DISSERTATION

RESPIRATORY MORBIDITY IN SUSCEPTIBLE POPULATIONS: THE ROLE OF JOINT EXPOSURE TO MULTIPLE ENVIRONMENTAL CHEMICALS AND POLLUTANTS

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ABSTRACT

RESPIRATORY MORBIDITY IN SUSCEPTIBLE POPULATIONS: THE ROLE OF JOINT EXPOSURE TO MULTIPLE ENVIRONMENTAL CHEMICALS AND POLLUTANTS

Exposure to ambient pollution from environmental chemicals and pollutants has been associated with a range of adverse respiratory outcomes; susceptible populations are disproportionately affected. Children with asthma are particularly at risk for adverse respiratory effects of environmental agents. The recent increase in US and worldwide pediatric asthma prevalence has encouraged new lines of inquiry focusing on environmental factors, rather than genetic factors, as the main etiologic agent in asthma-related morbidity; the complex relationship between individuals and their environment requires improved characterization and quantification.

The study of potential joint effects from multiple environmental chemical stressors is particularly relevant for chronic diseases with strong environmental antecedents, including asthma. As children with asthma tend to have spatially and temporally heterogeneous exposure to multiple domains of environmental chemicals determined by regional characteristics, study approaches with an understanding of the complexities of exposure (i.e. exposure data that are high dimensional and strongly correlated) of the health impact of multiple pollutants are required.

In this dissertation, we sought to evaluate the association between multipollutant exposures to ambient environmental chemicals and pollutants (ECP) and respiratory morbidity in uniquely exposed populations. By implementing health risk-based multipollutant epidemiologic

approaches, we targeted potential synergistic effects within multiple domains of ambient ECP, with specific attention to criteria air pollutants and agricultural pesticides.

Chapter 1 describes background information relevant to the dissertation while providing context for the multipollutant approaches and highlighting the specific objectives; Chapters 2 – 4 summarize methodology, study findings and aim-specific discussion; and the final chapter provides a discussion of overall findings, strengths, limitations, and suggestions for future research.

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DEDICATION

For Demilade, her mum, her grandparents, each equally my inspiration, pride and joy.

As important, for you dad, I am a double doctor because you believed in me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

The Burden of Pediatric Asthma: Environmental Exposures

Pediatric asthma is a heterogeneous chronic inflammatory disease of the airways. This airway inflammation is characterized by airway hyperresponsiveness, airflow limitation and other respiratory symptoms which contribute to the heterogeneous clinical presentation, severity, and pathophysiology of asthma [1,2]. The majority of pediatric asthma is believed to be attributable to atopy, characterized by airway hyperresponsiveness secondary to excessive antibody responses to allergens [3]. While the description of resulting symptoms defines the typical mechanisms of allergic asthma, it has become increasingly apparent and understood that asthma is not a single disease, but a syndrome consisting of an array of variable disease subtypes with similar clinical features [4,5].

Although the concepts that underlie the disease pathogenesis have evolved in the past 35 years, asthma prevalence has consistently trended upwards and is frequently described as the one of the most common chronic disease among children worldwide [6–8]. In the US, the prevalence of asthma remains high [9,10]; after a period of fairly steady increase from the 1980s to the mid-1990s, the proportion of children with current asthma (defined by the combination of positive responses to “ever diagnosed with asthma by a health professional” with “still having asthma”) has remained between eight and ten percent in the past decade [10].

Recently, national survey data from the CDC indicate a plateau in asthma prevalence [10]. However, high levels of avoidable morbidity and mortality remain. Uncontrolled or poorly controlled asthma, including complications and the general effects of symptoms, results in

significant direct and indirect costs to family and society in the form of healthcare utilization expenses (direct), and lost school days, disability and reduced productivity (indirect) [9,11]. The median annual medical cost of asthma in 2012 was estimated at more than $900 per child, and over 700,000 children visited the ER or were hospitalized due to asthma [9]. In addition, in 2013, over 13 million missed school days were reported due to asthma [9,10].

