Phthalate exposure, respiratory outcomes, and biomarkers of oxidative stress in children with asthma

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Abstract

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Phthalates are a class of ubiquitous synthetic chemicals found in commonly used products, leading to widespread human exposure. A growing body of evidence demonstrates the adverse association between phthalates and asthma, a leading chronic condition among children. Evidence from *in vitro* and *ex vivo* studies suggests that phthalates induce pro-inflammatory mediators and other immune responses characteristic of asthma, and evidence in animal models suggests phthalates may act as adjuvants in allergic airway reactions, with some support for oxidative stress as an underlying pathophysiological mechanism. Epidemiological studies suggest phthalate exposure is associated with asthma outcomes, though the majority of studies do not investigate the role of phthalates as asthma triggers in children diagnosed with asthma.

This dissertation reports on a study that accessed participant data from the Home Air in Agriculture Pediatric Intervention Trial (HAPI) to examine longitudinal relationships between phthalate exposure, respiratory outcomes, and biomarkers of oxidative stress in a cohort of children with asthma. In 2015-2019, participants provided urine specimens and completed comprehensive health outcome assessments at up to 4 individual time points over a year of follow-up. Collected at these time points were exposure measures for 11 urinary phthalate metabolites (plus one summed measure, ∑DEHP) as well as outcome measures for fractional exhaled nitric oxide (FENO), urinary leukotriene E_4 ($uLTE_4$), spirometry, the Asthma Control Test (ACT), a caregiver questionnaire of perceived symptoms, and urinary biomarkers of oxidative stress (a biomarker of lipid peroxidation via measure of 8-isoprostane and a biomarker of DNA/RNA oxidative damage via combined measure of 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), and 8-hydroxyguanine). Laboratory analyses on urine specimens to quantify all biomarkers of exposure and biomarkers of effect were carried out through the Children's Health Exposure Analysis Resource (CHEAR). We utilized linear mixed effects models and generalized linear mixed models to estimate associations between urinary phthalate metabolites and all outcomes. Our primary models controlled for specific gravity, age, sex, the HAPI intervention, body mass index (BMI), atopy, and season. We investigated effect modification of FENO by atopic status.

A total of 79 participants provided 281 observations in this study. Creatinine-corrected urinary phthalate metabolite concentrations were comparable to levels reported from National Health and Nutrition Examination Survey (NHANES) data (2015-2016). Geometric means (geometric standard deviation) of phthalate metabolite

concentrations ranged from 1.4 μg/g creatinine (2.5 μg/g creatinine) for MEHP to 25.4 μg/g creatinine (2.5 μg/g creatinine) for MEP. In linear mixed effects models, FENO concentrations generally increased as urinary phthalate metabolite concentrations increased, although not all associations were statistically significant. For a doubling (100% increase) in urinary MECPP, MEHP, MEP, and MNBP, FENO increased by 7.9% (95% CI: 0.7 – 15.7), 6.4% (95% CI: 0.0 – 14.1), 6.4 (95% CI: 0.7 – 13.3), and 8.7% (95% CI: 1.4 – 16.5), respectively. We did not observe statistically significant interaction with atopy. All phthalate measures (including ∑DEHP) demonstrated statistically significant, positive relationships with $uLTE_4$, with effect sizes ranging from a 6.4% increase in $uLTE_4$ (95% CI: 2.8 – 10.2) for a doubling in MCIOP to a 17.3% increase in $uLTE_4$ (95% CI: 12.5 – 22.3) for a doubling in MNBP. There were no statistically significant associations between urinary phthalates and caregiver report of child asthma symptoms in the two weeks prior, FEV_1, or ACT score. The oxidative stress urinary biomarker, 8-isoprostane, increased as urinary phthalate metabolite concentrations increased. Effect sizes ranged from a 7.9% (95% CI: 2.8% - 12.5%) increase in 8-isoprostane for doubling of MCIOP, to 19.8% (95% CI: 13.3% - 26.6%) increase in 8-isoprostane for doubling of MNBP. The DNA/RNA oxidative damage biomarker (combined measure of 8-OHdG, 8-OHG, and 8-hydroxyguanine) generally increased as urinary phthalate metabolite concentrations increased, though only associations with MCINP and MEP were significant in our primary models. For each doubling of MCINP and MEP, the DNA/RNA oxidative damage biomarker increased by 5.7% (95% CI: 0% – 11.7%) and 5.7% (95% CI: 0.7% – 10.9%), respectively.

In conclusion, in a cohort of children with asthma, we observed significant associations between urinary phthalate metabolites and concurrent biomarkers of inflammation and oxidative stress but not with clinical metrics such as symptoms in weeks or month prior or spirometric measurement of obstruction (FEV_1). The most consistent (across phthalate metabolites) and pronounced effects were observed with $uLTE_4$ and 8-isoprostane. Future studies of phthalate exposure and asthma outcomes should investigate these biomarkers of effect further, as well as the pathophysiological mechanisms they represent, in order to shed light on the role of phthalates as exacerbators of childhood asthma.

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LIST OF ACRONYMS

8-OHdG	8-hydroxydeoxyguanosine
8-OHG	8-hydroxyguanosine
ACT	Asthma Control Test
ATS	American Thoracic Society
BBzP	Butylbenzyl phthalate
BMI	Body mass index
C-ACT	Childhood Asthma Control Test
CDC	Centers for Disease Control and Prevention
CHAMACOS	Center for Health Assessment of Mothers and Children of Salinas
CHEAR	Children's Health Exposure Analysis Resource
CV	Coefficient of variation
CysLTs	Cysteinyl leukotrienes
DBP	Di-n-butyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
∑DEHP	DEHP metabolites summed measure (MECPP, MEHHP, MEHP, and MEOHP)
DEP	Diethyl phthalate
DIBP	Di-isobutyl phthalate
DIDP	Di-isodecyl phthalate
DINP	Di-isononyl phthalate
DNA	Deoxyribonucleic acid
DOP	Di-n-octyl phthalate
ELISA	Enzyme-linked immunosorbent assay
ERS	European Respiratory Society
FENO	Fractional exhaled nitric oxide

FEV_1	Forced expiratory volume in 1 second
GLMM	Generalized linear mixed effects model
GM	Geometric mean
GSD	Geometric standard deviation
HAPI	Home Air in Agriculture Pediatric Intervention Trial
HEPA	High efficiency particulate air
HERMOSA	The Health and Environmental Research on Makeup of Salinas Adolescents
HPLC	High performance liquid chromatography
ICC	Intraclass correlation coefficient
IgE	Immunoglobulin E
IL	Interleukin
iNOS	Inducible nitric oxide (NO) synthase
LC	Liquid chromatography
LTE_4	Leukotriene E_4
LMM	Linear mixed effects model
MBZP	Mono-benzyl phthalate
MCINP	Mono-carboxy isononyl phthalate
MCIOP	Mono-carboxy isooctyl phthalate
MCPP	Mono (3-carboxypropyl) phthalate
MECPP	Mono-(2-ethyl-5-carboxypentyl) phthalate
MEHHP	Mono (2-ethyl-5-hydroxyhexyl) phthalate
MEHP	Mono ethyl hexyl phthalate
MEOHP	Mono (2-ethyl-5-oxohexyl) phthalate
MEP	Monoethyl phthalate
MIBP	Mono-isobutyl phthalate

MNBP	Mono-n-butylphthalate
MS	Mass spectrometry
NH_3	Ammonia
NHANES	National Health and Nutrition Examination Survey
NIH	National institutes of Health
NIST	National Institute of Standards and Technology
NO	Nitric oxide
OR	Odds ratio
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGHS	Prostaglandin endoperoxide synthase
PM	Particulate matter
$PM_{2.5}$	Particulate matter with diameter less than 2.5 μm
PM_{10}	Particulate matter with diameter less than 10 μm
ppb	Parts per billion
PVC	Polyvinyl chloride
QC	Quality control
RNA	Ribonucleic acid
SD	Standard deviation
SRM	Standard reference material
TESIE	Toddlers Exposure to SVOCs in Indoor Environments
TNF-α	Tumor necrosis factor alpha
$uLTE_4$	Urinary leukotriene E_4
YVFWC	Yakima Valley Farmworker Clinic

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DEDICATION

To my family.

CHAPTER 1. INTRODUCTION AND SPECIFIC AIMS

1.1 INTRODUCTION

Phthalates are diesters of 1,2-benzenedicarboxylic acid (phthalic acid), a class of ubiquitous synthetic chemical compounds predominately used as plasticizers in manufacturing processes to impart desirable properties, such as durability and flexibility. Their use extends to a wide range of consumer products, personal care products, medical equipment, and building materials [1]. The manufacture of phthalates and their application as plasticizers can be traced back to the 1920's, and their proliferation over the past century is attributed in large part to their desirable properties in comparison to previously available plasticizers, including being relatively odorless and inexpensive to synthesize [2,3]. Phthalate production rose steadily beginning with their use as plasticizers in the manufacturing of a newly commercialized compound, polyvinyl chloride (PVC), in the 1930's. Developments in plasticizer and polymer technologies resulted in greater performance of PVC across an array of applications for durable goods and materials, which led to more widespread consumer adoption of PVC in the 1950s. The rise in the popularity of PVC increased the demand and production volume of plasticizers, especially phthalates [4]. In the decades since, phthalates have emerged as one of the most highly produced chemical classes worldwide and are among the most prominent plasticizers produced annually.

Exposure to phthalates is widespread, with metabolites present in the urine of over 90% of children in the United States [5]. There are several common phthalate esters in use (sometimes referred to as parent compounds) each with selected metabolites that serve as biomarkers for exposure in urine (Table 1). Phthalates migrate from the products

they are found in and are present in indoor dust at high concentrations [6,7]. The presence of phthalates in home, daycare, and school environments poses a high risk for childhood exposures [8–10]. Dominant routes of exposure in children vary by age and individual phthalate esters [11], but occur via ingestion, inhalation, and dermal absorption [6,12,13], and children are at a higher risk of exposure due to behavioral and physiological factors [14].

Interest in the association between phthalates and asthma has grown steadily in the last decade [15–21]. Given that phthalates are consistently found in dust, airway exposure via dust inhalation may be a risk factor for airway disease. Phthalate ingestion and dermal absorption may also induce airway and immune effects [22], and although the relative significance of each exposure route on allergic airway disease needs further study [19], systemic exposure from multiple routes is considered relevant to respiratory outcomes. Evidence suggests phthalates may contribute to childhood asthma [16,17,19,20]. Few studies attempt to elucidate the potential for phthalate exposures to serve as an environmental trigger of pediatric asthma exacerbation among individuals who have already been diagnosed with asthma. This data gap is important given identification and control of known environmental triggers of asthma exacerbation have become important components of consensus guidelines on pediatric asthma management [23,24].

Several lines of evidence suggest that further study is warranted to evaluate phthalates as triggers of asthma. In experimental *in vitro* and *ex vivo* studies, phthalates have been shown to induce pro-inflammatory mediators including cytokines and other immune cells that are associated with airway responses in asthma [16,19]. In animal

studies, phthalates have demonstrated the ability to act as adjuvants in allergic airway reactions in mouse models pertinent to asthma [22,25–27], and this adjuvant effect appears to involve oxidative stress [28–31].

Epidemiological studies in children have demonstrated associations between various urinary phthalate metabolites and lung function [32,33], report of respiratory symptoms such as cough and wheeze [34], and report of doctor diagnosed asthma [35]. While the existing epidemiological evidence suggests a role for phthalates in the pathogenesis and severity of asthma, the evidence overall is comprised primarily of studies utilizing outcomes that indicate presence or absence of disease, and many rely on cross-sectional analyses [18]. Very few epidemiological studies report on phthalate exposure, pathophysiologic factors of disease, and asthma exacerbation exclusively in study populations of children with asthma [36]. We sought to examine phthalates as potential triggers of asthma exacerbation in an understudied cohort of Latinx farm worker children utilizing a strong study design including repeated measures of diverse outcomes informative to asthma exacerbation, including biomarkers of inflammation and oxidative stress, clinical measures for asthma responses including lung function testing and validated questionnaires, and caregiver report of symptoms.

The Home Air in Agriculture Pediatric Intervention Trial (HAPI) recruited children with asthma in Yakima Valley, Washington in the United States between 2015 and 2019 [37]. The HAPI study randomly assigned participants to one of two groups: (i) those only receiving an asthma education program, or (ii) those receiving two high-efficiency particulate air (HEPA) air cleaners in their homes in addition to the asthma education program. HAPI collected data from each participant household for one year, including

urine specimens and a multi-pronged asthma health outcome assessment at up to 4 time points per participant. This longitudinal study presents an excellent opportunity to evaluate the associations between short term variation in urinary phthalate metabolites, which reflect phthalate exposure across all routes of exposure, and key outcomes related to changes in asthma health status among children with asthma, as well as potentially related mechanistic markers.

We present the segment of the study pertaining to aim 1, an investigation of urinary phthalate metabolites and their association with asthma outcomes, in Chapter 2. The segment of the study that pertains to the associations between urinary phthalate metabolites and biomarkers of oxidative stress is presented in Chapter 3. Concluding remarks reflecting all components are presented in Chapter 4. The dissertation specific aims are listed below and in Figure 1.

1.2 SPECIFIC AIMS

Aim 1: Determine associations between concentrations of urinary phthalate metabolites and indicators of asthma exacerbation, including fractional exhaled nitric oxide (FENO) and reported symptom days in 2 weeks prior (primary outcomes), as well as secondary outcomes (same day Asthma Control Test score, spirometry measures, and urinary leukotriene e4 ($uLTE_4$)).

Aim 2: To estimate the associations between urinary biomarkers of phthalate exposure and urinary biomarkers of oxidative stress representing lipid peroxidation (8-isoprostane) and DNA/RNA oxidative damage (combined measure of 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), and 8-hydroxyguanine).

This study is poised to generate novel data to inform knowledge gaps on phthalate exposure and child asthma morbidity, as well as biomarkers of oxidative stress, in an understudied and vulnerable population of Latinx farm worker children with asthma.

1.3 TABLES AND FIGURES

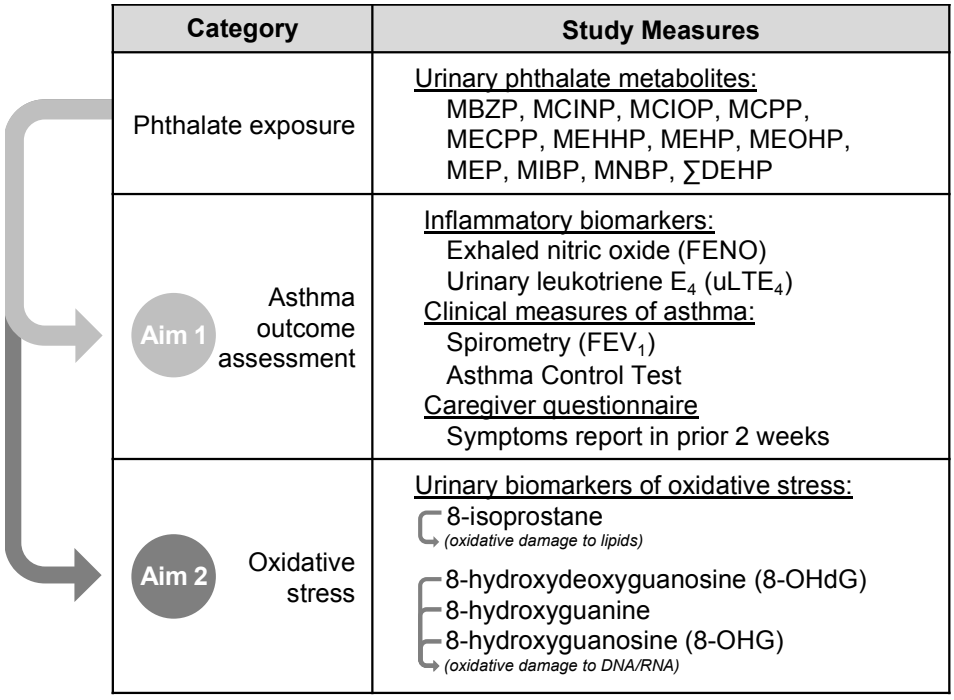
Table 1. Phthalate esters and corresponding phthalate metabolites measured in this study*

Phthalate ester	Abbreviations		Corresponding phthalate metabolite	Abbreviations	
Butylbenzyl phthalate	BBzP		mono-benzyl phthalate	MBZP	
Di-isodecyl phthalate	DIDP	(or DDP)	mono-carboxy isononyl phthalate	MCINP	(or MCNP)
Di-isononyl phthalate	DINP	(or DNP)			
Di-isononyl phthalate	DINP	(or DNP)	mono-carboxy isooctyl phthalate	MCIOP	(or MCOP)
Di-n-octyl phthalate	DOP		mono (3-carboxypropyl) phthalate	MCPP	
Di-n-butyl phthalate	DBP				
Di(2-ethylhexyl) phthalate	DEHP		mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP	
			mono (2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	
			mono ethyl hexyl phthalate	MEHP	
			mono (2-ethyl-5-oxohexyl) phthalate	MEOHP	
Diethyl phthalate	DEP		monoethyl phthalate	MEP	
Di-isobutyl phthalate	DIBP		mono-isobutyl phthalate	MIBP	
Butylbenzyl phthalate	BBzP		mono-n-butylphthalate	MNBP	(or MBP)
Di-n-butyl phthalate	DBP				

*listed alphabetically by metabolite abbreviation

Adapted from references: [12,14]

Figure 1. Study aims and corresponding measures



CHAPTER 2. PHTHALATE EXPOSURE AND MEASURES OF ASTHMA EXACERBATION IN A RURAL AGRICULTURAL COHORT OF LATINX CHILDREN IN YAKIMA VALLEY, WASHINGTON

INTRODUCTION

Phthalates are a class of synthetic chemicals primarily used as plasticizers in manufactured materials, most prominently in polyvinyl chloride (PVC), with a multitude of additional applications in building materials, medical devices, food and beverage packaging, personal care products, and consumer products in general [38]. The ubiquity of phthalates and their ability to migrate from polymers contributes to their proliferation as indoor environmental and dietary contaminants [7,39,40]. Sources and routes of exposure in humans vary by individual phthalate esters, but exposure is universal and occurs continuously through ingestion, inhalation, and dermal routes, with children at risk for increased exposure compared to adults due to increased surface area to volume ratio, increased respiratory rate, and increased hand to mouth behaviors compared to adults [11,14].

