± 0.9	5.4 ± 0.9
Serum vitamin D level (ng/ml)	27.4 ± 0.5	27.2 ± 0.4
Vitamin D insufficiency (<30 ng/ml)	1600 (63.8)	3900 (67.0)
Family history of asthma	736 (28.4)	1866 (30.0)
Fractional exhaled nitric oxide (ppb)	16.3 ± 0.6	16.4 ± 0.4
Use oral or inhaled steroid in past 2 days	69 (2.7)	163 (3.1)
%predicted FEV ₁	103.6 ± 0.4	103.9 ± 0.4
%predicted FVC	106.0 ± 0.4	106.0 ± 0.4
%predicted FEV ₁ /FVC	97.7 ± 0.2	97.4 ± 0.2
ΣPAHs (ng/L) ^a	10262 ± 567	11725 ± 1929
Σ2-PAHs (ng/L) ^b	8851 ± 538	10182 ± 1872
Σ3,4-PAHs (ng/L) ^c	1387 ± 53	1431 ± 115
Urinary creatinine (mg/dL)	119.7 ± 2.2	109.3 ± 4.8 [†]

Results are shown as mean ± standard error (SE) for continuous variables, and as N (%) for binary variables. PAHs= polycyclic aromatic hydrocarbons

^aΣPAHs = sum of all urinary PAH metabolites, n=355 for not included

^bΣ2-PAHs = sum of urinary PAHs with 2 benzene rings, n=359 for not included

^cΣ3,4-PAHs = sum of urinary PAHs with 3 or 4 benzene rings, n=362 for not included

[†]P<0.05 for comparison of children who were and were not included in the analysis

E-Table 2 – Urinary PAH and lung function measures (as z-scores) in asthmatic children, by vitamin D status

	Vitamin D $\geq$ 30 ng/ml (n = 52)	Vitamin D < 30 ng/ml (n = 205)
Outcomes	β (95% confidence interval)	
$\Sigma_{\text{mol}}\text{PAHs}^{\text{a}}$		
FEV ₁ z-score	0.04 (-0.31, 0.40)	-0.41 (-0.69, -0.14)[‡]
FVC z-score	-0.14 (-0.54, 0.25)	-0.22 (-0.55, 0.12)
FEV ₁ /FVC z-score	0.32 (-0.11, 0.74)	-0.31 (-0.54, -0.07)[†]
$\Sigma_{\text{mol}}\text{2-PAHs}^{\text{b}}$		
FEV ₁ z-score	0.04 (-0.23, 0.31)	-0.39 (-0.66, -0.13)[‡]
FVC z-score	-0.13 (-0.41, 0.15)	-0.22 (-0.54, 0.10)
FEV ₁ /FVC z-score	0.30 (-0.09, 0.70)	-0.27 (-0.49, -0.05)[†]
$\Sigma_{\text{mol}}\text{3,4-PAHs}^{\text{c}}$		
FEV ₁ z-score	-0.15 (-0.92, 0.62)	-0.42 (-0.88, 0.04)
FVC z-score	-0.09 (-0.96, 0.78)	-0.13 (-0.60, 0.64)
FEV ₁ /FVC z-score	-0.17 (-0.64, 0.29)	-0.49 (-0.81, -0.18)[‡]

All models adjusting for health insurance coverage, BMI z-score, use of oral or inhaled steroids in the past 2 days, serum cotinine, and urinary creatinine level.

^a $\Sigma_{\text{mol}}\text{PAHs}$ = sum of all urinary PAH metabolites

^b $\Sigma_{\text{mol}}\text{2-PAHs}$ = sum of urinary PAHs with 2 benzene rings

^c $\Sigma_{\text{mol}}\text{3,4-PAHs}$ = sum of urinary PAHs with 3 or 4 benzene rings

[†]P<0.05, [‡]P<0.01