This asthma burden is particularly significant because of prevalence and outcome disparities by race/ethnicity and socioeconomic status. This disparities emanate from both individual level risk factors in addition to patient and family interaction with social and structural factors in the communities [12–14]. According to data from the CDC's National Health Interview Survey, male children (versus female), Black children and children of Puerto Rican descent (versus White children), and children living in households with income of < 100% (versus ≥ 250%) of Federal Poverty Level (FPL) had higher asthma prevalence and associated morbidity and mortality indices, including but not limited to asthma-related exacerbations, hospitalizations, and death [9].

Mechanisms

The natural history of pediatric asthma is typically characterized by acute and episodic events/worsening (exacerbation) against an underlying persistent airway inflammation and airway remodeling that may be associated with varying degrees of respiratory symptoms and lung function decline [1]. In general, these mechanisms occur in accordance with chronic inflammatory pathways in which environmental trigger factors combine with the genetic underpinnings that manifest as the underlying phenotype of the individual patient [1,2,15].

Findings from even the earliest pathogenic models of asthma indicate an association with environmental factors [15]. Environmental factors associated with asthma morbidity encompass a wide range of biological and chemical components in the indoor and outdoor atmosphere.

The deleterious effects of indoor air pollutants on pediatric asthma morbidity, though beyond the scope of this thesis, are of important public health concern. Pediatric asthma exacerbations have been linked to indoor exposures arising from tobacco smoke, combustion products, including particulate matter and volatile organic chemicals, dampness and mold, and other biological allergens such as cockroach and mouse allergens [16–18].

With regard to the ambient environment, hazardous outdoor environmental exposures include a range of gaseous chemicals (including ozone and oxides of nitrogen and sulphur), particulate matter (including other products of biomass and diesel combustion like polycyclic aromatic hydrocarbons), dust (including from non-combustion sources) and organic pollutants like pesticides and aeroallergens (such as those derived from fungal spores and grass pollen) [19].

Although the role of outdoor environmental risk factors in the development of new-onset asthma remains unclear, several studies have identified associations between morbidity among children with existing asthma and exposure to ambient environmental chemicals and pollutants (ECP) [19,20]. A number of epidemiological and experimental studies have implicated both short- and long-term exposure to several ambient ECP in increases in pediatric asthma morbidity, assessed as asthma-related hospitalizations and emergency room visits, increases in asthma symptoms, medication use and exacerbations, or decrements in lung function [21–28]. From a mechanistic perspective, ECPs are believed to induce oxidative injury to the airways, leading to inflammation, remodeling, and increased risk of sensitization, through involvement of both the innate and

adaptive immune system [19,29,30]. The oxidative damage to airway epithelial cells stimulates the influx of inflammatory cells, including eosinophils, neutrophils, mast cells, macrophages and T-cells, and results in the release of cellular mediators. The resulting increase in airway inflammation is linked to both airway hyperresponsiveness and airway obstruction for exposed individuals with asthma [1,31]. Conversely, the chronic inflammation resulting from persistent exposure is associated with the process of airway remodeling, another important feature of asthma pathogenesis [32,33]. Overall, the airway hyperresponsiveness, obstruction and remodeling are jointly responsible for the classic symptoms and signs associated with asthma morbidity.

Pediatric Asthma and Regional Ambient Air Exposures

Across different regions in the US, the concentrations and composition of ambient ECP vary widely. These variations reflect unique characteristics of local pollution sources including local and long-range sources like motor vehicular traffic emissions, combustion, agriculture and industrial activities, domestic fuel burning, and natural sources like dust, pollen and aeroallergens [13,34–36]; as well as differing geography, topography and weather conditions. Consequently, there is likely heterogeneity in the risk association that can be attributed to ambient exposures across these different regions. It is increasingly important to focus attention on delineating populations that may be at higher risk for adverse ECP effects regardless of population density or geographical location and identifying which exposures (and patterns/mechanisms of exposures) are most relevant for adverse asthma outcomes. Two region-based determinants of exposure are of particular interest:

The "Urban" Effect

One of the most important determinants of epidemiological studies considering the health effects of ECP in the last few decades has been the nature of the exposure characteristics in urban regions [19]. Urbanization has frequently been highlighted as a driver behind the association between ambient air pollution and asthma exacerbation [6,19,37]. Urbanization, in the context of exposure to an "urban", or "inner-city" environment, typically refers to geographic locations defined as census tracts in large cities [12,16,38]. However, the urban concept also encompasses a socioeconomic and demographic component, often including households below the poverty line with these regions indirectly conveying low socioeconomic status and minority race/ethnic groups in addition to population density [16]. Though there are numerous studies that focus on household or indoor exposure in urban environments [18,39,40], ambient exposures characteristic of urban regions have also been the focus of research. These ambient exposures tend to be related to motor vehicle traffic (from both primary and secondary pollutants) or industrial pollution sources [16,19]. While urban exposures present a substantial challenge to pediatric asthma morbidity, harmful ambient exposure characteristics are not limited to urban regions. Several ECP exposures in non-urban settings have been reported as harmful for children with asthma [13], moreso in populations with similar socioeconomic and demographic characteristics as urban regions [13,41]. Although the ambient air quality in rural areas are affected by fewer sources of urban pollution, several unique macro-environmental factors negatively influence respiratory health among rural-dwelling children with asthma [13]. These include emissions from industrial-scale agricultural operations [28,35], high concentrations of pollen and other common aeroallergens [42–44], and from biomass combustion for cooking and heating, a common practice that primarily affects

indoor air, but regional outdoor air quality as well [6,45]. Overall, the ECP-pediatric asthma morbidity associations in rural settings, compared to urban settings, pose a distinct, largely understudied, yet substantial public health problem.

The "Agriculture" Effect

Several studies have linked living in agricultural settings to reduced asthma incidence and morbidity, presumably shaped by exposure to allergens, hygiene (and the "hygiene hypothesis"), nutrition and local air pollution [46,47]. However, most research supporting this theory has been conducted in rural European farming communities, largely different from agricultural regions in the contemporary United States [48–51]. Further, the belief that rurality equates to residing on farms is unfortunately a common misconception in the US; most rural-dwelling children do not live on farms [13,52]. Moreover, living near farms or agricultural regions is not particular to children in rural communities, but characteristic of children across the rural-urban continuum.

Agricultural settings poses some unique ambient environmental exposures related to agricultural production. In addition to exposure characteristics related to regional urbanicity, agricultural communities may also have emissions from industrial-scale operations including windblown dust [36], pesticide drift from large crop-growing operations [35,44], and air pollution released from animal feeding operations [13,28,35]; all of which have been demonstrated to contribute to respiratory morbidity. These relationships have received far less attention among susceptible populations like children with asthma. Further, the nature of the health impacts from interactions between agricultural exposures and urban- or rural-specific ECP are largely uncharacterized.

Specific components of ECP most likely to cause pediatric asthma morbidity are briefly described below:

$PM_{2.5}$

$PM_{2.5}$ is a complex mixture of extremely small (diameter of less than 2.5 micrometers) particles and liquid droplets in the atmosphere originating from a variety of natural or anthropogenic sources, including windblown dust, gasoline- and diesel-powered motor vehicles, biomass combustion, industrial activities, wildfires and waste disposal [53]. Inhaled $PM_{2.5}$ deposits throughout the respiratory tract, and can penetrate to the lower respiratory system and contribute to oxidative stress and stimulation of inflammatory responses [25,54]. Numerous epidemiological studies provide a body of substantial evidence that suggests that ambient levels of $PM_{2.5}$ exacerbate existing pediatric asthma [8]; these associations have been observed in both cases of short- and long-term exposure [21,27,55,56], and for a variety of outcome measures including increased clinical symptoms and healthcare use [19]. Moreso, among children with or without asthma, improved lung function has been shown to be strongly associated with lower levels of $PM_{2.5}$ [57].