Numerous studies indicate phthalate exposure is linked to allergic disease and asthma, though underlying mechanisms are unclear [16,18,19]. *In vitro* and *ex vivo* studies suggest phthalates may induce inflammatory mediators (e.g., interleukin-4 (IL-4), IL-6, IL-8, and tumor necrosis factor alpha (TNF-α)) [41–43] and modulate other immune cells (e.g., macrophages and dendritic cells) involved in respiratory outcomes related to asthma, while mouse models demonstrate a role for phthalates as adjuvants in allergic airway reactions [19]. Epidemiologic studies in children have demonstrated positive

associations between urinary phthalate metabolites and increased asthma risk [18], airway inflammation [36,44], and reduced lung function [36]. Several knowledge gaps persist, especially with respect to potential for phthalate-induced exacerbation of symptoms among children with preexisting asthma diagnoses. Airway inflammation is a key characteristic of asthma, and biomarkers of inflammation in exhaled breath can function as informative measures of asthma pathophysiology in children [45]. Fractional exhaled nitric oxide (FENO) is a biomarker of eosinophilic airway inflammation that offers utility as an objective measure in child asthma assessment with predictive potential for the risk of asthma exacerbation [46,47]. Urinary biomarkers also inform assessment of airway and systemic inflammation [48]. Cysteinyl leukotrienes (CysLTs) are a class of inflammatory lipid mediators leading to the metabolic end product urinary leukotriene E_4 ($uLTE_4$), a biomarker correlated with airway inflammation and asthma exacerbation [49]. Longitudinal studies utilizing an array of both objective (e.g., biomarkers of effect, lung function testing) and caregiver- or self-report measures are needed to investigate phthalates as triggers of airway inflammation and asthma symptoms among children with asthma, especially within understudied racial, geographic, and socioeconomic groups. Studies have reported elevated urinary phthalate metabolite concentrations among Latinx children [50,51], and such populations in rural, low-income settings may experience unique factors related to asthma and corresponding stressors [52,53].

In this longitudinal study of Latinx children with asthma in a primarily rural agricultural community in lower Yakima Valley, WA, we use repeated measures to examine the relationship between eleven individual urinary phthalate metabolites (mono-benzyl phthalate (MBZP), mono-carboxy isononyl phthalate (MCINP), mono-carboxy

isooctyl phthalate (MCIOP), mono (3-carboxypropyl) phthalate (MCPP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono ethyl hexyl phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), monoethyl phthalate (MEP), mono-isobutyl phthalate (MIBP), and mono-n-butylphthalate (MNBP)) and a comprehensive respiratory health assessment consisting of established clinical tools for assessing asthma status (spirometry [54], FENO [46], the Asthma Control Test (ACT) [55], as well as caregiver report of symptoms and a biomarker of inflammation that has shown promise in pediatric asthma research, $uLTE_4$) [49].

MATERIALS AND METHODS

Study participants and setting

This study accessed participant samples and data from the Home Air in Agriculture Pediatric Intervention Trial (HAPI), a randomized trial of high-efficiency particulate air (HEPA) air cleaners with asthma education vs. education only [37]. The HAPI intervention was designed to examine the effectiveness of the HEPA air cleaners in rural homes to reduce particulate matter (PM) and ammonia concentrations as well as to examine health outcomes among participants. The original study was not designed to address phthalates specifically. Air cleaners would not be expected to reduce phthalate exposure assessed via urinary phthalate metabolites in this study, as diet is the dominant driver of total phthalate exposure in children aged 3-11 years [11]. Furthermore, children's urinary phthalate metabolites levels have not been affected by air purifiers in other settings [56]. Enrollment occurred on a rolling basis beginning in 2015 and data collection concluded in 2019. The present study uses data from the total sample of 79 HAPI participants who

provided at least a single urine specimen. Details regarding recruitment and follow up are provided in Masterson et al. 2020 [37]. Briefly, participants were recruited through the Yakima Valley Farmworker Clinic (YVFWC), and eligibility criteria included: (i) child's age six to twelve years, (ii) poorly controlled asthma identified through a screening questionnaire (iii) absence of smokers in the home, and (iv) residential proximity (i.e., within ½ miles) to crop and/or dairy production. Human subjects research approval was granted by the University of Washington Institutional Review Board and the YVFWC research review committee.

The HAPI study followed each participant for one year, with data and sample collection at four separate time points. Same-day exposure and outcome assessments occurred at each time point. The first time point took place at enrollment, while the second, third, and fourth occurred at follow up visits approximately two, six, and 12 months later, respectively. After the second time point, participant households were randomized to one of two study arms: (i) asthma education, or (ii) asthma education plus two indoor portable HEPA air cleaners, one placed in the child's sleeping area and the other in the main living area.

Urine sample collection and analysis

Spot urine samples were collected at each time point in phthalate-free polyethylene containers and transported on dry ice to a field laboratory where specific gravity was measured using a handheld refractometer and samples were stored at a temperature of -20ºC. At the field laboratory, 24 trip blanks were prepared using high performance liquid chromatography (HPLC) water (VWR, Radnor, Pennsylvania, USA).

Urine samples and blanks were randomized and shipped along with 5 aliquots of National Institute of Standards and Technology (NIST) standard reference material (SRM) 3673 (Organic Contaminants in Non-Smokers' Urine) to the Icahn School of Medicine at Mount Sinai laboratory hub of the National Institutes of Health (NIH) Children's Health Exposure Analysis Resource (CHEAR), where they were analyzed for the target metabolites using isotope-dilution liquid chromatography with tandem mass spectrometry following Centers for Disease Control and Prevention (CDC) methods [57–59]. CHEAR laboratory hubs include their own internal quality assurance and quality control (QC) protocols, including analysis of pooled samples to assess analytical precision.

Respiratory health assessment

The respiratory health assessment related to child asthma morbidity included measurement of forced expiratory volume in one second (FEV_1), FENO, caregiver report of symptom days in the prior 2 weeks and age-appropriate administration of the ACT (child ACT for age 4-11 years, adult ACT for age 12 year and older). In addition, a urinary biomarker of inflammation, $uLTE_4$, was measured as prior studies have demonstrated that levels of this biomarker of systemic inflammation correlate with asthma exacerbation [49]. The assessments are described briefly here but are described in detail elsewhere [37].

Measurement of FENO was assessed using the NIOX Vero, (Aerocrine Inc., Stockholm, Sweden) in accordance with standardized procedures recommended by both the American Thoracic Society (ATS) and European Respiratory Society (ERS) [60]. FENO is associated with eosinophilic airway inflammation [47]. FENO values less than

20 ppb in children <12 years of age are interpreted to be low and indicate that eosinophilic inflammation is less likely, while values greater than 35 ppb in children are interpreted to be high and indicative of eosinophilic inflammation [46].

FEV_1 was measured via spirometry using the EasyOne spirometer (NDD Technologies, Andover, MA) in accordance with recommended procedures from the ATS and ERS [54,61,62].

An aliquot of the same urine sample that was used for the urinary phthalate metabolite analyses was designated for the detection of $uLTE_4$ and submitted to the Icahn School of Medicine at Mount Sinai CHEAR laboratory hub, where all laboratory analysis of urine samples were conducted [57]. Quantification of $uLTE_4$ was carried out via an enzyme-linked immunosorbent assay (No. 501060; Cayman Chemical, Ann Arbor, Michigan, USA). To report $uLTE_4$ and urinary phthalate metabolites in creatinine-corrected units to allow comparison with prior research [49], urine creatinine was measured using a validated colorimetric method also via CHEAR.

As a part of a broader questionnaire, at each urine sample collection time point, the primary caregiver was asked: “During the past two weeks, how many days did the child have any asthma symptoms, such as wheezing, coughing, tightness in the chest, shortness of breath, waking up at night because of asthma symptoms, or slowing down of usual activities because of asthma?”.

Field staff also deployed the ACT, a validated composite measure of asthma control recommended by several guidelines for assessment of asthma control in both children and adults [55,63]. Children up to eleven years of age were given the Childhood Asthma Control Test (C-ACT), a 7-item questionnaire which contains four questions for

the child that solicit perceptions of asthma severity, limitation of physical activity, cough, and sleep disturbances, while the caregiver answered three questions pertaining to daytime symptoms, daytime wheeze, and sleep disturbances with a 4-week recall period [64]. Responses to the first four questions are scored 0-3 and the latter three questions 0-5 for a summed score range of 0-27. Higher scores represent responses indicating less frequent signs and symptoms (better asthma control). Children twelve years of age or older were given the 5-item ACT, which consists of four questions for the child that solicit perception of their asthma's impact on schoolwork, shortness of breath, sleep disturbances, and rescue medication use, and a question on overall perception of asthma control, all for the prior 4-week recall period. Each response is scored 1-5 for a total summed score range of 5-25. ACT and C-ACT scores of 19 or less are commonly used to identify poorly controlled asthma [65]. We computed a percentage-scale score to standardize the ACT and C-ACT results by dividing the score observed by the total possible score.

Covariate assessment

Various participant-specific characteristics related to asthma health were also collected in the HAPI study [37]. These included skin prick testing to determine atopy based on at least one positive among 6 common aeroallergens, determination of use of asthma controller medications, and assessment of additional home environment and demographic factors (e.g., annual household income).

Statistical analysis

Variables of interest were evaluated for normality by visualization of distributions and urinary phthalate metabolite, FENO, and $uLTE_4$ concentrations were natural log transformed. Pearson correlation coefficients were calculated between pairs of the eleven individual urinary phthalate metabolites. The four urinary metabolites of di-2-ethylhexyl phthalate (DEHP), MECPP, MEHHP, MEHP, and MEOHP, were grouped by summing their individual molar concentrations and then converting this value to represent a single summed DEHP metabolite variable, ∑DEHP [5]. For urinary phthalate metabolite concentrations < LOD, the machine values were used. In descriptive analyses, we addressed urine dilution among phthalate metabolite concentrations by calculating uncorrected and specific gravity-corrected concentrations. In descriptive analyses of $uLTE_4$, creatinine-corrected values were calculated to allow comparison with other published data. In regression models, specific gravity was included as a covariate to correct for urine dilution, and values of urinary phthalate metabolites and $uLTE_4$ were not corrected in these models. We examined FEV_1 in our model specifications as both FEV_1 z-scores and FEV_1 as a percentage of predicted values (FEV_1 percent predicted) for child age, height, and sex [66].

To estimate associations between urinary phthalate metabolite concentrations and respiratory health measures, and account for the longitudinal study design with repeated measures from participants, we used linear mixed effects models (LMMs) for continuous outcomes and generalized linear mixed effect models (GLMMs) for dichotomized outcomes. Model specifications included fixed effects for the predictor of interest and corresponding covariates, and a by-participant random intercept. The overall model equation is as follows:

$$Y_{ij} = \beta_0 + \beta_1 * ph_{ij} + \sum \beta_k * X_{ijk} + \gamma_i + \epsilon \quad (1)$$

In equation (eq.) 1, Y_{ij} denotes the *j*-th observation on the *i*-th participant, ph_{ij} denotes the phthalate metabolite, X_{ijk} denotes the covariates, and γ_i denotes the random intercept. We fit LMMs for natural log-transformed FENO, FEV_1 z-scores, FEV_1% predicted, natural log-transformed $uLTE_4$, and a transformed percentage-scale ACT score outcome. The ACT transformation addresses the heterogeneity of variance associated with the fixed upper and lower limits of the percentage scale. This ACT outcome was calculated using a logit-like transformation as follows:

$$log\left(\frac{Y-5}{(100-(Y-5))}\right) \quad (2)$$

In eq. 2, *Y* denotes the percentage-scale score on the ACT or C-ACT, calculated as the quotient of the observed score divided by the maximum possible score. In the analysis of our symptom questionnaire, we fit GLMMs for this dichotomized outcome variable indicating whether the caregiver provided an affirmative response (any symptom days) to any of four questions regarding asthma symptoms in the prior 2 weeks. In all outcome models, we specified fixed effects for a single predictor of interest (a given urinary phthalate metabolite or ∑DEHP) and all model covariates, and we specified a random intercept for participants.

Four separate models were fit for each of the twelve separate exposure variables (eleven individual natural log-transformed urinary phthalate metabolites and natural log-transformed ∑DEHP) and the respiratory outcomes: (1) a crude model, (2) a minimally adjusted model, (3) a fully adjusted model (primary), and (4) an extended adjustment

model. Participant and demographic factors were selected *a priori* as potential confounder or precision variables with respect to the relationship being modeled.

The crude models consisted of only the predictor of interest (natural log-transformed urinary phthalate metabolite or natural log-transformed ∑DEHP) as the independent variable and the respiratory outcome as the dependent variable, with specific gravity as a covariate to correct for urine dilution. The minimally adjusted models added participant factors associated with asthma outcomes: age [67], sex [68], and HAPI intervention status as covariates. HAPI intervention status was coded as a binary variable to indicate whether or not a given observation is both (i) of the intervention group and (ii) post-randomization such that in-home air cleaners would be present at the observation's time point of data collection. The fully adjusted models added additional asthma-related factors: baseline clinic visit body mass index (BMI)-for-age percentile [69] (continuous percentile generated from CDC growth charts [70]), atopy [71], and meteorological season as a nominal variable with four levels: (i) March, April, May, (ii) June, July, and August, (iii) September, October, November, and (iv) December, January, and February [71]. Extended adjustment models added annual household income (as an ordinal variable with four levels: (i) <$14,999, (ii) $15,000-$29,999, (iii) $30,000-$60,000, and (iv) >$60,000) [72], baseline assessment of controller medication utilization as a binary variable, and the visit (e.g., of the 4 time points possible).

As an exception, models estimating effects on FENO, FEV_1 z-score, and FEV_1% predicted additionally included the time of day of the measurement as a covariate in the extended model. In addition, to examine potential effect modification of atopy in FENO models, we conducted an additional analysis including an interaction term.

All statistical analyses were conducted in R version 3.6.2 (The R Foundation for Statistical Computing), with package “lme4” for LMMs and “glmer” for GLMMs. Analyses were interpreted as statistically significant at p-value < 0.05.

RESULTS

Participant characteristics

A total of 79 HAPI participants were included in the present study (Table 1). The mean age at enrollment was 9 years (SD: 2.1 years), and 29 participants (36.7%) were female. All participants identified as Hispanic/Latinx, and 61 (77.2%) came from households where the primary spoken language was Spanish. The mean BMI-for-age percentile at enrollment was 79% (SD: 26%). Use of controller medication was documented at baseline for 69 participants (87.3%). The number of atopic children was 48 (60.8%). The HAPI randomization resulted in 39 participants (49.4%) in the intervention group and 40 (50.6%) in the control group, resulting in 65 observations coinciding with possession of HEPA air cleaners and 216 observations with no air cleaners. Approximately half (39 (49.4%)) of the participants’ homes were located in a town setting, 18 (22.8%) were located in a rural area with no farm, and 19 (24.1%) were located on a farm. An annual household income level of <$14,999 was reported by 12 households (15.2%), with another 31 households (39.2%) at the $15,000-$29,999 level, 27 households (34.2%) at the $30,000-$60,000 level, and 5 households (6.3%) exceeded $60,000 in annual income.

Urinary phthalate metabolite concentrations

A total of 281 urine samples were collected and analyzed for target phthalate metabolites: 54 participants provided 4 urine samples each, 19 provided three samples each, two provided two samples each, and 4 provided one sample each. A total of 77 urine samples were collected at timepoint 1, 75 at timepoint 2, 73 at timepoint 3, and 56 at timepoint 4. The number of observations of each exposure and outcome variable, including urinary phthalate metabolites, are listed in Table S1 both by visit and in total. Analyte-specific limits of detection, calculated as ± 33% of laboratory blank concentrations, ranged from 0.05 ng/mL for MCINP, MCIOP, and MCPP, to 0.2 ng/mL for MBZP, MECPP, and MEP. Trip and laboratory blanks were all < LOD. Mean (N=5) recoveries of the analyte-specific NIST 3673 reference values ranged from 88% (coefficient of variation (CV) 4%) for MIBP to 102% (CV 12%) for MNBP, indicating good analytical accuracy, while % CVs calculated using the CHEAR QC pools ranged from 3-8%, indicating good analytical precision.

The target phthalate metabolites were detected in 100% of samples, except for MEHP which was detected in 99%. Table 2 presents descriptive statistics of the unadjusted and creatinine-corrected concentrations. The unadjusted geometric mean (geometric SD) concentrations ranged from 1.4 ng/mL (2.6 ng/mL) for MEHP to 24.7 ng/mL (2.8 ng/mL) for MEP, while the creatinine-corrected concentrations ranged from 1.4 µg/g creatinine (2.5 µg/g creatinine) for MEHP to 25.4 µg/g creatinine (2.5 µg/g creatinine) for MEP. Specific gravity-corrected concentrations are available in Table S3. Pearson correlation coefficients were weakest for MCINP and MEP (0.22) and generally strongest among DEHP metabolites (e.g., MEHHP and MEOHP (0.99)) (Table S2).

Respiratory outcomes

Table 3 summarizes the respiratory outcomes measures. A total of 258 FENO measurements were available from 76 participants. The mean FENO values were 22.9 parts per billion (ppb) (SD: 25.5), falling between "low" and "high" inflammation cut-offs for children (<20 and >35, respectively) [46]. A total of 274 $uLTE_4$ measures were available from all 79 participants, with a mean of 1.43 μg/g creatinine (SD: 0.56). Questionnaire results regarding caregiver report of symptom days in the prior 2 weeks were also available from all 79 participants at all time points, with 162 instances (57.6%) of a caregiver providing an affirmative response regarding perceived symptoms. Results were available from 279 age-appropriate administrations of the ACT from all 79 participants, with 75 (26.8%) of these administrations resulting in a score that suggested asthma was poorly controlled in the child. The mean percent score across all ACT administrations was 80.3% (SD: 12.8%). FEV_1 was available in 231 observations from 76 participants, with a mean value of 1.81 L (SD: 0.57). Mean FEV_1 z score and % predicted were -0.53 and 93.6 respectively.