Ozone

Ozone (O_3) is a strong oxidizing agent formed in the troposphere through a complex series of reactions involving the action of sunlight on pollutant precursors such as nitrogen dioxide and volatile organic compounds, especially in warmer temperatures [19]. The dominant anthropogenic origin of O_3 is the combustion of fossil fuels [53,58], although downwind transport of ozone and ozone precursors is common [59]. Ozone exposure has been shown to result in airway inflammation, airway hyper-responsiveness, and decrements in lung function. The impact of ambient ozone pollution on respiratory morbidity endpoints (including pediatric asthma

exacerbation) has also been subject to intense study, resulting in substantial evidence of detrimental effects following both short-term and chronic exposures [26,58,60].

Nitrogen dioxide

In ambient conditions, nitrogen oxides are formed when atmospheric oxidants react with nitric oxide emitted from the combustion of fossil fuels from stationary sources (heating, power generation) and in motor vehicles [19,61]. Like O_3, nitrogen dioxide (NO_2) is an irritant gas and is believed to cause airway inflammation upon inhalation. NO_2 has also been shown to be associated with pediatric asthma exacerbations and reduced lung function in epidemiologic studies [62–64], although limited effects have been observed in experimental studies [19]. Further, the high degree of covariation between NO_2 and other outdoor air pollutants makes it difficult to interpret and infer on study results [53].

Pesticides

Pesticides are chemical products used to eliminate or control unwanted insects, plants, molds, and rodents [65]. In agricultural settings where crop and animal production activities produce airborne contaminants, agricultural pesticides are a public health concern due to their deleterious effects on health. Among the commonly studied pesticides, organophosphates (OP) and carbamates have been demonstrated to potentiate cholinergic action on airway smooth muscle and mucus-secreting epithelial cells, resulting in airway hyperactivity and increased mucus secretion [66]. Children are particularly at greater risk of adverse effects of pesticides because of developmental and physiologic factors [65,67,68]. Several studies have shown an association between agricultural

pesticides and symptoms of respiratory disease [69,70] or asthma exacerbation [71,72], frequently among adults in occupational settings and less so among children.

Ambient Air Exposure Mixtures: Multidomain, Multipollutant Health Effects

The effects of short- and long-term exposure to specific ambient air exposures on respiratory health and pulmonary function in children have been widely reported, especially among children with asthma. These studies have contributed to the evidence for deleterious health effects to a wide range of pollutants and chemicals including criteria air pollutants like particulate matter, ozone, and nitrogen dioxide [19,63], and non-criteria pollutants like ammonia [28,44], polycyclic aromatic hydrocarbons [73,74] and pesticides [22,75].

Most of these studies examine only single-pollutant effects on health outcomes; environmental epidemiology research has traditionally been limited to the analysis of the health effects of exposure to a single chemical or a group of similar chemicals at one time [76–78]. Although these studies are essential and have shaped the current regulatory atmosphere for ambient air pollutants, researchers also recognize that under normal ambient conditions, human ambient exposures almost always occur as a mixture. Therefore, the general consensus is moving toward an approach that embraces understanding the health impact of multiple environmental exposures [76,77,79,80].

Despite this shift toward a multipollutant approach, the preponderance of recent studies still center on examining the health effects resulting solely from exposures in one pollutant domain, usually looking at criteria pollutants [81–83]. Other multipollutant studies have exclusively assess the impacts from metals [84], polychlorinated biphenyls [85,86], and phthalates [87]. Very few

studies have implemented a multipollutant approach exploring the health effects of simultaneous exposure to diverse chemical classes within multiple exposure domains. The need for a more flexible (albeit refined) approach to multipollutant exposure assessment beyond unilateral domains of exposure necessitates the "multidomain" approach. The multidomain approach extends the multipollutant epidemiological framework to evaluate if exposure to multiple chemical classes are individually or jointly associated with specific health endpoints and may reflect more real-world exposures and account for the unique differences in types and sources of pollutants based on geography, urbanization, and social and demographic characteristics.

Regardless of approach, the foundation of any mixtures analysis is dependent on the research question. Using the framework suggested by recent reviews [88–90], we summarize the types of questions related to environmental exposure mixtures in epidemiology as follows:

1. *Considering the health effects of individual chemicals within a mixture*

   Quantifying the association between individual exposures/groups of highly correlated/related exposures with a common source and human health outcomes is pertinent to reducing the public health burden of disease. These research questions represent the simplest model forms and underlie most of the traditional approaches towards mixtures. Typically, separate (multiple) single-exposure models are used to quantify the association between each individual component (environmental agent) of the mixture and the health outcome, and inference on the health effects of the "mixture" are subsequently based on results from the single-exposure models. For example, Patel *et al.* assessed the role of multiple environmental chemical factors on lipid levels using several individual linear regression models, and postulated the implications of the broad class of

chemicals on lipid levels based on their findings [91]. The authors report identification and validation of their hypotheses with regard to complex relationships between serum lipid levels, and favorable and unfavorable environmental chemical factors, accounting for false discovery rate.

2. *Considering the interactions between chemicals within a mixture*

Another consideration is the interaction between components of the mixture; these types of studies explore the effects of the sum or product of component pollutants of mixtures. Interaction in this sense refers to interdependence of the effects of two or more environmental agents or exposures, and can be characterized as synergistic or antagonistic, depending on the direction of the combined effect [92]. For example, a study assessing the neurotoxic effects of exposure to metals using linear mixed-effects models with (product) interaction terms to determine increased lead toxicity among young children with high manganese co-exposure [93]. The authors found increased lead toxicity among children with high manganese co-exposure, providing potential evidence of an interaction between exposures.

3. *Considering the overall health effect of cumulative environmental exposure mixture*

A third question epidemiological studies can address is with regard to the estimation of the overall health effect of cumulative chemical exposure. These studies are usually based on prior research or strongly developed hypotheses guided by knowledge of a common pathophysiological/mechanistic pathway or toxicological evidence, and often go beyond simple 'copollutant' structures (as confounders) or basic additive effects [88,89]. For

example, Valeri *et al*. used Bayesian methods to examine the joint effect of prenatal exposure to metal mixtures on pediatric neurodevelopmental outcomes in Bangladesh [84]. In this study, the authors discovered evidence of neurotoxicity of ubiquitous metal mixtures (arsenic, lead, and manganese), as well as potential synergism between arsenic and manganese.

Adequate definition of the research question underlies the development or application of appropriate statistical methods that appropriately estimate the association between environmental exposure mixtures and respiratory health. In this dissertation, we structure our research questions to understand health impacts of environmental exposures in the context of the "total environment" approach [94] which is geared towards a more robust characterization of a range of concurrent exposures unique to a region or population.

Statistical methods for estimating health effects of multiple environmental exposures

In recent years, the environmental epidemiology research field has evolved to promote a research framework that considers the health effects of mixtures of ECP exposures or simultaneous effects of multiple ECP exposures, and that these research questions require more sophisticated methods than traditional "naïve" multipollutant models [90,95–98].

The mixtures workshop by the National Institutes of Environmental Health Sciences (NIEHS) in 2015 brought together a confluence of scientists including toxicologists, epidemiologists, biostatisticians, regulators, and exposure scientists, to explore statistical methods for better characterization of health-related effects of environmental chemical mixtures [98]. This workshop provided a number of proposed statistical methods which were summarized as classification and

prediction, exposure–response surface estimation, variable selection, and variable shrinkage strategies [98].