Associations between urinary phthalate metabolites and respiratory outcomes

Estimated regression coefficients of urinary phthalate metabolites and respiratory outcomes are shown in Table 4a-c (expanded regression output in Tables S4 – S9). Log FENO concentrations generally increased as log urinary phthalate metabolite concentrations increased, although not all associations were statistically significant (Table 4a). For 100% (i.e., doubling) increase in MECPP, MEHP, MEP, and MNBP, concentrations of FENO increased by 7.9% (95% CI: 0.7 – 15.7), 6.4% (95% CI: 0.0 –

14.1), 6.4% (95% CI: 0.7 – 13.3), and 8.7% (95% CI: 1.4 – 16.5), respectively, in the primary model. In the extended model, MECPP, MEHP and MEP remained significantly associated with FENO, with comparable effect sizes to the primary model. We did not observe statistically significant interaction of the metabolites by atopy (Table S10), although a subset of interaction coefficients were relatively large, suggesting the possibility of more pronounced associations among participants with atopy.

In the $uLTE_4$ models, all phthalate metabolite models (including ∑DEHP) demonstrated statistically significant, positive associations (Table 4a). In the primary models, effect sizes ranged from a 6.4% increase in $uLTE_4$ (95% CI: 2.8 – 10.2) for a 100% increase in MCIOP to a 17.3% increase in $uLTE_4$ (95% CI: 12.5 – 22.3) for a 100% increase in MNBP. Findings were similar in the extended models.

In models of caregiver report of any symptom days in the prior two weeks, none of the phthalate exposure metrics were significantly associated in primary models. MCIOP was marginally significant in the minimally adjusted model (odds ratio (OR) 1.37 (95% CI: 1.00 – 1.86 (Table 4c).

No urinary phthalate metabolites were statistically significantly associated with FEV_1 z-score, FEV_1 percent predicted, or standardized ACT score (Table 4b).

DISCUSSION

In this longitudinal study of phthalate exposure and respiratory outcomes in a population of Latinx children with asthma in a rural, agricultural community in central Washington, we found that urinary phthalate metabolite concentrations were associated with objective biomarkers of inflammation (FENO, $uLTE_4$) that have been linked with

asthma exacerbation in children [46,49]. No association with same day measure of pulmonary obstruction (FEV_1) were observed nor did we observe associations with caregiver report of symptoms in the prior 2 weeks, or with ACT scores, a composite measure based on child and/or caregiver report of asthma related symptoms and disruptions in the prior month.

The cohort of study represents a relatively understudied group (rural Latinx children with asthma). We observed urinary phthalate metabolite concentrations in the cohort that were comparable to those reported for children of similar age in the National Health and Nutrition Examination Survey (NHANES) populations from 2015-2016 (Table 2) [73]. Geometric means of unadjusted urinary phthalate metabolites from children in this study (*n* = 79) were slightly elevated for all phthalate metabolites except MCIOP and MEHP when compared to unadjusted values reported by NHANES from 2015-2016 for children aged 6 – 11 years. However, after correction for creatinine, concentrations in the present study were slightly lower than corresponding creatinine-corrected geometric means in NHANES, but largely near the lower end or within 95% confidence intervals. Median phthalate exposures in our study were lower across all 11 metabolites compared to those of younger participants in the Toddlers Exposure to SVOCs in Indoor Environments (TESIE) Study [50], and we observed lower concentrations of MEP and MBP but slightly elevated MIBP concentrations compared to older female participants in The Health and Environmental Research on Makeup of Salinas Adolescents (HERMOSA) Study [74], a cohort of Mexican American adolescents. Our approach to phthalate exposure assessment via measure of urinary monoester metabolites is widely accepted and preferred in studies of health effects vs. measurement of other products in urine (e.g.,

parent or other compounds) due in part to the increased bioactivity of monoester metabolites [75].

Few studies to date have reported on the association between urinary phthalate metabolites and FENO. The first was a cross-sectional analysis of 244 children (mean age 6.5 years, SD: 1.3) of Dominican and African American mothers living in the dense urban environment of New York City, USA [44]. The study, which was not conducted exclusively among children with asthma, reported positive associations between MEP, MNBP, and MBZP and FENO. This matches our observation of MEP and MNBP as significant predictors of FENO. A more recent longitudinal study of 56 children (mean age 8.6 years, SD: 2.5) with asthma living near Seoul, South Korea, reported associations between MEHHP and MEOHP and elevated FENO [36]. Although these specific DEHP metabolites were not significantly associated with FENO in our study, we did observe statistically significant associations across FENO models with DEHP's primary metabolite, MEHP, as the predictor of interest. The Korean study did not measure MEHP.

DEHP and its metabolite MEHP are among the most well-studied phthalates with respect to adverse allergy and asthma outcomes, and animal studies primarily in BALB/c mice suggest DEHP can induce eosinophilic immune responses and act as an adjuvant in allergic airway reactions [16,19]. Eosinophilic airway inflammation is a characteristic of atopic asthma [76], motivating our assessment of interaction between concentrations of phthalate metabolites and atopy in the association with FENO vs. our other outcome measures. We did not observe significant interaction, although our sample size limited power for assessment across strata. The study in New York City children also reported no interaction between atopy (as assessed by serum specific immunoglobulin E (IgE))

and phthalate metabolites with FENO. Phthalates are associated with increased release of immune mediators related to eosinophilic airway inflammation [19], but potential mechanisms linking exposure to elevated FENO are not well understood and eosinophilic airway responses may occur in non allergic individuals with asthma [77]. Phthalates increase production of TNF-α [43,78], a proinflammatory cytokine known to upregulate inducible nitric oxide (NO) synthase (iNOS), the enzyme responsible for NO synthesis from L-arginine in the respiratory tract [79]. Phthalates also increase expression of IL-4 and act as adjuvants in expression of IL-13 [26,43], both T helper 2 (Th2) inflammatory cytokines said to drive FENO [79].

Although $uLTE_4$ has been utilized as an informative asthma outcome measure [49], we are not aware of any epidemiologic studies examining its relationship with phthalate exposure. Our consistent findings of significant associations between all urinary phthalate metabolites tested and $uLTE_4$ provide new insight into the relevance of this biomarker of effect in studies of phthalate exposure and childhood asthma outcomes. Mechanisms underlying the associations we observed are unclear. LTE_4 synthesis occurs through arachidonic acid metabolism via the 5-lipooxygenase enzymatic pathway [49], and *in vitro* and *ex vivo* studies suggest phthalates may be involved in this process as demonstrated by their ability to increase release of arachidonic acid [80] and their interplay with the lipoxygenase pathway [78]. Multi-pronged assessments of airway and systemic inflammation via diverse biomarkers may assist in understanding the underlying pathophysiological relationships between phthalates and asthma outcomes. While FENO is interpreted as an indicator of eosinophilic airway inflammation related to allergic

asthma, $uLTE_4$ is a more generalized biomarker of inflammation. It has been suggested that a high ratio of $uLTE_4$ to FENO may be a sign of nonallergic airway inflammation [49].

We did not observe statistically significant associations with FEV_1 measures, unlike prior studies conducted in pediatric populations in Canada [81], Korea [36], and Taiwan [32], which observed significant associations between reduced FEV_1 and MCPP, MEHHP, and MEP, respectively. None of the 11 metabolites tested in our study were significant predictors of FEV_1. Among these comparison studies, only the study in Korea was comparable to ours in evaluating the effect in a cohort of children *with asthma*, and it was the only study of these which included repeated measures in design, though just 12 of 34 children provided repeated samples for a total of 47 observations in their analysis. Furthermore, fewer total observations for spirometry measures were available compared to other asthma outcome measures in our study (Table S1).

The significant associations we found between urinary phthalate metabolites and biomarkers of inflammation (FENO and $uLTE_4$) contrasted with the null associations we found with caregiver- or self-reported symptom reports. This could be due to several factors. Questionnaire data are vulnerable to potential errors in recall and individual differences in recognition of symptoms and disruption, which may vary by respondent and by time of assessment. In addition, the referent period for our assessment of asthma health concerns over a timeframe of 2 weeks (symptom day reports) or four weeks (ACT) prior, was assessed in relation to urinary biomarker concentrations derived from a single urine specimen from each time point. As such our measures of effect that were derived from urine samples taken the same day may be more relevant for capturing the influence of short-term fluctuations in phthalate exposure on airway response. Urinary phthalate

metabolites generally reflect exposure that occurs in the prior 24 hours [11], and temporal reliability is moderate, with intraclass correlation coefficients (ICCs) for spot urine samples varying widely between individual metabolites [75]. Outcomes that summarize asthma experience in last two weeks and one month may be less sensitive to capture the influence of phthalate exposure assessed as a single measure at the end of that referent time frame of our survey-based assessments.

There are some limitations to our study. In using urinary biomarkers to represent total exposure, we cannot provide insight on route of exposure or biologically active doses at sites of interest. The significance of individual routes of phthalate exposure (e.g., inhalation vs. ingestion) with respect to asthma pathogenesis and exacerbation is not well understood [19], and there could be links between routes due to the potential for inhaled phthalates to be ultimately ingested via mucociliary clearance. Measures of parent phthalate concentrations in indoor environments via air and dust sampling, as well as in building materials [82], and detailed activity information such as dietary practices and personal care product use are needed to inform body burden from specific routes of exposure and asthma related health impacts. Furthermore, as described above, our exposure assessment may have been temporally mismatched to the available questionnaire-based outcome assessments.

Our statistical approach analyzed each phthalate metabolite as a predictor in separate regression models. Thus, we report on multiple statistical comparisons tested, and do not consider exposure to phthalate mixtures (e.g., through aggregate analyses such as weighted quantile sums [83]). However, metabolite-specific models are widely accepted,

provide important phthalate-specific data, and do not rely on assumptions of similar biological activity across phthalates and their metabolites [75].

We attempted to control for unmeasured confounding due to indoor air contaminants such as particulate matter, based on whether an outcome metric was assessed when a HEPA air cleaner was in the home or not. HEPA air cleaners have been shown in the HAPI study to effectively reduce PM with diameter less than 2.5 μm ($PM_{2.5}$) based on pre and one year later two-week integrated sampling for $PM_{2.5}$ concentrations in these children's homes. We did not have repeated measures of indoor $PM_{2.5}$ concurrent with each urinary specimen used in our study to examine phthalate exposure, however, we evaluated potential for confounding using the integrated two-week average data. We observed largely negative correlations between fine PM measures and urinary phthalate metabolites in this study (Table S11), and overall the correlations between urinary phthalates and prior two-week average $PM_{2.5}$ concentrations in these homes were very low (-0.32 – 0.12). We also examined data from the HAPI study regarding two-week integrated coarse PM and PM_{10} (assessed contemporaneously with $PM_{2.5}$) and similarly saw very weak correlation with phthalates, reducing concern for meaningful residual confounding after controlling for the intervention.

There are several strengths of our study. First, few studies of phthalate exposure and asthma have been conducted in rural settings where asthma morbidity may be comparable or worse than for urban counterparts and for which exposure to phthalates has not been well characterized [84–87]. Yakima County, Washington is a rural area of industrial scale agriculture with both elevated child poverty and asthma hospitalization rates compared to the rest of Washington and the United States [84,88,89]. Second, this

is the first study to our knowledge to use repeated measures for both phthalate exposure and asthma morbidity in a rural population with measures for $uLTE_4$, a relatively novel and promising biomarker of airway inflammation and a measure sensitive to subclinical airway changes [49]. Third, our repeated measure design conducted in a cohort of children with asthma provides opportunity to inform the relationship of exposure to phthalates and *asthma exacerbation* – an area of inquiry with scant human data [36,44]. To date, research on phthalates and asthma has almost exclusively focused on association between *occurrence of disease* (e.g. asthma/no asthma) with phthalate exposure.

In conclusion, we provide novel evidence with a strong repeated measures design to understand associations between urinary phthalate metabolites and asthma exacerbation, utilizing a suite of measures commonly used to assess asthma status among children with asthma. The goal of childhood asthma treatment is control of symptoms and reduction in disruptions in sleep, school, and activities, and control of the underlying pathophysiological processes, namely inflammation. Short term changes in environmental conditions may trigger the inflammatory response and subsequent symptoms and perceptions of disease. Our study adds to emerging data that suggest phthalates may promote asthma exacerbations [36]. Additional studies characterizing phthalate exposure and its association with respiratory outcomes in children with asthma are needed. Future studies that incorporate well characterized co-exposure to common allergens in order to investigate potential adjuvant effects [90] would further advance this field, as well as studies adequately powered to assess effect modification in important subgroups (e.g., atopy). Additional biomarkers of effect may assist in elucidating potential

mechanisms, such as oxidative stress, a well-characterized factor in asthma responses [91], which may play a role in the adjuvant pathway of phthalate-induced asthma responses [28], and more generally in the toxic effects of phthalate exposures that are related to asthma [15].

TABLES

Table 1. Study population and household characteristics at baseline (*N*=79)

Characteristic	N (%)		
	All	Atopy	
	(N=79)	No (N=29)	Yes (N=48)
Sex (female)	29 (36.7%)	12 (41.4%)	15 (31.2%)
Age at enrollment[a]	9.0 (2.1)	8.40 (2.0)	9.41 (2.2)
Ethnicity[b]			
Hispanic/Latinx	77 (100%)	29 (100%)	48 (100%)
BMI[a, c]	20.9 (5.5)	19.9 (5.5)	21.5 (5.5)
BMI-for-age percentile at enrollment[a, c]	79 (26)	79 (22)	78 (28)
Baseline controller medication[b]	69 (87.3%)	25 (86.2%)	42 (87.5%)
HAPI intervention group	39 (49.4%)	14 (48.3%)	23 (47.9%)
Primary household language			
English	18 (22.8%)	4 (13.8%)	13 (27.1%)
Spanish	61 (77.2%)	25 (86.2%)	35 (72.9%)
Home location[c]			
On farm	19 (24.1%)	5 (17.2%)	14 (29.2%)
Rural, no farm	18 (22.8%)	9 (31.0%)	7 (14.6%)
Town	39 (49.4%)	14 (48.3%)	25 (52.1%)
Annual household income[d]			
<$14,999	12 (15.2%)	4 (13.8%)	8 (16.7%)
$15,000-$29,999	31 (39.2%)	15 (51.7%)	14 (29.2%)
$30,000-$60,000	27 (34.2%)	9 (31.0%)	18 (37.5%)
>$60,000	5 (6.3%)	0 (0%)	5 (10.4%)

[a]Mean ± SD.

[b](N=77)

[c](N=76)

[d](N=75)

Table 2. Distributions of unadjusted and adjusted urinary phthalate metabolite concentrations with NHANES comparisons

Phthalate metabolite	Present study[a]		Unadjusted values (μg/L)								Creatinine-corrected values (μg/g creatinine)							
			Present study[a]				NHANES (6-11 years, 2015-2016)[b]				Present study[a]				NHANES (6-11 years, 2015-2016)[b]			
	LOD (μg/L)	%>LOD	GM	GSD	50th%	90th%	GM	(95% CI)	50th% (95% CI)	90th% (95% CI)	GM	GSD	50th%	90th%	GM	(95% CI)	50th% (95% CI)	90th% (95% CI)
MBZP	0.2	100%	12.4	3.3	13.0	56.4	10.7	(8.6-13.3)	10.9 (8.4-13.8)	59.2 (41.6-73.2)	12.7	2.8	12.5	47.3	13.8	(11.3-16.8)	12.0 (10.0-15.6)	57.9 (49.0-85.7)
MCINP	0.05	100%	2.3	2.4	2.1	6.5	2.3	(2.0-2.6)	2.4 (2.0-2.8)	6.7 (5.9-8.0)	2.3	2.3	2.2	7.0	2.9	(2.7-3.2)	2.8 (2.5-3.0)	6.7 (5.9-8.3)
MCIOP	0.05	100%	9.5	2.8	8.0	40.9	11.1	(9.2-13.4)	10.2 (8.5-12.0)	51.9 (34.3-71.5)	9.7	2.6	7.9	40.6	14.3	(12.0-16.9)	11.5 (9.6-14.3)	59.6 (39.6-90.4)
MCPP	0.05	100%	2.4	2.5	2.3	7.3	1.8	(1.6-2.0)	1.8 (1.5-2.0)	6.8 (4.7-7.9)	2.5	2.2	2.3	6.6	2.3	(2.1-2.6)	2.2 (1.8-2.6)	6.7 (5.5-7.1)
MECPP	0.2	100%	16.9	2.4	16.9	50.8	14.6	(12.9-16.6)	14.9 (13.3-16.7)	42.5 (35.3-54.8)	17.3	2.1	16.1	45.8	18.9	(17.5-20.4)	17.5 (16.6-19.1)	48.3 (35.6-57.5)
MEHHP	0.1	100%	10.3	2.6	11.0	34.4	8.8	(7.8-9.9)	9.0 (7.9-10.0)	27.5 (20.0-35.9)	10.6	2.1	10.2	26.9	11.3	(10.5-12.3)	10.5 (9.6-11.6)	28.8 (21.7-35.2)
MEHP	0.1	99%	1.4	2.6	1.3	4.9	1.4	(1.3-1.6)	1.3 (1.0-1.6)	4.2 (3.6-4.8)	1.4	2.5	1.4	4.3	1.8	(1.6-2.0)	1.8 (1.6-2.1)	4.7 (4.1-5.2)
MEOHP	0.15	100%	6.8	2.6	7.0	23.0	6.0	(5.4-6.7)	6.1 (5.3-6.8)	19.0 (14.7-21.5)	7.0	2.1	6.7	18.2	7.7	(7.1-8.3)	7.3 (6.6-8.0)	19.4 (14.6-22.9)
MEP	0.2	100%	24.7	2.8	24.3	92.2	24.5	(18.6-32.3)	22.1 (16.3-31.1)	115.0 (70.4-203.0)	25.4	2.5	22.8	75.8	31.6	(25.6-39.0)	26.5 (21.8-33.6)	120.0 (77.8-205.0)
MIBP	0.1	100%	13.5	2.6	24.3	92.2	11.2	(9.3-13.5)	11.6 (10.2-13.7)	39.2 (31.1-52.1)	13.8	2.2	12.8	38.6	14.5	(12.6-16.6)	13.5 (11.9-15.0)	42.5 (28.4-49.6)
MNBP	0.1	100%	15.7	2.5	16.6	44.4	14.4	(13.1-15.8)	15.4 (13.4-17.3)	40.6 (36.0-43.0)	16.1	1.9	16.2	35.7	18.5	(17.4-19.7)	18.6 (17.2-19.8)	40.1 (35.5-44.3)
∑DEHP	NR	NR	35.3	2.5	35.9	108.7	NR	NR	NR	NR	36.2	2.1	33.4	90.1	NR	NR	NR	NR