Similarly, a small collection of epidemiologic research articles have also attempted to summarize the available methods for the health risk-based multipollutant approach [76,83,89,90,99,100]. For example, Davalos et al. [83], conducted a review of air pollution epidemiology studies and categorized available methods based on pollutant mixture relationship specification. Stafoggia et al. [90], also recently provided a classified summary of recently proposed statistical methods to handle multipollutant mixtures and multiple exposures; their approach to classification was broadly based on statistical techniques involved in the method. As with proceedings from the NIEHS workshop, there was no consensus regarding what method is appropriate in either of these studies. The literature suggests that the choice of health risk-based multipollutant approach should be guided by the research question and goals, available data and statistical resources, and with contextual perception of the methodological strengths and limitations [77,83,88,99]. Nevertheless, the main statistical challenges in health risk-based multipollutant approaches include:

1. The ability to address collinearity or correlated variable.
2. The ability to handle interaction between mixture component, including complex and possibly non-additive interaction.
3. The ability to correctly describe the nature of the association between the exposures and the health outcome, in the presence of multidimensionality or non-linear relationships.

Table 1.1 provides a summary of statistical methods using classification provided by the NIEHS workshop "Statistical Approaches for Assessing Health Effects of Environmental Chemical Mixtures in Epidemiology Studies" [98].

As previously stated, the choice of statistical method is largely dependent on the study question (including knowledge of the exposure-outcome and exposure-exposure associations), and characteristics of the available data. For example, one approach considered for mixtures analysis is recursive partitioning using classification and regression trees (CART); this method allows for the analysis of a large number of component exposure variables (even more than the number of observations), detection of complex interactions, and handle multicollinearity [83,101]. However, CART models are limited in their inability to produce exposure-effect estimates, and are not useful for research questions that require quantitative coefficients (the magnitude or direction of the association) [101,102].

To our knowledge, the limited number of studies that considered a multipollutant approach towards assessment of pediatric respiratory health effects of ambient ECP employed traditional regression methods (including co-pollutants as co-exposures in regression models [26,103,104], or/and interaction terms [81]), dimension reducing cluster analysis [105], or classification and prediction methods (CART-based recursive partitioning [101], and automated algorithms [106]). Health risk-based multidomain exposure studies are a reasonably novel approach and hence much more limited; to our knowledge, no epidemiological study has used the multidomain approach to assess pediatric respiratory health effects of ambient ECP.

TABLE 1.1 SUMMARY OF STATISTICAL TOOLS USED TO STUDY ENVIRONMENTAL EXPOSURE MIXTURES

NIEHS Workshop Category*	Supervised	Short characterization	Application in environmental epidemiology mixtures	Research Question Answered		
				Individual[a]	Interactions[b]	Overall health effect[c]
Classic linear regression (ordinary least squares)	No	The classic linear regression models are used to explore/describe the relationship between a response and a set of variables, with model parameters typically typically estimated from a sample of observations using the ordinary least squares (OLS) criterion.	-	x	x	x
Dimension Reduction	No	These methods transfom mixture concentrations into a smaller set of variables that are then used to represent exposures to various pollutant combinations or sources.	[97,98]			x
Dimension Reduction	No		[99,100]			x
Dimension Reduction	Yes		[101]			x
Dimension Reduction	Yes		[96]	x		x
Dimension Reduction	Yes		[102]	x		x
Variable selection and shrinkage strategies	Yes	Methods for variable selection aim to identify the "best" sub-set of component exposure variables either based on their mutual correlation (unsupervised) or on their relation with the study outcome (supervised approach). Shrinkage then usually involves fitting a regression model under some	[103,104]	x		x
Variable selection and shrinkage strategies	Yes		[104]	x		x
Variable selection and shrinkage strategies	Yes		[105]	x		x
Variable selection and shrinkage strategies	Yes		[83,106]	x	x	
Classification and prediction	Yes	These methods include parametric and nonparametric techniques which categorize patterns of exposure based on algorithms that identify observations with similarities to exposure components or the health outcome.	[107]	x	x	
Classification and prediction	Yes		[108]	x	x	
Classification and prediction	Yes		[109]	x	x	
Classification and prediction	Yes		[110]	x	x	
Classification and prediction	Yes		[111,112]	x	x	x
Exposure–response surface estimation	Yes	In these methods, a response is regressed on a weighted sum of measures between exposure mixtures observations. A kernel function is used to define the measures, and its specification in turn defines the properties and form of the response surface.	[93,113]	x	x	x