[a] *N* = 281 urine specimens from 79 participants

[b] Values drawn from *National Health and Nutrition Examination Survey (NHANES) Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2019* (U.S. Centers for Disease Control and Prevention) [73]

NR = not reported

Table 3. Summary of respiratory outcome measures

Respiratory outcome	*N*[a]	Participants	Mean (SD)
FENO (ppb)	258	76	22.9 (25.5)
FEV_1	231	76	
FEV_1 (L)			1.81 (0.57)
FEV_1 z-score			-0.53 (1.2)
FEV_1 percent predicted (%)			93.6 (13.9)
$uLTE_4$ (µg/g creatinine)	274	78	1.43 (0.56)
Caregiver report of symptoms in prior 2 weeks	281	79	
No			119 (42.3%)[b]
Yes			162 (57.6%)[b]
ACT	279	79	
controlled[c]			204 (73.1%)[b]
poorly controlled[c]			75 (26.8%)[b]
percent score (%)[d]			80.3 (12.8)

[a]Number of observations with matched urinary phthalate metabolite concentrations

[b]Expressed as *N* (%)

[c]Scores $\leq$ 19 = poorly controlled

[d]Score on age-appropriate ACT divided by total possible (27 on C-ACT and 25 on ACT)

Table 4a. Estimated model coefficients of the relationship between natural log-transformed urinary phthalate metabolites and natural log-transformed biomarkers of inflammation

	Biomarker of inflammation											
	FENO[a]						$uLTE_4$[b]					
	Minimally adjusted		Fully adjusted (primary)		Extended adjustment		Minimally adjusted		Fully adjusted (primary)		Extended adjustment	
Metabolite	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
MBZP	0.06	-0.03 – 0.16	0.06	-0.03 – 0.16	0.05	-0.05 – 0.15	**0.15**	**0.11 – 0.20**	**0.15**	**0.10 – 0.20**	**0.15**	**0.10 – 0.20**
MCINP	0.01	-0.09 – 0.11	0.01	-0.09 – 0.10	0.01	-0.09 – 0.11	**0.13**	**0.07 – 0.19**	**0.13**	**0.08 – 0.19**	**0.13**	**0.08 – 0.19**
MCIOP	0.06	-0.02 – 0.14	0.06	-0.02 – 0.14	0.06	-0.02 – 0.15	**0.09**	**0.04 – 0.14**	**0.09**	**0.04 – 0.14**	**0.12**	**0.07 – 0.17**
MCPP	0.08	-0.01 – 0.18	0.09	-0.01 – 0.19	0.09	-0.02 – 0.19	**0.10**	**0.05 – 0.16**	**0.11**	**0.05 – 0.17**	**0.12**	**0.06 – 0.18**
MECPP	**0.11**	**0.00 – 0.21**	**0.11**	**0.01 – 0.21**	**0.12**	**0.01 – 0.23**	**0.16**	**0.10 – 0.22**	**0.17**	**0.11 – 0.23**	**0.18**	**0.12 – 0.25**
MEHHP	0.08	-0.02 – 0.17	0.08	-0.02 – 0.17	0.08	-0.02 – 0.18	**0.15**	**0.10 – 0.20**	**0.16**	**0.11 – 0.21**	**0.17**	**0.12 – 0.23**
MEHP	**0.11**	**0.02 – 0.21**	**0.09**	**0.00 – 0.19**	**0.10**	**0.00 – 0.20**	**0.08**	**0.03 – 0.13**	**0.09**	**0.04 – 0.15**	**0.12**	**0.06 – 0.17**
MEOHP	0.08	-0.02 – 0.18	0.08	-0.01 – 0.18	0.08	-0.02 – 0.18	**0.17**	**0.11 – 0.22**	**0.17**	**0.12 – 0.23**	**0.19**	**0.13 – 0.24**
MEP	0.08	-0.01 – 0.16	**0.09**	**0.01 – 0.18**	**0.09**	**0.00 – 0.18**	**0.13**	**0.09 – 0.18**	**0.14**	**0.09 – 0.19**	**0.13**	**0.08 – 0.18**
MIBP	**0.10**	**0.01 – 0.20**	0.07	-0.03 – 0.17	0.06	-0.05 – 0.17	**0.14**	**0.08 – 0.19**	**0.16**	**0.10 – 0.21**	**0.15**	**0.09 – 0.21**
MNBP	**0.13**	**0.02 – 0.23**	**0.12**	**0.02 – 0.22**	0.11	-0.00 – 0.22	**0.22**	**0.16 – 0.28**	**0.23**	**0.17 – 0.29**	**0.24**	**0.18 – 0.30**
∑DEHP	0.09	-0.01 – 0.19	0.09	-0.01 – 0.19	0.1	-0.01 – 0.21	**0.16**	**0.10 – 0.22**	**0.17**	**0.11 – 0.23**	**0.19**	**0.13 – 0.25**

[a] Minimally adjusted model covariates: specific gravity, age, sex, intervention
Fully adjusted: minimal model plus BMI, atopy, season
Extended adjustment: full model plus income, time of day, baseline medication, visit

[b] Minimally adjusted model covariates: specific gravity, age, sex, intervention
Fully adjusted: minimal model plus BMI, atopy, season
Extended adjustment: full model plus income, baseline medication, visit

Table 4b. Estimated model coefficients of the relationship between natural log-transformed urinary phthalate metabolites and clinical measures of asthma status

	Clinical measure of asthma status																	
	FEV_1 z-score[a]						FEV_1 % predicted[b]						ACT Score[c]					
	Minimally adjusted		Fully adjusted (primary)		Extended adjustment		Minimally adjusted		Fully adjusted (primary)		Extended adjustment		Minimally adjusted		Fully adjusted (primary)		Extended adjustment	
Metabolite	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
MBZP	0	-0.14 – 0.15	0.03	-0.12 – 0.17	-0.01	-0.17 – 0.16	0.02	-1.68 – 1.71	0.32	-1.43 – 2.06	-0.1	-2.09 – 1.89	0.04	-0.05 – 0.13	0.04	-0.05 – 0.14	0	-0.09 – 0.09
MCINP	-0.04	-0.19 – 0.11	-0.03	-0.18 – 0.12	-0.04	-0.19 – 0.12	-0.45	-2.23 – 1.33	-0.37	-2.17 – 1.44	-0.42	-2.31 – 1.46	0	-0.11 – 0.11	0.01	-0.10 – 0.12	0	-0.11 – 0.11
MCIOP	0.05	-0.06 – 0.17	0.05	-0.07 – 0.17	0.01	-0.12 – 0.15	0.62	-0.79 – 2.04	0.52	-0.93 – 1.97	0.14	-1.47 – 1.75	-0.02	-0.11 – 0.08	-0.01	-0.10 – 0.09	0.01	-0.08 – 0.10
MCPP	0	-0.14 – 0.15	0	-0.15 – 0.15	-0.03	-0.20 – 0.14	0.03	-1.72 – 1.78	-0.01	-1.83 – 1.81	-0.37	-2.36 – 1.62	-0.04	-0.15 – 0.07	-0.03	-0.14 – 0.09	-0.04	-0.15 – 0.07
MECPP	-0.01	-0.17 – 0.15	0.01	-0.15 – 0.18	-0.05	-0.23 – 0.14	-0.18	-2.09 – 1.72	0.1	-1.85 – 2.04	-0.61	-2.80 – 1.58	0.02	-0.10 – 0.14	0.01	-0.11 – 0.12	-0.03	-0.15 – 0.08
MEHHP	-0.02	-0.17 – 0.12	-0.01	-0.15 – 0.14	-0.07	-0.24 – 0.10	-0.37	-2.10 – 1.37	-0.13	-1.89 – 1.63	-0.91	-2.92 – 1.11	0.03	-0.07 – 0.14	0.02	-0.09 – 0.13	-0.01	-0.11 – 0.10
MEHP	-0.05	-0.20 – 0.10	-0.03	-0.18 – 0.13	-0.09	-0.26 – 0.08	-0.67	-2.41 – 1.08	-0.37	-2.17 – 1.42	-1.11	-3.13 – 0.91	0.02	-0.08 – 0.12	0.01	-0.09 – 0.12	0.01	-0.09 – 0.11
MEOHP	-0.03	-0.17 – 0.12	-0.01	-0.16 – 0.14	-0.07	-0.24 – 0.10	-0.38	-2.14 – 1.39	-0.15	-1.95 – 1.65	-0.88	-2.94 – 1.18	0.04	-0.07 – 0.15	0.03	-0.07 – 0.14	0	-0.11 – 0.11
MEP	-0.09	-0.22 – 0.04	-0.09	-0.22 – 0.05	-0.06	-0.20 – 0.08	-1.11	-2.64 – 0.42	-1.06	-2.64 – 0.52	-0.76	-2.48 – 0.96	0.04	-0.06 – 0.14	0.05	-0.05 – 0.14	0.02	-0.08 – 0.11
MIBP	-0.05	-0.19 – 0.10	-0.01	-0.16 – 0.14	-0.06	-0.24 – 0.12	-0.59	-2.32 – 1.14	-0.17	-1.98 – 1.64	-0.7	-2.81 – 1.42	0.07	-0.04 – 0.17	0.09	-0.02 – 0.21	-0.01	-0.13 – 0.10
MNBP	-0.1	-0.26 – 0.06	-0.07	-0.24 – 0.09	-0.13	-0.32 – 0.06	-1.22	-3.16 – 0.72	-0.92	-2.90 – 1.05	-1.57	-3.80 – 0.66	0.01	-0.10 – 0.13	0.01	-0.11 – 0.13	-0.06	-0.18 – 0.06
∑DEHP	-0.02	-0.18 – 0.14	0	-0.16 – 0.16	-0.06	-0.25 – 0.12	-0.32	-2.21 – 1.56	-0.05	-1.97 – 1.87	-0.83	-2.99 – 1.34	0.03	-0.09 – 0.14	0.01	-0.10 – 0.13	-0.02	-0.14 – 0.09

[a] Minimally adjusted model covariates: specific gravity, age, sex, intervention
Fully adjusted: minimal model plus BMI, atopy, season
Extended adjustment: full model plus income, time of day, baseline medication, visit

[b] Minimally adjusted model covariates: specific gravity, age, sex, intervention
Fully adjusted: minimal model plus BMI, atopy, season
Extended adjustment: full model plus income, time of day, baseline medication, visit

[c] Minimally adjusted model covariates: specific gravity, age, sex, intervention
Fully adjusted: minimal model plus BMI, atopy, season
Extended adjustment: full model plus income, baseline medication, visit

Table 4c. Estimated odds ratio of the relationship between natural log-transformed urinary phthalate metabolites and caregiver report of symptoms in the prior 2 weeks

	Caregiver report of symptoms[c]					
	Minimally adjusted		Fully adjusted (primary)		Extended adjustment	
Metabolite	OR	95% CI	OR	95% CI	OR	95% CI
MBZP	0.91	0.68 – 1.21	0.91	0.68 – 1.22	0.98	0.70 – 1.37
MCINP	1.26	0.88 – 1.82	1.28	0.88 – 1.86	1.34	0.89 – 1.99
MCIOP	**1.37**	**1.00 – 1.86**	1.35	0.97 – 1.86	1.23	0.86 – 1.74
MCPP	1.05	0.74 – 1.51	1.04	0.72 – 1.52	1	0.66 – 1.50
MECPP	1.2	0.83 – 1.75	1.23	0.83 – 1.81	1.13	0.76 – 1.69
MEHHP	1.03	0.73 – 1.45	1.05	0.74 – 1.49	0.96	0.65 – 1.43
MEHP	1.12	0.81 – 1.55	1.11	0.79 – 1.56	1.02	0.72 – 1.45
MEOHP	1.04	0.74 – 1.47	1.06	0.74 – 1.51	0.98	0.66 – 1.46
MEP	0.82	0.60 – 1.11	0.85	0.62 – 1.17	0.91	0.64 – 1.29
MIBP	0.82	0.58 – 1.17	0.85	0.58 – 1.24	1	0.64 – 1.55
MNBP	1.01	0.70 – 1.48	1.06	0.71 – 1.56	1.15	0.76 – 1.73
∑DEHP	1.13	0.78 – 1.63	1.15	0.78 – 1.68	1.07	0.70 – 1.63

[c] Minimally adjusted model covariates: specific gravity, age, sex, intervention
Fully adjusted: minimal model plus BMI, atopy, season
Extended adjustment: full model plus income, baseline medication, visit

Table S1. Number of observations for study measures at each time point

	Study time point during follow-up year				
Measure	1 (Baseline)	2 (month 2/3)	3 (month 6)	4 (month 11/12)	Total
Urine Samples	77	75	73	56	281
Urinary phthalate metabolites	77	75	73	56	281
Urinary Leukotriene E4 ($uLTE_4$)	74	73	73	54	274
Fractional Exhaled Nitric Oxide (FENO)	61	72	70	55	258
Spirometry	62	62	62	45	231
Symptom Questionnaire	77	75	73	56	281
Asthma Control Test	76	75	73	55	279

Table S2. Pearson correlation matrix of urinary phthalate metabolites (281 samples from 79 participants)

	MNBP	*MBZP*	*MCINP*	*MCIOP*	*MCPP*	*MECPP*	*MEHHP*	*MEHP*	*MEOHP*	*MEP*	*MIBP*
MNBP		0.68	0.37	0.38	0.53	0.6	0.67	0.56	0.69	0.48	0.66
MBZP			0.38	0.37	0.48	0.54	0.57	0.5	0.59	0.42	0.47
MCINP				0.63	0.52	0.49	0.49	0.4	0.5	0.22	0.27
MCIOP					0.76	0.57	0.54	0.45	0.55	0.31	0.29
MCPP						0.61	0.61	0.46	0.61	0.33	0.43
MECPP							0.94	0.79	0.95	0.42	0.53
MEHHP								0.82	0.99	0.42	0.59
MEHP									0.82	0.32	0.49
MEOHP										0.43	0.6
MEP											0.36
MIBP											

Table S3. Distribution of urinary phthalate metabolites with specific gravity adjustment (ng/mL) (281 samples from 79 participants)

phthalate metabolite	GM	GSD	50th %	90th %
MBZP	14.5	2.9	14.0	55.9
MCINP	2.7	2.2	2.5	7.4
MCIOP	11.2	2.7	8.8	42.8
MCPP	2.8	2.2	2.7	7.2
MECPP	19.8	2.2	19.3	47.6
MEHHP	12.1	2.3	12.1	32.3
MEHP	1.6	2.5	1.6	5.1
MEOHP	8.0	2.3	7.9	21.2
MEP	29.1	2.7	28.6	89.4
MIBP	15.9	2.4	16.3	45.1
MNBP	18.4	2.1	18.9	56.9
∑DEHP	41.4	2.2	39.3	104.1

Table S4. All FENO model output

	crude model[A]				minimally adjusted model[B]				fully adjusted model[C]				extended adjustment model[D]			
	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat
MBZP	0.05	0.05	-0.04 – 0.15	1.09	0.06	0.05	-0.03 – 0.16	1.32	0.06	0.05	-0.03 – 0.16	1.34	0.05	0.05	-0.05 – 0.15	1.02
MCINP	0.01	0.05	-0.09 – 0.11	0.15	0.01	0.05	-0.09 – 0.11	0.22	0.01	0.05	-0.09 – 0.10	0.12	0.01	0.05	-0.09 – 0.11	0.21
MCIOP	0.05	0.04	-0.03 – 0.12	1.12	0.06	0.04	-0.02 – 0.14	1.42	0.06	0.04	-0.02 – 0.14	1.49	0.06	0.04	-0.02 – 0.15	1.44
MCPP	0.06	0.05	-0.03 – 0.16	1.29	0.08	0.05	-0.01 – 0.18	1.74	0.09	0.05	-0.01 – 0.19	1.79	0.09	0.05	-0.02 – 0.19	1.66
MECPP	0.09	0.05	-0.01 – 0.20	1.82	**0.11 ***	**0.05**	**0.00 – 0.21**	**2.05**	**0.11 ***	**0.05**	**0.01 – 0.21**	**2.07**	**0.12 ***	**0.06**	**0.01 – 0.23**	**2.09**
MEHHP	0.07	0.05	-0.02 – 0.16	1.43	0.08	0.05	-0.02 – 0.17	1.6	0.08	0.05	-0.02 – 0.17	1.6	0.08	0.05	-0.02 – 0.18	1.58
MEHP	**0.11 ***	**0.05**	**0.01 – 0.20**	**2.25**	**0.11 ***	**0.05**	**0.02 – 0.21**	**2.38**	**0.09 ***	**0.05**	**0.00 – 0.19**	**1.97**	**0.10 ***	**0.05**	**0.00 – 0.20**	**2.05**
MEOHP	0.07	0.05	-0.02 – 0.16	1.44	0.08	0.05	-0.02 – 0.18	1.65	0.08	0.05	-0.01 – 0.18	1.71	0.08	0.05	-0.02 – 0.18	1.63
MEP	0.08	0.04	-0.00 – 0.17	1.9	0.08	0.04	-0.01 – 0.16	1.74	**0.09 ***	**0.04**	**0.01 – 0.18**	**2.14**	**0.09 ***	**0.05**	**0.00 – 0.18**	**1.99**
MIBP	0.1	0.05	-0.00 – 0.19	1.94	**0.10 ***	**0.05**	**0.01 – 0.20**	**2.06**	0.07	0.05	-0.03 – 0.17	1.31	0.06	0.06	-0.05 – 0.17	1.1
MNBP	**0.11 ***	**0.05**	**0.01 – 0.22**	**2.17**	**0.13 ***	**0.05**	**0.02 – 0.23**	**2.39**	**0.12 ***	**0.05**	**0.02 – 0.22**	**2.35**	0.11	0.06	-0.00 – 0.22	1.92
∑DEHP	0.08	0.05	-0.02 – 0.18	1.63	0.09	0.05	-0.01 – 0.19	1.83	0.09	0.05	-0.01 – 0.19	1.83	0.1	0.05	-0.01 – 0.21	1.83

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, time of day of FENO, baseline controller medication use, visit

** p<0.05, ** p<0.01, *** p<0.001*

Table S5. All $uLTE_4$ model output

	crude model[A]				minimally adjusted model[B]				fully adjusted model[C]				extended adjustment model[D]			
	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat
MBZP	**0.16 *** **	**0.02**	**0.11 – 0.21**	**6.39**	**0.15 *** **	**0.02**	**0.11 – 0.20**	**6.24**	**0.15 *** **	**0.02**	**0.10 – 0.20**	**6.12**	**0.15 *** **	**0.03**	**0.10 – 0.20**	**5.75**
MCINP	**0.14 *** **	**0.03**	**0.08 – 0.19**	**4.79**	**0.13 *** **	**0.03**	**0.07 – 0.19**	**4.56**	**0.13 *** **	**0.03**	**0.08 – 0.19**	**4.58**	**0.13 *** **	**0.03**	**0.08 – 0.19**	**4.66**
MCIOP	**0.10 *** **	**0.02**	**0.05 – 0.14**	**4.01**	**0.09 *** **	**0.02**	**0.04 – 0.14**	**3.81**	**0.09 *** **	**0.02**	**0.04 – 0.14**	**3.69**	**0.12 *** **	**0.03**	**0.07 – 0.17**	**4.47**
MCPP	**0.10 *** **	**0.03**	**0.04 – 0.16**	**3.37**	**0.10 *** **	**0.03**	**0.05 – 0.16**	**3.48**	**0.11 *** **	**0.03**	**0.05 – 0.17**	**3.48**	**0.12 *** **	**0.03**	**0.06 – 0.18**	**3.76**
MECPP	**0.16 *** **	**0.03**	**0.10 – 0.22**	**5.3**	**0.16 *** **	**0.03**	**0.10 – 0.22**	**5.32**	**0.17 *** **	**0.03**	**0.11 – 0.23**	**5.64**	**0.18 *** **	**0.03**	**0.12 – 0.25**	**5.9**
MEHHP	**0.15 *** **	**0.03**	**0.10 – 0.21**	**5.6**	**0.15 *** **	**0.03**	**0.10 – 0.20**	**5.62**	**0.16 *** **	**0.03**	**0.11 – 0.21**	**5.8**	**0.17 *** **	**0.03**	**0.12 – 0.23**	**6.21**
MEHP	**0.09 ** **	**0.03**	**0.03 – 0.14**	**3.24**	**0.08 ** **	**0.03**	**0.03 – 0.13**	**3.02**	**0.09 *** **	**0.03**	**0.04 – 0.15**	**3.29**	**0.12 *** **	**0.03**	**0.06 – 0.17**	**4.08**
MEOHP	**0.17 *** **	**0.03**	**0.12 – 0.22**	**6.18**	**0.17 *** **	**0.03**	**0.11 – 0.22**	**6.16**	**0.17 *** **	**0.03**	**0.12 – 0.23**	**6.32**	**0.19 *** **	**0.03**	**0.13 – 0.24**	**6.73**
MEP	**0.14 *** **	**0.02**	**0.09 – 0.19**	**5.95**	**0.13 *** **	**0.02**	**0.09 – 0.18**	**5.53**	**0.14 *** **	**0.03**	**0.09 – 0.19**	**5.41**	**0.13 *** **	**0.03**	**0.08 – 0.18**	**5.3**
MIBP	**0.14 *** **	**0.03**	**0.09 – 0.20**	**5.11**	**0.14 *** **	**0.03**	**0.08 – 0.19**	**5**	**0.16 *** **	**0.03**	**0.10 – 0.21**	**5.31**	**0.15 *** **	**0.03**	**0.09 – 0.21**	**4.7**
MNBP	**0.23 *** **	**0.03**	**0.17 – 0.29**	**7.79**	**0.22 *** **	**0.03**	**0.16 – 0.28**	**7.59**	**0.23 *** **	**0.03**	**0.17 – 0.29**	**7.71**	**0.24 *** **	**0.03**	**0.18 – 0.30**	**7.72**
∑DEHP	**0.16 *** **	**0.03**	**0.11 – 0.22**	**5.52**	**0.16 *** **	**0.03**	**0.10 – 0.22**	**5.52**	**0.17 *** **	**0.03**	**0.11 – 0.23**	**5.8**	**0.19 *** **	**0.03**	**0.13 – 0.25**	**6.16**

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, visit

** p<0.05, ** p<0.01, *** p<0.001*

Table S6. All ACT model output

	crude model[A]				minimally adjusted model[B]				fully adjusted model[C]				extended adjustment model[D]			
	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat
MBZP	0.03	0.05	-0.06 – 0.12	0.67	0.04	0.05	-0.05 – 0.13	0.86	0.04	0.05	-0.05 – 0.14	0.94	0	0.05	-0.09 – 0.09	-0.06
MCINP	-0.01	0.06	-0.12 – 0.10	-0.14	0	0.06	-0.11 – 0.11	0.07	0.01	0.06	-0.10 – 0.12	0.2	0	0.05	-0.11 – 0.11	-0.01
MCIOP	-0.03	0.05	-0.12 – 0.07	-0.56	-0.02	0.05	-0.11 – 0.08	-0.33	-0.01	0.05	-0.10 – 0.09	-0.15	0.01	0.05	-0.08 – 0.10	0.2
MCPP	-0.05	0.06	-0.17 – 0.06	-0.96	-0.04	0.06	-0.15 – 0.07	-0.65	-0.03	0.06	-0.14 – 0.09	-0.51	-0.04	0.06	-0.15 – 0.07	-0.66
MECPP	0	0.06	-0.11 – 0.12	0.06	0.02	0.06	-0.10 – 0.14	0.32	0.01	0.06	-0.11 – 0.12	0.08	-0.03	0.06	-0.15 – 0.08	-0.55
MEHHP	0.02	0.06	-0.09 – 0.13	0.41	0.03	0.05	-0.07 – 0.14	0.57	0.02	0.06	-0.09 – 0.13	0.38	-0.01	0.05	-0.11 – 0.10	-0.17
MEHP	0.01	0.05	-0.09 – 0.11	0.17	0.02	0.05	-0.08 – 0.12	0.35	0.01	0.05	-0.09 – 0.12	0.23	0.01	0.05	-0.09 – 0.11	0.24
MEOHP	0.03	0.06	-0.08 – 0.14	0.5	0.04	0.05	-0.07 – 0.15	0.75	0.03	0.06	-0.07 – 0.14	0.62	0	0.05	-0.11 – 0.11	0.02
MEP	0.01	0.05	-0.09 – 0.10	0.19	0.04	0.05	-0.06 – 0.14	0.8	0.05	0.05	-0.05 – 0.14	0.89	0.02	0.05	-0.08 – 0.11	0.38
MIBP	0.07	0.05	-0.04 – 0.18	1.24	0.07	0.05	-0.04 – 0.17	1.27	0.09	0.06	-0.02 – 0.21	1.64	-0.01	0.06	-0.13 – 0.10	-0.2
MNBP	0.01	0.06	-0.11 – 0.12	0.11	0.01	0.06	-0.10 – 0.13	0.22	0.01	0.06	-0.11 – 0.13	0.15	-0.06	0.06	-0.18 – 0.06	-1.02
∑DEHP	0.01	0.06	-0.10 – 0.13	0.21	0.03	0.06	-0.09 – 0.14	0.44	0.01	0.06	-0.10 – 0.13	0.22	-0.02	0.06	-0.14 – 0.09	-0.37

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, visit

** p<0.05, ** p<0.01, *** p<0.001*

Table S7. All FEV_1 z-score model output

	crude model[A]				minimally adjusted model[B]				fully adjusted model[C]				extended adjustment model[D]			
	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat
MBZP	-0.01	0.07	-0.15 – 0.14	-0.08	0	0.07	-0.14 – 0.15	0.04	0.03	0.07	-0.12 – 0.17	0.37	-0.01	0.09	-0.17 – 0.16	-0.09
MCINP	-0.05	0.08	-0.20 – 0.10	-0.67	-0.04	0.08	-0.19 – 0.11	-0.51	-0.03	0.08	-0.18 – 0.12	-0.42	-0.04	0.08	-0.19 – 0.12	-0.46
MCIOP	0.05	0.06	-0.07 – 0.17	0.79	0.05	0.06	-0.06 – 0.17	0.9	0.05	0.06	-0.07 – 0.17	0.76	0.01	0.07	-0.12 – 0.15	0.21
MCPP	0.01	0.07	-0.14 – 0.16	0.13	0	0.07	-0.14 – 0.15	0.04	0	0.08	-0.15 – 0.15	0.01	-0.03	0.09	-0.20 – 0.14	-0.36
MECPP	0.01	0.08	-0.15 – 0.16	0.06	-0.01	0.08	-0.17 – 0.15	-0.12	0.01	0.08	-0.15 – 0.18	0.16	-0.05	0.09	-0.23 – 0.14	-0.5
MEHHP	-0.01	0.07	-0.16 – 0.13	-0.16	-0.02	0.07	-0.17 – 0.12	-0.33	-0.01	0.08	-0.15 – 0.14	-0.07	-0.07	0.09	-0.24 – 0.10	-0.83
MEHP	-0.04	0.07	-0.19 – 0.10	-0.58	-0.05	0.07	-0.20 – 0.10	-0.66	-0.03	0.08	-0.18 – 0.13	-0.33	-0.09	0.09	-0.26 – 0.08	-1
MEOHP	-0.01	0.08	-0.16 – 0.13	-0.18	-0.03	0.08	-0.17 – 0.12	-0.34	-0.01	0.08	-0.16 – 0.14	-0.08	-0.07	0.09	-0.24 – 0.10	-0.78
MEP	-0.08	0.06	-0.21 – 0.04	-1.29	-0.09	0.07	-0.22 – 0.04	-1.37	-0.09	0.07	-0.22 – 0.05	-1.27	-0.06	0.07	-0.20 – 0.08	-0.82
MIBP	-0.05	0.07	-0.20 – 0.09	-0.68	-0.05	0.07	-0.19 – 0.10	-0.64	-0.01	0.08	-0.16 – 0.14	-0.16	-0.06	0.09	-0.24 – 0.12	-0.65
MNBP	-0.1	0.08	-0.27 – 0.06	-1.22	-0.1	0.08	-0.26 – 0.06	-1.2	-0.07	0.08	-0.24 – 0.09	-0.89	-0.13	0.1	-0.32 – 0.06	-1.35
∑DEHP	-0.01	0.08	-0.16 – 0.15	-0.09	-0.02	0.08	-0.18 – 0.14	-0.26	0	0.08	-0.16 – 0.16	0.02	-0.06	0.09	-0.25 – 0.12	-0.69

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, time of day of spirometry, visit

** p<0.05, ** p<0.01, *** p<0.001*

Table S8. All FEV_1 percent predicted model output

	crude model[A]				minimally adjusted model[B]				fully adjusted model[C]				extended adjustment model[D]			
	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat	β	std. error	CI	t-stat
MBZP	-0.1	0.87	-1.80 – 1.61	-0.11	0.02	0.87	-1.68 – 1.71	0.02	0.32	0.89	-1.43 – 2.06	0.35	-0.1	1.02	-2.09 – 1.89	-0.1
MCINP	-0.6	0.91	-2.38 – 1.18	-0.66	-0.45	0.91	-2.23 – 1.33	-0.49	-0.37	0.92	-2.17 – 1.44	-0.4	-0.42	0.96	-2.31 – 1.46	-0.44
MCIOP	0.53	0.72	-0.88 – 1.94	0.74	0.62	0.72	-0.79 – 2.04	0.86	0.52	0.74	-0.93 – 1.97	0.71	0.14	0.82	-1.47 – 1.75	0.17
MCPP	0.09	0.89	-1.65 – 1.83	0.11	0.03	0.89	-1.72 – 1.78	0.03	-0.01	0.93	-1.83 – 1.81	-0.01	-0.37	1.02	-2.36 – 1.62	-0.36
MECPP	-0.01	0.97	-1.91 – 1.89	-0.01	-0.18	0.97	-2.09 – 1.72	-0.19	0.1	0.99	-1.85 – 2.04	0.1	-0.61	1.12	-2.80 – 1.58	-0.55
MEHHP	-0.21	0.88	-1.94 – 1.52	-0.24	-0.37	0.88	-2.10 – 1.37	-0.41	-0.13	0.9	-1.89 – 1.63	-0.15	-0.91	1.03	-2.92 – 1.11	-0.88
MEHP	-0.59	0.89	-2.33 – 1.15	-0.66	-0.67	0.89	-2.41 – 1.08	-0.75	-0.37	0.92	-2.17 – 1.42	-0.41	-1.11	1.03	-3.13 – 0.91	-1.08
MEOHP	-0.24	0.9	-2.00 – 1.53	-0.26	-0.38	0.9	-2.14 – 1.39	-0.42	-0.15	0.92	-1.95 – 1.65	-0.16	-0.88	1.05	-2.94 – 1.18	-0.83
MEP	-1.02	0.77	-2.53 – 0.49	-1.32	-1.11	0.78	-2.64 – 0.42	-1.42	-1.06	0.81	-2.64 – 0.52	-1.31	-0.76	0.88	-2.48 – 0.96	-0.87
MIBP	-0.63	0.89	-2.37 – 1.11	-0.71	-0.59	0.88	-2.32 – 1.14	-0.67	-0.17	0.92	-1.98 – 1.64	-0.18	-0.7	1.08	-2.81 – 1.42	-0.65
MNBP	-1.25	0.99	-3.20 – 0.70	-1.26	-1.22	0.99	-3.16 – 0.72	-1.23	-0.92	1.01	-2.90 – 1.05	-0.92	-1.57	1.14	-3.80 – 0.66	-1.38
∑DEHP	-0.15	0.96	-2.03 – 1.72	-0.16	-0.32	0.96	-2.21 – 1.56	-0.34	-0.05	0.98	-1.97 – 1.87	-0.05	-0.83	1.11	-2.99 – 1.34	-0.75

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, time of day of spirometry, visit

** p<0.05, ** p<0.01, *** p<0.001*

Table S9. All Caregiver symptom report model output

	crude model[A]				minimally adjusted model[B]				fully adjusted model[C]				extended adjustment model[D]			
	OR	std. error	CI	t-stat	OR	std. error	CI	t-stat	OR	std. error	CI	t-stat	OR	std. error	CI	t-stat
MBZP	0.95	0.14	0.72 – 1.26	-0.34	0.91	0.15	0.68 – 1.21	-0.66	0.91	0.15	0.68 – 1.22	-0.6	0.98	0.17	0.70 – 1.37	-0.12
MCINP	1.32	0.18	0.93 – 1.89	1.53	1.26	0.19	0.88 – 1.82	1.26	1.28	0.19	0.88 – 1.86	1.3	1.34	0.2	0.89 – 1.99	1.42
MCIOP	**1.40 ***	**0.15**	**1.04 – 1.89**	**2.22**	**1.37 ***	**0.16**	**1.00 – 1.86**	**1.99**	1.35	0.16	0.97 – 1.86	1.8	1.23	0.18	0.86 – 1.74	1.13
MCPP	1.16	0.18	0.83 – 1.64	0.87	1.05	0.18	0.74 – 1.51	0.29	1.04	0.19	0.72 – 1.52	0.23	1	0.21	0.66 – 1.50	-0.02
MECPP	1.25	0.19	0.87 – 1.79	1.2	1.2	0.19	0.83 – 1.75	0.98	1.23	0.2	0.83 – 1.81	1.04	1.13	0.2	0.76 – 1.69	0.62
MEHHP	1.05	0.17	0.75 – 1.47	0.29	1.03	0.17	0.73 – 1.45	0.16	1.05	0.18	0.74 – 1.49	0.25	0.96	0.2	0.65 – 1.43	-0.18
MEHP	1.13	0.16	0.83 – 1.55	0.78	1.12	0.17	0.81 – 1.55	0.69	1.11	0.17	0.79 – 1.56	0.58	1.02	0.18	0.72 – 1.45	0.12
MEOHP	1.08	0.17	0.77 – 1.51	0.44	1.04	0.18	0.74 – 1.47	0.22	1.06	0.18	0.74 – 1.51	0.32	0.98	0.2	0.66 – 1.46	-0.09
MEP	0.86	0.15	0.64 – 1.14	-1.06	0.82	0.16	0.60 – 1.11	-1.31	0.85	0.16	0.62 – 1.17	-0.97	0.91	0.18	0.64 – 1.29	-0.53
MIBP	0.85	0.18	0.60 – 1.19	-0.95	0.82	0.18	0.58 – 1.17	-1.09	0.85	0.19	0.58 – 1.24	-0.86	1	0.22	0.64 – 1.55	-0.02
MNBP	1.02	0.19	0.71 – 1.46	0.09	1.01	0.19	0.70 – 1.48	0.08	1.06	0.2	0.71 – 1.56	0.28	1.15	0.21	0.76 – 1.73	0.67
∑DEHP	1.16	0.18	0.81 – 1.67	0.83	1.13	0.19	0.78 – 1.63	0.63	1.15	0.19	0.78 – 1.68	0.71	1.07	0.22	0.70 – 1.63	0.31

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, visit

** p<0.05, ** p<0.01, *** p<0.001*

Table S10. Fully adjusted model* output for interaction term and metabolite				
urinary phthalate metabolite	fully adjusted model w/ interaction term (metabolite x atopy) (73 participants, 249 obs.)			
	β (metabolite)	*p*-value (metabolite)	β (interaction term)	*p*-value (interaction term)
MBZP	0.04	0.57	0.03	0.71
MCINP	-0.01	0.89	0.02	0.82
MCIOP	0.02	0.78	0.06	0.47
MCPP	0.06	0.48	0.05	0.58
MECPP	0.07	0.42	0.06	0.55
MEHHP	0.05	0.53	0.04	0.62
MEHP	0	0.96	0.13	0.13
MEOHP	0.06	0.42	0.03	0.71
MEP	0.09	0.17	0	0.99
MIBP	0.08	0.29	-0.02	0.82
MNBP	0.1	0.20	0.04	0.70
∑DEHP	0.06	0.48	0.05	0.57
*Fully adjusted model: FENO ~ metabolite*atopy, specific gravity, age, sex, intervention, BMI, season				

Table S11. Pearson correlation coefficients for urinary phthalate metabolites with $PM_{2.5}$, PM_{10}, and coarse fraction pre- (timepoint 2) and post- (timepoint 4) intervention

	Time point 2 (HAPI visit 3)			Time point 4 (HAPI visit 6)		
urinary phthalate metabolites	$PM_{2.5}$	PM_{10}	coarse fraction	$PM_{2.5}$	PM_{10}	coarse fraction
MBZP	-0.05	-0.09	-0.01	-0.14	-0.13	-0.25
MCINP	0.12	0.10	0.08	-0.14	-0.15	-0.09
MCIOP	-0.05	0.02	0.13	0.02	-0.18	-0.14
MCPP	0.02	0.01	0.16	-0.18	-0.20	-0.17
MECPP	-0.07	0.02	0.15	-0.27	-0.03	-0.03
MEHHP	-0.08	0.00	0.14	-0.27	-0.07	-0.06
MEHP	-0.04	0.00	0.07	-0.31	-0.14	-0.18
MEOHP	-0.10	0.00	0.14	-0.30	-0.07	-0.08
MEP	-0.11	0.06	0.22	-0.03	0.07	0.15
MIBP	-0.11	-0.08	0.13	-0.02	-0.14	-0.20
MNBP	-0.11	-0.05	-0.01	-0.32	-0.13	-0.08
∑DEHP	-0.07	0.01	0.14	-0.28	-0.05	-0.04

CHAPTER 3. BIOMARKERS OF PHTHALATE EXPOSURE AND OXIDATIVE STRESS: A LONGITUDINAL STUDY IN CHILDREN WITH ASTHMA

1. INTRODUCTION

There is growing concern that phthalate exposure may influence asthma, including symptoms such as wheeze and measures of airway inflammation characteristic of asthma such as fractional exhaled nitric oxide (FENO) [16–19]. Human exposure to phthalates is widespread [11], promoting the need to understand its role in asthma, a leading chronic condition with significant health and economic burden [92,93]. Some pathophysiologic mechanisms attributable to phthalates are well characterized in humans including anti-androgenic and weak estrogenic properties [94,95], while others, such as increased oxidative stress coinciding with high phthalate exposure, are not well understood. Studies in children, adolescents, and pregnant women demonstrate associations between several urinary phthalate metabolites and distinct biomarkers of oxidative stress in blood and urine, including markers indicative of lipid peroxidation and oxidative damage to nucleic acids [35,96–103]. Oxidative stress is a well-described underlying mechanism of asthma pathophysiology and could provide a mechanistic link between the observed relationship of phthalates to asthma outcomes [15,16,91].

Urine samples provide a non-invasive opportunity to simultaneously measure biomarkers of phthalate exposure and oxidative stress in epidemiological studies [11,104]. Compounds excreted in urine reflect distinct measures of oxidative modifications to biological molecules including lipids and nucleic acids. A proxy of

oxidative damage to lipids, the isoprostanes are a group of eicosanoids resulting from non-enzymatic peroxidation of arachidonic acid [105]. Among the isoprostanes, 8-isoprostane (also referred to as 8-iso-prostalgandin $F_{2\alpha}$) is a urinary oxidative stress biomarker commonly used in epidemiological studies [106]. Oxidant-induced damage of the guanine base of nucleic acids result in the release of oxidized guanine species via repair mechanisms that are excreted in urine, including 8-hydroxydeoxyguanosine (8-OHdG) as well as 8-hydroxyguanosine (8-OHG) and 8-hydroxyguanine [107]. Measurement of these three oxidized species in aggregate as a single biomarker provides an assessment of oxidative damage to DNA (8-OHdG and 8-hydroxyguanine) and RNA (8-OHG).

This longitudinal study of children with asthma in a primarily rural agricultural community in Washington State uses repeated measures to estimate associations between eleven individual urinary phthalate metabolites, as well as a summed measure for di-2-ethylhexyl phthalate (DEHP), with two urinary biomarkers of oxidative stress: (i) 8-isoprostane and (ii) DNA/RNA oxidative damage via combined measure of 8-OHdG, 8-OHG, and 8-hydroxyguanine.

2. MATERIALS AND METHODS

2.1 Study participants and setting

This study accessed participant samples and data from the Home Air in Agriculture Pediatric Intervention Trial (HAPI), a randomized trial of high-efficiency particulate air (HEPA) air cleaners with asthma education vs. education only [37]. The HEPA air cleaners utilized were designed to reduce particulate matter (PM) and ammonia (NH_3)

concentrations in the participating households, although findings of the study only demonstrated reduction of PM with diameter less than 2.5 μm ($PM_{2.5}$) and no effect on NH_3 (Riederer, under review). The HEPA intervention and educational components were not designed to address phthalates specifically. Enrollment occurred on a rolling basis beginning in 2015 and data collection activities concluded in 2019. The present study uses data from the total sample of 79 HAPI participants who provided at least a single urine specimen. Participants were recruited through the Yakima Valley Farmworker Clinic (YVFWC) asthma education program, and eligibility criteria included: (i) child's age of six to twelve years, (ii) poorly controlled asthma identified through a screening questionnaire, (iii) absence of smokers in the child's home, and (iv) residential proximity (i.e., within 0.5 miles) to agricultural production (crop or dairy). Human subjects research approval was granted by the University of Washington Institutional Review Board and the YVFWC research review committee.

2.2 Study design

The HAPI study followed each participant for one year, with data and sample collection occurring at four separate time points; enrollment and approximately two, six, and 12 months follow-up, respectively. After the two-month follow-up, participant households were randomized to one of two study arms: (i) asthma education, or (ii) asthma education plus two indoor portable HEPA air cleaners placed in the child's sleeping area and main living area.

2.3 Urine sample collection and measurement of urinary phthalate metabolites

Spot urine samples were collected in phthalate-free polyethylene containers and transported on dry ice to a field laboratory where specific gravity was measured using a handheld refractometer and samples were stored at -20ºC. At the field laboratory, 24 trip blanks were prepared. Urine samples and blanks were randomized and shipped along with 5 aliquots of National Institute of Standards and Technology (NIST) standard reference materials (SRMs) 3676 (Organic Contaminants in Non-Smokers' Urine) to the Icahn School of Medicine at Mount Sinai laboratory hub of the National Institutes of Health (NIH) Children's Health Exposure Analysis Resource (CHEAR), where they were analyzed for eleven urinary phthalate metabolites (mono-benzyl phthalate (MBZP), mono-carboxy isononyl phthalate (MCINP), mono-carboxy isooctyl phthalate (MCIOP), mono (3-carboxypropyl) phthalate (MCPP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono ethyl hexyl phthalate (MEHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), monoethyl phthalate (MEP), mono-isobutyl phthalate (MIBP), and mono-n-butylphthalate (MNBP). The target metabolites were quantified using isotope-dilution liquid chromatography with tandem mass spectrometry (LC/MS/MS) following Centers for Disease Control and Prevention (CDC) methods [57–59]. CHEAR laboratory hubs include their own additional internal quality assurance and quality control (QC) protocols, including analysis of pooled samples to assess analytical precision. CHEAR provided quantification of creatinine using an established colorimetric method [108]. Creatinine-correction was calculated by dividing a given analyte's concentration in ng/mL (µg/L) by creatinine in mg/dL, and multiplying by 100 to yield units in µg/g creatinine. Specific gravity-correction was calculated by subtracting 1 from the median specific gravity value in the study population, dividing the

difference by the specific gravity value in a given urine sample minus 1, and then multiplying this quotient by the observed analyte concentration (e.g., a urinary phthalate metabolite in ng/mL).

2.4 Measurement of oxidative stress urinary biomarkers

An aliquot of the urine sample used for phthalate metabolite analysis was designated for the detection of oxidative stress biomarkers and also submitted to the Icahn School of Medicine at Mount Sinai CHEAR laboratory hub [57]. Quantification of 8-isoprostane was carried out via an enzyme-linked immunosorbent assay (ELISA) kit (No. 516351; Cayman Chemical, Ann Arbor, Michigan, USA). Quantification of DNA and RNA oxidative damage was carried out via a DNA/RNA Oxidative Damage (High Sensitivity) ELISA Kit (No. 589320; Cayman Chemical, Ann Arbor, Michigan, USA), which utilizes an antibody that detects 8-OHdG, 8-OHG, and 8-hydroxyguanine (manufacturer cross reactivity listed as 100%, 38%, and 23%, respectively).

2.5 Covariate assessment

The clinic-based enrollment visit involved baseline assessment of various participant characteristics related to child asthma health [37]. This included skin prick testing to determine atopy based on at least one positive among 6 common aeroallergens, and interviewing caregivers on medication use including use of asthma controller medications. Data on additional home environment and demographic factors (e.g., annual household income) were collected at the enrollment and subsequent in-home visits.

2.6 Statistical analysis

Variables of interest were evaluated for normality by visualization of distributions. Urinary phthalate metabolites, 8-isoprostane, and DNA/RNA oxidative damage measures were natural log transformed in regression analyses. Pearson correlation coefficients were used to analyze correlations between pairs of the eleven individual urinary phthalate metabolites. Following Zota et al. (2014), the four urinary metabolites of DEHP, MECPP, MEHHP, MEHP, and MEOHP, were grouped by summing their individual molar concentrations and then converting this value to represent a single summed DEHP metabolite variable, ∑DEHP [5]. In descriptive analyses, we addressed variable urine dilution among phthalate metabolite concentrations by calculating creatinine-corrected and specific gravity-corrected concentrations, to enable comparisons of analytes from the present study to other published data. In regression models, specific gravity was included as a covariate to correct for urine dilution, and values of urinary phthalate metabolites, 8-isoprostane, and DNA/RNA oxidative damage were not corrected as variables in these models.

Each participating child contributed at least one and up to four individual urine samples for phthalate and oxidative stress measurements depending on completion of the four study visits over their follow-up year. To estimate the associations between individual urinary phthalate metabolite concentrations (and ∑DEHP) and oxidative stress biomarkers, and account for the longitudinal study design, linear mixed effects models (LMMs) were fit for both 8-isoprostane and the DNA/RNA oxidative damage. To examine associations between phthalate concentrations and each outcome, we used a staged

approach. Four separate models were fit for each of the twelve separate exposure variables (eleven individual natural log-transformed urinary phthalate metabolites and natural log-transformed ∑DEHP) and the two oxidative stress biomarker outcomes: (i) a crude model, (ii) a minimally adjusted model, (iii) a fully adjusted (primary) model, and (iv) an extended adjustment model. Participant and demographic factors were selected *a priori* as potential confounder or precision variables for the modeled association. The crude models consisted of only the predictor of interest (natural log-transformed urinary phthalate metabolite or natural log-transformed ∑DEHP) as the independent variable and the oxidative stress biomarker outcome as the dependent variable, with specific gravity as a covariate to correct for urine dilution. The minimally adjusted models added age, sex, and HAPI intervention status as covariates. HAPI intervention status was coded as a binary variable to indicate whether or not a given observation reflected an intervention group participant post randomization (i.e., during period of receipt of the HEPA air cleaner). The fully adjusted models added child's study entry body mass index (BMI)-for-age percentile (continuous percentile generated from CDC growth charts) [70], atopy (skin prick test positive yes/no), and meteorological season as a nominal variable with four levels: (i) March, April, May, (ii) June, July, and August, (iii) September, October, November, and (iv) December, January, and February. Extended adjustment models added annual household income as an ordinal variable with four levels: (i) <$14,999, (ii) $15,000-$29,999, (iii) $30,000-$60,000, and (iv) >$60,000, and participant use of controller medication at baseline (yes/no). All statistical analyses were conducted in R version 3.6.2 (The R Foundation for Statistical Computing), with package "lme4" for LMEs.

3. RESULTS

3.1 Participant characteristics

All 79 enrolled HAPI participants were included in the present study (Table 1). The mean age at enrollment was 9 years (SD: 2.1 years), and 29 participants (36.7%) were female. All identified as Hispanic/Latinx, and 61 (77.2%) came from households where the primary spoken language was Spanish. The mean BMI-for-age percentile at enrollment was 79% (SD: 26%). Controller medication use was documented at baseline based on the record of prescribed medications for 69 participants (87.3%). The number of atopic children was 48 (60.8%). The HAPI randomization resulted in 39 participants (49.4%) in the intervention group and 40 (50.6%) in the control group. Approximately half (39 (49.4%)) of the participants' homes were located in one of the small towns of the lower Yakima Valley, 18 (22.8%) were located in a rural setting of this area with no farm, and 19 (24.1%) were located on a farm property. An annual household income level of <$14,999 was reported by 12 households (15.2%), with another 31 households (39.2%) at the $15,000-$29,999 level, 27 households (34.2%) at the $30,000-$60,000 level, and 5 households (6.3%) at the >$60,000 level.

3.2 Urinary phthalate metabolite concentrations

A total of 281 urine samples were collected and analyzed for target phthalate metabolites: 54 participants provided 4 urine samples each, 19 provided 3 samples each, 2 provided 2 samples each, and 4 provided one sample each. A total of 77 urine samples were collected at timepoint 1, 75 at timepoint 2, 73 at timepoint 3, and 56 at timepoint 4.

Analyte-specific limits of detection, calculated as ± 33% of laboratory blank concentrations, ranged from 0.05 ng/mL for MCINP, MCIOP, and MCPP, to 0.2 ng/mL for MBZP, MECPP, and MEP. Trip and laboratory blanks were all < LOD. Mean (N=5) recoveries of the analyte-specific NIST 3673 reference values ranged from 88% (coefficient of variation (CV) 4%) for MIBP to 102% (CV 12%) for MNBP, indicating good analytical accuracy, while % CVs calculated using the CHEAR QC pools ranged from 3-8%, indicating good analytical precision.

The target phthalate metabolites were detected in 100% of samples, except for MEHP which was detected in 99%. For urinary phthalate metabolite concentrations < LOD, the machine values were used. Table 2 presents descriptive statistics of the unadjusted and creatinine-corrected concentrations. The unadjusted geometric mean (GM) (geometric SD (GSD)) concentrations ranged from 1.4 ng/mL (2.6 ng/mL) for MEHP to 24.7 ng/mL (2.8 ng/mL) for MEP, while the creatine-corrected concentrations ranged from 1.4 µg/g creatinine (2.5 µg/g creatinine) for MEHP to 25.4 µg/g creatinine (2.5 µg/g creatinine) for MEP. Specific gravity-adjusted concentrations are available in Table S2. Pearson correlation coefficients were weakest between MCINP and MEP (0.22) and generally the strongest among the four DEHP metabolites (e.g., MEHHP and MEOHP (0.99)) (Table S1).

3.3 Oxidative stress outcomes

Table 3 summarizes the distributions of unadjusted (ng/mL) and creatinine-corrected (µg/g creatinine) oxidative stress outcome measures. Measures for oxidative damage to lipids (8-isoprostane) and oxidative damage to DNA/RNA (via combined

measure of 8-OHdG, 8-OHG, and 8-hydroxyguanine) were available from all participants in 277 and 271 urine specimens, respectively. The GM value for 8-isoprostane was 1.30 µg/g creatinine (GSD: 1.6 µg/g creatinine), and its median and 90th percentile were 1.38 µg/g creatinine and 2.34 µg/g creatinine, respectively. The GM value for the DNA/RNA oxidative damage biomarker was 123.4 µg/g creatinine (GSD: 2.1 µg/g creatinine), and its median and 90th percentile were 130.4 µg/g creatinine and 295.4 µg/g creatinine, respectively

3.4 Associations between urinary phthalate metabolites and 8-isoprostane

Estimated regression coefficients of urinary phthalate metabolites and oxidative stress outcomes are shown in Table 4. Log 8-isoprostane concentrations increased as log urinary phthalate metabolite concentrations increased, and all associations were statistically significant across the 12 exposure variables ($p<0.01$). In the primary adjusted model, effect sizes ranged from a 7.9% (95% CI: 2.8% - 12.5%) increase in 8-isoprostane for a doubling of MCIOP, to 19.8% (95% CI: 13.3% - 26.6%) increase in 8-isoprostane for a doubling of MNBP (Table S4). Effect sizes were similar in the minimally adjusted, fully adjusted, and extended adjustment models.

3.5 Associations between urinary phthalate metabolites and a biomarker of DNA/RNA oxidative damage (8-OHdG, 8-OHG, and 8-hydroxyguanine)

Log concentrations of the urinary DNA/RNA oxidative damage biomarker (combined measure of 8-OHdG, 8-OHG, and 8-hydroxyguanine) generally increased as log urinary phthalate metabolite concentrations increased, though only associations with

MCINP and MEP were statistically significant in primary models ($p<0.05$) (Table 4). For each doubling of MCINP and MEP, the DNA/RNA oxidative damage biomarker increased by 5.7% (95% CI: 0% – 11.7%) and 5.7% (95% CI: 0.7% – 10.9%), respectively (Table S6). The extended adjustment model yielded statistically significant relationships of comparable effect sizes.

4. DISCUSSION

In this novel study of repeated measures of phthalate exposure and oxidative stress among children with asthma, we observed positive, statistically significant associations between urinary phthalates and both biomarkers of oxidative modifications of lipids (urinary 8-isoprostane) and oxidative damage to nucleic acids (combined urinary measure of 8-OHdG, 8-OHG, and 8-hydroxyguanine). 8-isoprostane demonstrated the most consistent effects across all tested phthalate metabolites and the largest magnitude of effect size. Data from epidemiological studies of children's phthalate exposure and corresponding oxidative stress levels in the U.S. are scarce, as are studies of these biomarkers of exposure and effect in children with asthma. Despite establishment of oxidative stress as a component of the pathophysiologic process underlying asthma development and severity [91], this longitudinal study is the first to our knowledge to use multiple repeated measures for children's phthalate exposure and two separate biomarkers for oxidative stress in a cohort of children with asthma in the United States. The results provide additional insights into the potential role of phthalate exposure as exacerbators of asthma in childhood through their role in inducing oxidative stress.

Our study population is generally understudied (rural Latinx children with asthma), and the urinary phthalate metabolite concentrations observed in this cohort were comparable to those reported among U.S. children aged 6-11 years in the National Health and Nutrition Examination Survey (NHANES) (Table 2) [73]. Overall, geometric means of unadjusted urinary phthalate metabolites from children in this study (*n* = 79) were slightly elevated for all phthalate metabolites except MCIOP and MEHP compared to unadjusted values reported by NHANES from 2015-2016 for children aged 6-11 years, and creatinine-corrected concentrations in our study were slightly lower than the corresponding NHANES geometric means [73].

Comparing our observed distributions of urinary biomarkers of oxidative stress to other studies presents challenges. Longitudinal studies with repeated measures of 8-isoprostane in similar populations (children aged 6-12 years and diagnosed with asthma in rural settings) were not identified in the published literature. Disparate analytical methods for quantification and reporting of 8-isoprostane also may influence comparisons [109], although there is debate on whether these analytical differences are significant enough to preclude comparisons [110]. A somewhat comparable study population, the CHAMACOS (Center for Health Assessment of Mothers and Children of Salinas) cohort of Mexican-American children in an agricultural region of California, reported urinary 8-isoprostane in children at age 5 (*n* = 256), 9 (*n* = 250), and 14 (*n* = 258), with mean (SD) values of 4.6 (7.7), 4.2 (6.7), and 6.0 (12.1) ng/mg creatinine, respectively [111]. These values are notably higher than the 90th percentile (2.34 µg/g (ng/mg) creatinine) observed in our study. The CHAMACOS study, which was not restricted to children with asthma, also reported a mean (SD) BMI of 20.9 (4.7) for children at age 9, similar to our study

population at the same age, mean (SD) BMI 20.9 (5.5). Elevated levels of 8-isoprostane have been reported in overweight and obese populations of both children and adults [106,112,113], as well as in populations with asthma compared to healthy controls [114]. Background reference ranges specific to children for population-level comparisons of our biomarker for DNA/RNA oxidative damage (combined measure of aggregate 8-OHdG, 8-OHG, and 8-hydroxyguanine) are not readily available, and we would expect our values to be higher than studies that quantify only 8-OHdG. A recent meta-analysis of 24 studies of healthy non-smoking adults using immunoassay analytical techniques for quantification [107] reported a median urinary 8-OHdG of 11.5 ng/mg creatinine (IQR: 5.9 – 21.6 ng/mg creatinine), an order of magnitude lower than our median of 128.8 µg/g creatinine (Table 3).

Few studies have examined relationships between urinary markers of phthalate exposure and urinary biomarkers of both 8-isoprostane and DNA/RNA oxidative damage among children in the United States, and to our knowledge this is the first longitudinal study to evaluate these measures in a cohort of children with asthma. A cross-sectional study of 751 individuals (mean age of 21.5 years, 217 individuals aged between 12-19 years) in Taiwan reported positive statistically significant associations for MMP with both 8-isoprostane and 8-OHdG, but not for the five other phthalate metabolite measures in their study (MBZP, MEP, MIBP, MNBP, and a summed measure for MEHP + MEHHP + MEOHP), all of which were individual significant predictors of 8-isoprostane in our study [98]. Among metabolites not summed, only MBZP exposure was higher in our study (GM 12.7 µg/g creatinine versus 1.93 µg/g creatinine); it is possible that differences including a cohort not defined by having asthma, a single timepoint cross-sectional design, earlier

sample collection time period (1992-2000) [5], and inclusion of participants of older age may all contribute to inconsistent findings to our study. Two other similarly designed, one time point cross-sectional studies reported statistically significant associations between specific metabolites and 8-OHdG. One reported positive associations between 8-OHdG and MEHP, MEHHP, MEOHP, MNBP, MBZP, MIBP, and MEP in 418 Belgian children recruited in 2008, 2009, and 2013 with a mean age of 14.8 years [35], while the other reported positive associations between 8-OHdG and MMP, MIBP, MNBP, MBZP, MCPP, MCMHP, MECPP, MEHHP, MEOHP, and ∑DEHP in 109 children recruited in 2017 aged 4 – 6 years in Saudi Arabia [96]. A longitudinal study of phthalates and 8-OHdG in children was conducted in Korea in 2011 among 287 healthy infants with urine samples collected at 3, 9, 12, and 15 months of age [97]. This study reported positive statistically significant associations between 8-OHdG and MEHHP, MEOHP, MIBP, and MNBP. Repeated measures are better suited to capturing temporal variability of urinary biomarkers of oxidative stress that may associate with changes in a short term biomarker such as phthalates [115]. The half-life of phthalates in human urine is less than 24 hours [11], and metabolites measured in spot urine samples are thought to reflect exposure from the preceding hours or days [75]. Similarly, 8-isoprostane and 8-OHdG are both stable and reliable biomarkers of oxidative stress when measured in spot urine samples with intraclass correlation coefficients (ICCs) of 0.76 and 0.96, respectively, as seen in a recent study of intra- and inter-individual variability of oxidative stress biomarkers [115].

While a role for oxidative stress in asthma pathogenesis and severity has been fairly well characterized [91,116], evidence for phthalates influencing asthma outcomes through oxidative stress pathways remains limited. Studies in animal models suggest

oxidative stress is involved in respiratory outcomes related to asthma that are induced by DEHP [28], di-n-butyl phthalate (DBP) [29], di-isononyl phthalate (DINP) [30], and di-isodecyl phthalate (DIDP) [31]. The Belgian study described above included a mediation analysis based on 8-OHdG, urinary phthalate metabolites, and report of doctor-diagnosed asthma [35]. The study found significant associations between both MBP and ∑DEHP with doctor-diagnosed asthma, between all target phthalates with 8-OHdG, but not between 8-OHdG and doctor-diagnosed asthma. The inability to demonstrate a mediating effect in that study may be attributable to its cross-sectional design which may be inadequate for characterizing the exposure during the critical window of influence on asthma development.

Our study has some limitations. The HAPI study's intervention resulted in an air cleaner in use for 25 of 79 participants included in the present study at timepoints 3 and 4 (65 of 281 observations). However, we address this in our regression models by including presence of air cleaners as a covariate, and we would not expect air cleaners to affect the phthalate exposures in this study as diet is the dominant driver of phthalate exposure in this age group [11]. Additionally, air purifiers have failed to demonstrate significant changes in child urinary phthalate metabolites [56]. Both oxidative stress biomarkers in this study were quantified via ELISA and it is possible these immunoassays overestimate analyte concentrations due to antibody cross-reactivity when compared to mass spectrometry (MS)-based methods [104], though this would not affect our inferential findings, and furthermore, a meta-analysis observed no significant differences in 8-isoprostane quantities analyzed with different methods [110]. Although the oxidative stress biomarkers measured in this study are not specific to asthma and may also be

associated with other health outcomes mediated by oxidative stress, the associations observed in this cohort of children with asthma suggest further study in this population is needed.

Although 8-isoprostane has long been regarded as a reliable biomarker of oxidative stress in humans [117], its interpretation as solely a marker of lipid peroxidation has been supplanted by use of the 8-isoprostane-to-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) ratio [118,119]. This is due to an alternative (enzymatic) 8-isoprostane generation pathway involving inflammation, catalyzed by prostaglandin endoperoxide synthase (PGHS), which may inflate the role of oxidative stress in investigations interpreting the non-ratio approach [110,118]. Without quantifying $PGF_{2\alpha}$, we could not determine the proportion of 8-isoprostane in our study attributable to inflammation (catalyzed by PGHS) vs oxidative stress (non-enzymatic, free radical oxidation). Nevertheless, the strong associations demonstrated in this study across all phthalate metabolites with 8-isoprostane suggest this marker may underlie the relationship between phthalates and asthma exacerbation in children with asthma. Further investigation of the relationship between phthalates and oxidative stress in children with asthma is warranted, including attempts to distinguish distinct upstream pathophysiological mechanisms involving either oxidative stress and inflammation. The 8-isoprostane/$PGF_{2\alpha}$ ratio approach was used to clarify associations with 17 phthalate metabolites in a recent study of pregnant women (*n* = 758) [103], and suggests that most metabolites differentially associate only with oxidative stress-derived 8-isoprostane, while MINP (DINP metabolite) and MCINP (DIDP metabolite) were associated only with inflammation-derived 8-isoprostane. Similar studies are needed in children with asthma, including evaluations of aeroallergen exposure to investigate the

role of phthalate-induced oxidative stress in the adjuvant effect demonstrated by studies in mouse models of asthma [28–30].

In conclusion, this longitudinal study of children with asthma observed significant, positive associations between repeated measures of urinary biomarkers of phthalate exposure and biomarkers of oxidative stress. Further study of these biomarkers of effect are needed in child populations with asthma in order to clarify the role of phthalate exposure in driving oxidative stress and contributing to asthma morbidity.

TABLES

Table 1. Study population and household characteristics (*N*=79)*

Characteristic	*N* (%)
Sex (female)	29 (36.7%)
Age at enrollment	9.0 (2.1)[a]
Ethnicity[b]	
Hispanic/Latinx	77 (100%)
BMI-for-age percentile at enrollment[c]	79 (26)[a]
Atopic[b]	48 (60.8%)
Baseline controller medication[b]	69 (87.3%)
HAPI intervention recipient	39 (49.4%)
Primary household language	
English	18 (22.8%)
Spanish	61 (77.2%)
Home location[c]	
On farm	19 (24.1%)
Rural, no farm	18 (22.8%)
Town	39 (49.4%)
Annual household income[d]	
<$14,999	12 (15.2%)
$15,000-$29,999	31 (39.2%)
$30,000-$60,000	27 (34.2%)
>$60,000	5 (6.3%)

[a] Mean ± SD.

[b] (N=77)

[c] (N=76)

[d] (N=75)

*Adapted from *Masterson et al. 2020* [37]

Table 2. Distributions of unadjusted and adjusted urinary phthalate metabolite concentrations with NHANES comparisons

Phthalate metabolite	Present study[a]		Unadjusted values (μg/L)								Creatinine-corrected values (μg/g creatinine)							
	LOD (μg/L)	%>LOD	Present study[a]				NHANES (6-11 years, 2015-2016)[b]				Present study[a]				NHANES (6-11 years, 2015-2016)[b]			
			GM	GSD	50th%	90th%	GM	(95% CI)	50th% (95% CI)	90th% (95% CI)	GM	GSD	50th%	90th%	GM	(95% CI)	50th% (95% CI)	90th% (95% CI)
MBZP	0.2	100%	12.4	3.3	13.0	56.4	10.7	(8.6-13.3)	10.9 (8.4-13.8)	59.2 (41.6-73.2)	12.7	2.8	12.5	47.3	13.8	(11.3-16.8)	12.0 (10.0-15.6)	57.9 (49.0-85.7)
MCINP	0.05	100%	2.3	2.4	2.1	6.5	2.3	(2.0-2.6)	2.4 (2.0-2.8)	6.7 (5.9-8.0)	2.3	2.3	2.2	7.0	2.9	(2.7-3.2)	2.8 (2.5-3.0)	6.7 (5.9-8.3)
MCIOP	0.05	100%	9.5	2.8	8.0	40.9	11.1	(9.2-13.4)	10.2 (8.5-12.0)	51.9 (34.3-71.5)	9.7	2.6	7.9	40.6	14.3	(12.0-16.9)	11.5 (9.6-14.3)	59.6 (39.6-90.4)
MCPP	0.05	100%	2.4	2.5	2.3	7.3	1.8	(1.6-2.0)	1.8 (1.5-2.0)	6.8 (4.7-7.9)	2.5	2.2	2.3	6.6	2.3	(2.1-2.6)	2.2 (1.8-2.6)	6.7 (5.5-7.1)
MECPP	0.2	100%	16.9	2.4	16.9	50.8	14.6	(12.9-16.6)	14.9 (13.3-16.7)	42.5 (35.3-54.8)	17.3	2.1	16.1	45.8	18.9	(17.5-20.4)	17.5 (16.6-19.1)	48.3 (35.6-57.5)
MEHHP	0.1	100%	10.3	2.6	11.0	34.4	8.8	(7.8-9.9)	9.0 (7.9-10.0)	27.5 (20.0-35.9)	10.6	2.1	10.2	26.9	11.3	(10.5-12.3)	10.5 (9.6-11.6)	28.8 (21.7-35.2)
MEHP	0.1	99%	1.4	2.6	1.3	4.9	1.4	(1.3-1.6)	1.3 (1.0-1.6)	4.2 (3.6-4.8)	1.4	2.5	1.4	4.3	1.8	(1.6-2.0)	1.8 (1.6-2.1)	4.7 (4.1-5.2)
MEOHP	0.15	100%	6.8	2.6	7.0	23.0	6.0	(5.4-6.7)	6.1 (5.3-6.8)	19.0 (14.7-21.5)	7.0	2.1	6.7	18.2	7.7	(7.1-8.3)	7.3 (6.6-8.0)	19.4 (14.6-22.9)
MEP	0.2	100%	24.7	2.8	24.3	92.2	24.5	(18.6-32.3)	22.1 (16.3-31.1)	115.0 (70.4-203.0)	25.4	2.5	22.8	75.8	31.6	(25.6-39.0)	26.5 (21.8-33.6)	120.0 (77.8-205.0)
MIBP	0.1	100%	13.5	2.6	24.3	92.2	11.2	(9.3-13.5)	11.6 (10.2-13.7)	39.2 (31.1-52.1)	13.8	2.2	12.8	38.6	14.5	(12.6-16.6)	13.5 (11.9-15.0)	42.5 (28.4-49.6)
MNBP	0.1	100%	15.7	2.5	16.6	44.4	14.4	(13.1-15.8)	15.4 (13.4-17.3)	40.6 (36.0-43.0)	16.1	1.9	16.2	35.7	18.5	(17.4-19.7)	18.6 (17.2-19.8)	40.1 (35.5-44.3)
∑DEHP	NR	NR	35.3	2.5	35.9	108.7	NR	NR	NR	NR	36.2	2.1	33.4	90.1	NR	NR	NR	NR

[a] *N* = 281 urine specimens from 79 participants

[b] Values drawn from *National Health and Nutrition Examination Survey (NHANES) Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2019* (U.S. Centers for Disease Control and Prevention) [73]

NR = not reported

Table 3. Distributions of urinary biomarkers of oxidative stress: 8-isoprostane and DNA/RNA oxidative damage marker

	Unadjusted (ng/mL)				Adjusted (µg/g creatinine)			
Urinary biomarker	GM	GSD	50th %	90th %	GM	GSD	50th %	90th %
8-isoprostane[a]	1.28	2.05	1.43	2.87	1.30	1.62	1.38	2.34
DNA/RNA oxidative damage marker[b]	123.5	1.67	126.8	235.8	123.4	2.06	130.4	295.4

[a] N = 277

[b] N = 271

Table 4. Estimated linear mixed-effects model coefficients of the relationship between natural log-transformed urinary phthalate metabolites and oxidative stress biomarkers

	Oxidative stress biomarker											
	8-isoprostane[a]						DNA/RNA damage (8-OHdG, 8-OHG, and 8-hydroxyguanine)[b]					
	Minimally adjusted		Fully adjusted (primary)		Extended adjustment		Minimally adjusted		Fully adjusted (primary)		Extended adjustment	
Phthalate metabolites	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
MBZP	**0.21**	**0.14 – 0.27**	**0.22**	**0.15 – 0.28**	**0.21**	**0.14 – 0.28**	-0.01	-0.08 – 0.05	0.0	-0.07 – 0.07	0.01	-0.06 – 0.08
MCINP	**0.20**	**0.12 – 0.27**	**0.20**	**0.12 – 0.28**	**0.20**	**0.13 – 0.28**	0.07	-0.01 – 0.15	**0.08**	**0.00 – 0.16**	**0.08**	**0.00 – 0.17**
MCIOP	**0.11**	**0.05 – 0.18**	**0.11**	**0.04 – 0.17**	**0.13**	**0.06 – 0.20**	0.04	-0.03 – 0.10	0.06	-0.01 – 0.12	0.05	-0.02 – 0.12
MCPP	**0.16**	**0.08 – 0.23**	**0.16**	**0.08 – 0.24**	**0.16**	**0.08 – 0.25**	-0.01	-0.09 – 0.06	-0.0	-0.09 – 0.07	-0.01	-0.10 – 0.07
MECPP	**0.23**	**0.15 – 0.31**	**0.24**	**0.16 – 0.32**	**0.27**	**0.19 – 0.35**	0.05	-0.03 – 0.13	0.05	-0.03 – 0.13	0.05	-0.04 – 0.14
MEHHP	**0.22**	**0.15 – 0.29**	**0.23**	**0.16 – 0.30**	**0.25**	**0.18 – 0.33**	0.03	-0.05 – 0.10	0.03	-0.04 – 0.11	0.03	-0.05 – 0.11
MEHP	**0.14**	**0.07 – 0.21**	**0.16**	**0.08 – 0.23**	**0.19**	**0.11 – 0.26**	0.03	-0.04 – 0.10	0.04	-0.03 – 0.11	0.03	-0.05 – 0.11
MEOHP	**0.23**	**0.16 – 0.30**	**0.24**	**0.16 – 0.31**	**0.26**	**0.19 – 0.34**	0.02	-0.05 – 0.10	0.03	-0.05 – 0.10	0.02	-0.06 – 0.10
MEP	**0.13**	**0.07 – 0.20**	**0.13**	**0.06 – 0.20**	**0.13**	**0.06 – 0.20**	**0.07**	**0.00 – 0.14**	**0.08**	**0.01 – 0.15**	**0.09**	**0.02 – 0.16**
MIBP	**0.22**	**0.15 – 0.30**	**0.24**	**0.17 – 0.32**	**0.26**	**0.18 – 0.34**	0.0	-0.07 – 0.08	-0.0	-0.08 – 0.07	0.01	-0.08 – 0.10
MNBP	**0.25**	**0.18 – 0.33**	**0.26**	**0.18 – 0.34**	**0.28**	**0.19 – 0.36**	0.01	-0.07 – 0.10	0.02	-0.07 – 0.11	0.03	-0.06 – 0.12
∑DEHP	**0.24**	**0.16 – 0.31**	**0.25**	**0.17 – 0.33**	**0.28**	**0.19 – 0.36**	0.04	-0.04 – 0.12	0.04	-0.04 – 0.12	0.04	-0.05 – 0.13

[a] Minimally adjusted model covariates: specific gravity, age, sex, intervention
Fully adjusted (primary): minimal model plus BMI, atopy, season
Extended adjustment: full model plus income, baseline medication, visit

[b] Minimally adjusted model covariates: specific gravity, age, sex, intervention
Fully adjusted (primary): minimal model plus BMI, atopy, season
Extended adjustment: full model plus income, baseline medication, visit

* Bolding denotes statistical significance for 8-isoprostane (*p<0.01*) and DNA/RNA oxidative damage biomarker (*p<0.05*)

Table S1. Pearson correlation matrix of urinary phthalate metabolites (281 samples from 79 participants)

	MNBP	*MBZP*	*MCINP*	*MCIOP*	*MCPP*	*MECPP*	*MEHHP*	*MEHP*	*MEOHP*	*MEP*	*MIBP*
MNBP		0.68	0.37	0.38	0.53	0.6	0.67	0.56	0.69	0.48	0.66
MBZP			0.38	0.37	0.48	0.54	0.57	0.5	0.59	0.42	0.47
MCINP				0.63	0.52	0.49	0.49	0.4	0.5	0.22	0.27
MCIOP					0.76	0.57	0.54	0.45	0.55	0.31	0.29
MCPP						0.61	0.61	0.46	0.61	0.33	0.43
MECPP							0.94	0.79	0.95	0.42	0.53
MEHHP								0.82	0.99	0.42	0.59
MEHP									0.82	0.32	0.49
MEOHP										0.43	0.6
MEP											0.36
MIBP											

Table S2. Distribution of urinary phthalate metabolites with specific gravity adjustment (ng/mL) (281 samples from 79 participants)

phthalate metabolites	GM	GSD	50th %	90th %
MBZP	14.5	2.9	14.0	55.9
MCINP	2.7	2.2	2.5	7.4
MCIOP	11.2	2.7	8.8	42.8
MCPP	2.8	2.2	2.7	7.2
MECPP	19.8	2.2	19.3	47.6
MEHHP	12.1	2.3	12.1	32.3
MEHP	1.6	2.5	1.6	5.1
MEOHP	8.0	2.3	7.9	21.2
MEP	29.1	2.7	28.6	89.4
MIBP	15.9	2.4	16.3	45.1
MNBP	18.4	2.1	18.9	56.9
∑DEHP	41.4	2.2	39.3	104.1

Table S3. Regression coefficients and confidence intervals for all 8-isoprostane models*

	crude model[A]			minimally adjusted model[B]			fully adjusted (primary) model[C]			extended adjustment model[D]		
	β	CI low	CI high	β	CI low	CI high	β	CI low	CI high	β	CI low	CI high
MBZP	**0.21**	**0.14**	**0.27**	**0.21**	**0.14**	**0.27**	**0.22**	**0.15**	**0.28**	**0.21**	**0.14**	**0.28**
MCINP	**0.19**	**0.12**	**0.27**	**0.20**	**0.12**	**0.27**	**0.20**	**0.12**	**0.28**	**0.20**	**0.13**	**0.28**
MCIOP	**0.11**	**0.05**	**0.17**	**0.11**	**0.05**	**0.18**	**0.11**	**0.04**	**0.17**	**0.13**	**0.06**	**0.20**
MCPP	**0.14**	**0.07**	**0.22**	**0.16**	**0.08**	**0.23**	**0.16**	**0.08**	**0.24**	**0.16**	**0.08**	**0.25**
MECPP	**0.22**	**0.15**	**0.30**	**0.23**	**0.15**	**0.31**	**0.24**	**0.16**	**0.32**	**0.27**	**0.19**	**0.35**
MEHHP	**0.22**	**0.15**	**0.28**	**0.22**	**0.15**	**0.29**	**0.23**	**0.16**	**0.30**	**0.25**	**0.18**	**0.33**
MEHP	**0.14**	**0.07**	**0.21**	**0.14**	**0.07**	**0.21**	**0.16**	**0.08**	**0.23**	**0.19**	**0.11**	**0.26**
MEOHP	**0.22**	**0.15**	**0.29**	**0.23**	**0.16**	**0.30**	**0.24**	**0.16**	**0.31**	**0.26**	**0.19**	**0.34**
MEP	**0.12**	**0.06**	**0.19**	**0.13**	**0.07**	**0.20**	**0.13**	**0.06**	**0.20**	**0.13**	**0.06**	**0.20**
MIBP	**0.22**	**0.15**	**0.30**	**0.22**	**0.15**	**0.30**	**0.24**	**0.17**	**0.32**	**0.26**	**0.18**	**0.34**
MNBP	**0.25**	**0.17**	**0.33**	**0.25**	**0.18**	**0.33**	**0.26**	**0.18**	**0.34**	**0.28**	**0.19**	**0.36**
∑DEHP	**0.23**	**0.15**	**0.30**	**0.24**	**0.16**	**0.31**	**0.25**	**0.17**	**0.33**	**0.28**	**0.19**	**0.36**

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted (primary) model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, visit

*Bolding denotes statistical significance (*p<0.01*)

Table S4. Percent increase* in 8-isoprostane (and CIs) for 100% increase in phthalate metabolite**												
	crude model[A]			minimally adjusted model[B]			fully adjusted (primary) model[C]			extended adjustment model[D]		
	%	CI low	CI high	%	CI low	CI high	%	CI low	CI high	%	CI low	CI high
MBZP	**15.67**	**10.19**	**20.58**	**15.67**	**10.19**	**20.58**	**16.47**	**10.96**	**21.42**	**15.67**	**10.19**	**21.42**
MCINP	**14.08**	**8.67**	**20.58**	**14.87**	**8.67**	**20.58**	**14.87**	**8.67**	**21.42**	**14.87**	**9.43**	**21.42**
MCIOP	**7.92**	**3.53**	**12.51**	**7.92**	**3.53**	**13.29**	**7.92**	**2.81**	**12.51**	**9.43**	**4.25**	**14.87**
MCPP	**10.19**	**4.97**	**16.47**	**11.73**	**5.70**	**17.28**	**11.73**	**5.70**	**18.10**	**11.73**	**5.70**	**18.92**
MECPP	**16.47**	**10.96**	**23.11**	**17.28**	**10.96**	**23.97**	**18.10**	**11.73**	**24.83**	**20.58**	**14.08**	**27.46**
MEHHP	**16.47**	**10.96**	**21.42**	**16.47**	**10.96**	**22.26**	**17.28**	**11.73**	**23.11**	**18.92**	**13.29**	**25.70**
MEHP	**10.19**	**4.97**	**15.67**	**10.19**	**4.97**	**15.67**	**11.73**	**5.70**	**17.28**	**14.08**	**7.92**	**19.75**
MEOHP	**16.47**	**10.96**	**22.26**	**17.28**	**11.73**	**23.11**	**18.10**	**11.73**	**23.97**	**19.75**	**14.08**	**26.58**
MEP	**8.67**	**4.25**	**14.08**	**9.43**	**4.97**	**14.87**	**9.43**	**4.25**	**14.87**	**9.43**	**4.25**	**14.87**
MIBP	**16.47**	**10.96**	**23.11**	**16.47**	**10.96**	**23.11**	**18.10**	**12.51**	**24.83**	**19.75**	**13.29**	**26.58**
MNBP	**18.92**	**12.51**	**25.70**	**18.92**	**13.29**	**25.70**	**19.75**	**13.29**	**26.58**	**21.42**	**14.08**	**28.34**
∑DEHP	**17.28**	**10.96**	**23.11**	**18.10**	**11.73**	**23.97**	**18.92**	**12.51**	**25.70**	**21.42**	**14.08**	**28.34**

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted (primary) model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, visit

*Formula for percent increase in 8-isoprostane for 100% increase in phthalate metabolite: (((2^coefficient)-1)*100)

**Bolding denotes statistical significance (*p<0.01*)

Table S5. Regression coefficients and confidence intervals for all DNA/RNA oxidative damage models*												
	crude model[A]			minimally adjusted model[B]			fully adjusted (primary) model[C]			extended adjustment model[D]		
	β	CI low	CI high	β	CI low	CI high	β	CI low	CI high	β	CI low	CI high
MBZP	-0.01	-0.07	0.05	-0.01	-0.08	0.05	0.0	-0.07	0.07	0.01	-0.06	0.08
MCINP	0.07	-0.01	0.15	0.07	-0.01	0.15	**0.08**	**0.00**	**0.16**	**0.08**	**0.00**	**0.17**
MCIOP	0.04	-0.02	0.10	0.04	-0.03	0.10	0.06	-0.01	0.12	0.05	-0.02	0.12
MCPP	0.0	-0.08	0.08	-0.01	-0.09	0.06	-0.0	-0.09	0.07	-0.01	-0.10	0.07
MECPP	0.05	-0.03	0.13	0.05	-0.03	0.13	0.05	-0.03	0.13	0.05	-0.04	0.14
MEHHP	0.03	-0.04	0.10	0.03	-0.05	0.10	0.03	-0.04	0.11	0.03	-0.05	0.11
MEHP	0.03	-0.04	0.10	0.03	-0.04	0.10	0.04	-0.03	0.11	0.03	-0.05	0.11
MEOHP	0.02	-0.05	0.10	0.02	-0.05	0.10	0.03	-0.05	0.10	0.02	-0.06	0.10
MEP	0.06	-0.01	0.12	**0.07**	**0.00**	**0.14**	**0.08**	**0.01**	**0.15**	**0.09**	**0.02**	**0.16**
MIBP	0.0	-0.07	0.08	0.0	-0.07	0.08	-0.01	-0.08	0.07	0.01	-0.08	0.10
MNBP	0.01	-0.08	0.09	0.01	-0.07	0.10	0.02	-0.07	0.11	0.03	-0.06	0.12
∑DEHP	0.04	-0.04	0.12	0.04	-0.04	0.12	0.04	-0.04	0.12	0.04	-0.05	0.13

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted (primary) model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, visit

*Bolding denotes statistical significance (*p<0.05*)

Table S6. Percent change* in DNA/RNA oxidative damage biomarker (and CIs) for 100% increase in phthalate metabolite**

	crude model[A]			minimally adjusted model[B]			fully adjusted (primary) model[C]			extended adjustment model[D]		
	%	CI low	CI high	%	CI low	CI high	%	CI low	CI high	%	CI low	CI high
MBZP	0.00	-4.74	3.53	0.00	-5.39	3.53	0.00	-4.74	4.97	0.70	-4.07	5.70
MCINP	4.97	-0.69	10.96	4.97	-0.69	10.96	**5.70**	**0.00**	**11.73**	**5.70**	**0.00**	**12.51**
MCIOP	2.81	-1.38	7.18	2.81	-2.06	7.18	4.25	-0.69	8.67	3.53	-1.38	8.67
MCPP	0.00	-5.39	5.70	0.00	-6.05	4.25	0.00	-6.05	4.97	0.00	-6.70	4.97
MECPP	3.53	-2.06	9.43	3.53	-2.06	9.43	3.53	-2.06	9.43	3.53	-2.73	10.19
MEHHP	2.10	-2.73	7.18	2.10	-3.41	7.18	2.10	-2.73	7.92	2.10	-3.41	7.92
MEHP	2.10	-2.73	7.18	2.10	-2.73	7.18	2.81	-2.06	7.92	2.10	-3.41	7.92
MEOHP	1.40	-3.41	7.18	1.40	-3.41	7.18	2.10	-3.41	7.18	1.40	-4.07	7.18
MEP	4.25	-0.69	8.67	**4.97**	**0.00**	**10.19**	**5.70**	**0.70**	**10.96**	**6.44**	**1.40**	**11.73**
MIBP	0.00	-4.74	5.70	0.00	-4.74	5.70	0.00	-5.39	4.97	0.70	-5.39	7.18
MNBP	0.70	-5.39	6.44	0.70	-4.74	7.18	1.40	-4.74	7.92	2.10	-4.07	8.67
∑DEHP	2.81	-2.73	8.67	2.81	-2.73	8.67	2.81	-2.73	8.67	2.81	-3.41	9.43

[A] Crude model covariate: specific gravity

[B] Minimally adjusted model covariates: specific gravity, age, sex, intervention

[C] Fully adjusted (primary) model covariates: specific gravity, age, sex, intervention, bmi, atopy, season

[D] Extended adjustment model covariates: specific gravity, age, sex, intervention, bmi, atopy, season, income, baseline controller medication use, visit

*Formula for percent increase in DNA/RNA oxidative damage biomarker for 100% increase in phthalate metabolite: (((2^coefficient)-1)*100)

**Bolding denotes statistical significance (*p<0.05*)

CHAPTER 4. CONCLUSIONS

This dissertation represents an epidemiological investigation examining (i) the relationships between phthalate exposure and asthma outcomes (aim 1, Chapter 2), and (ii) the relationships between phthalate exposure and oxidative stress (aim 2, Chapter 3), in a population of children with asthma residing in rural, agricultural communities of Yakima Valley, Washington State in the United States, between 2015 and 2019. The findings of this dissertation suggest that phthalates are associated with asthma outcomes as measured by biomarkers of inflammation in urine and exhaled breath, in addition to associations with urinary biomarkers of oxidative stress. This study population is exposed to phthalate compounds at similar levels when compared to the most recently available national data on phthalate exposure for their age group.

In Chapter 2, we observed significant associations between MECPP, MEHP, MEP, and MNBP with FENO, a biomarker of eosinophilic airway inflammation that is associated with asthma exacerbations [46,47]. We did not observe statistically significant interaction in this relationship by atopic status. We also observed significant associations between all urinary phthalate metabolites tested (MBZP, MCINP, MCIOP, MCPP, MECPP, MEHHP, MEHP, MEOHP, MEP, MIBP, MNBP, and a summed measure of DEHP metabolites) and urinary leukotriene E_4 ($uLTE_4$), a biomarker of systemic inflammation that is associated with asthma exacerbations [49]. This is the first study to our knowledge to report on associations between phthalate exposure and $uLTE_4$, and the consistent, strong associations demonstrated in this study suggest that this inflammatory marker should be examined further in the context of phthalate exposure and asthma

outcomes. We did not observe significant associations between phthalate exposure and a clinical measure of lung function testing (spirometry) via the forced expiratory volume in 1 second (FEV_1) [62], neither in models using the FEV_1 z-score nor the FEV_1 percent predicted models. Similarly, we did not observe significant associations between urinary phthalate metabolites and the Asthma Control Test (ACT) [64], a composite measure derived from a validated questionnaire which evaluates the participant's level of asthma control in the prior 4 weeks. Finally, we did not observe significant associations between urinary phthalate metabolites and caregiver report of asthma symptoms in the prior 2 weeks, but for one metabolite, MCIOP, and only in the minimally adjusted model; this association was not significant in the primary model.

In Chapter 3, we observed significant associations between urinary phthalate metabolites and both urinary biomarkers of oxidative stress, 8-isoprostane, which reflects oxidative damage to lipids [106], and our measure for DNA/RNA oxidative damage (combined measure of 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-OHG), and 8-hydroxyguanine), a reflection of oxidative damage to nucleic acids [107]. All tested urinary phthalate metabolites (MBZP, MCINP, MCIOP, MCPP, MECPP, MEHHP, MEHP, MEOHP, MEP, MIBP, MNBP, and a summed measure of DEHP metabolites) demonstrated significant associations with 8-isoprostane, suggesting the importance of this biomarker in children with asthma, and a potential role for oxidative stress in the observed relationships between phthalate exposure and asthma outcomes. The urinary phthalate metabolites MCINP and MEP were both significantly associated with the biomarker for oxidative damage to DNA/RNA.

In aggregate, these results suggest that urinary phthalate metabolites are significant predictors of biomarkers of inflammation in both urine and exhaled breath as well as urinary biomarkers of oxidative stress that reflect oxidative damage to lipids and oxidative damage to nucleic acids, in children with asthma. These findings are in line with a growing body of evidence establishing phthalate exposure as a predictor of these biological markers implicated in the pathogenesis and severity of asthma, and importantly, shed light on the importance of these specific markers in the context of potential exacerbation of disease among children with asthma. The evidence supporting phthalate exposure's link to asthma is demonstrated by *in vitro* and *ex vivo* studies as well as experimental studies in animal models for asthma [16]. Epidemiological studies overall suggest phthalates may be associated with asthma pathogenesis and severity [18], but studies in populations of children who have developed asthma are needed. The present longitudinal study's consistent findings, particularly of the relationships with $uLTE_4$ and 8-isoprostane, are important contributions to this area of research, and should prompt further study of these biomarkers in the context of phthalate exposure and child asthma, as well as the pathophysiological mechanisms these markers represent.

Some minor limitations to this study should be noted. In the asthma health outcome assessment, the questionnaire-based outcomes may be temporally mismatched to our exposure assessment. Data generated through administration of the ACT as well as the caregiver symptom questionnaire reflect the prior 4 weeks and 2 weeks, respectively, and thus are suboptimal in examining urinary phthalate metabolites that are collected at the time of the questionnaire as predictors. Second, the interventional design in the HAPI study, which provided data for our study questions, required addressing the influence of

the intervention on results. We believe our addition of the air cleaner as a covariate in our regression models addresses this.

This dissertation boasts several strengths. First, a key strength to this dissertation is its study population, for two primary reasons: (i) all participants are from underserved and understudied rural Latinx communities, and (ii) all participants have preexisting diagnosed asthma, permitting the study of phthalates as triggers of asthma - an area of critical need in the epidemiological evidence base. A second strength, this study employs a longitudinal design, providing a stronger design to identify relationships between short-lived changes in phthalate exposure and short-lived perturbations in inflammation/oxidative stress and/or asthma morbidity compared with one time cross-sectional study designs. Third, this is the first study of its kind to evaluate the relationship between phthalate exposure and a urinary biomarker of inflammation associated with asthma exacerbation, $uLTE_4$. Fourth, this study combines a rich array of outcome assessment measures that represent various aspects of asthma pathophysiology, including both biological measures from urine and exhaled breath as well as objective measures of lung function and validated symptom survey based data.

Additional longitudinal studies of phthalate exposure and outcomes designed to assess asthma exacerbation in populations of children with asthma are needed. Such epidemiological studies should be designed to assess exposure to phthalates through both environmental measures and biological measures, and conduct extensive questionnaires based on diet, in order to permit evaluation of the roles of individual routes and sources of exposure on hypothesized relationships with outcomes related to asthma. Furthermore, studies should assess exposure to aeroallergens in order to investigate the

potential for phthalates to act as adjuvants in allergic airway reactions in asthma. To clarify the potential role of oxidative stress as a potential mechanism of phthalate-induced asthma exacerbation, studies should be equipped in data collection and analysis phases to investigate whether oxidative stress mediates any observed relationships. Finally, researchers should prioritize assessing exposure to phthalates and their alternatives, as well as mixtures of these compounds, to reflect the ongoing shifting nature of real-world exposure of children both in and out of the United States.

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