In a broader review of epidemiologic studies that examine the associations between air pollutants and health effects, we note two studies that employed the multidomain approach. Czarnota et al. used weighted quantile sums regression models (WQS), a variable selection and shrinkage startegy, to estimated the association between 27 correlated environmental chemicals (in different chemical domains) measured in residential carpet dust and non-Hodgkin lymphoma [107]. More recently, Zhang and colleagues assessed the joint effect of domains of environmental phenols, pesticides, and phthalate on obesity metrics using three models (traditional linear regression model, Bayesian Kernel Machine Regression (BKMR), and WQS) [108]. Both studies

note that the exposure patterns within environmental chemicals are complex with high correlation and complicated interactions. Their use of the multidomain approach represented a tailored startegy to assess the simultaneous health effects of multiple chemicals beyond the context of single-chemical risk or total exposure within specific chemical groups.

Given the complexities associated with understanding the association between health impacts of exposure to complex mixtures of ambient ECP, especially in regions with distinct geographical and urbanization characteristics, this dissertation presents the exploration of practical multidomain approaches towards disentangling health risk-based multipollutant research questions. *Specifically, we sought to understand the relationship between agricultural pesticides and ambient air pollutants with pediatric respiratory outcomes through the development and validation of multiple tailored epidemiological methods for multipollutant research.*

We implemented health risk-based multidomain, multipollutant approaches in three case studies reflecting signals of exposures along a rural/urban gradient:

- The Yakima Valley region of central Washington State is mostly rural and is characterized by a high density of agricultural operations including farmlands for crop production as well as animal confinement facilities.
- Fresno is located in the San Joaquin Valley (SJV) near the southern end of the Central Valley of California. The SJV is a richly productive agricultural region and is notorious for poor air quality, with high levels of urban-generated ozone and particulate pollution.

- The Colorado Front Range is a largely urban, densely populated area with a unique mixture of urban sources of air pollutants (i.e. traffic and light industrial), extensive oil and gas production and concentrated agriculture feed operations.

The Aggravating Factors of Asthma in a Rural Environment (AFARE) study

The Aggravating Factors of Asthma in a Rural Environment (AFARE) project was a longitudinal cohort study of pediatric asthma set in the Yakima Valley region of eastern Washington State [28,109]. The region consists of a high density of large-scale agricultural operations including farm lands for the production of apples, corn, grapes and other crops, and farm animal confinement facilities, predominantly large dairy operations, largely concentrated in the southern half of the valley. In 2016, residents in Yakima County have an estimated inflation-adjusted per capita income of $40,588 compared to $54,579 for Washington State overall, and $49,246 for the US [110]. More than a fifth (22.3%) of residents live below the poverty level (31.7% of all children), in comparison to 12.9% for the state (18.3% of WA state children). This region is also characterized by a high proportion of the individuals who identify as Hispanic/Latinx (46.3%), four times higher than the Washington State average [110]. The Yakima Valley agricultural industry is highly dependent on a labor force composed mainly of migrant and seasonal farmworkers, many of whom are first- and second- generation immigrants from Mexico and other Latin American countries [111].

The AFARE study collected longitudinal data to explore and identify environmental factors that exacerbate pediatric asthma in rural settings. School-age children with asthma were recruited into the study provided they had no serious illnesses other than asthma and intended to stay in the

region during the proposed study duration. As part of a sub-cohort to examine exposure to organophosphate pesticides among participants, 16 children (31.4% of total cohort population) who participated for at least 3 months during the period of urinary pesticide and air monitoring (between September 2011 and October 2012) were considered. A baseline health history survey was conducted at enrollment to determine demographic and health information, as well as clinical features of asthma status including medication use. All subjects also underwent skin prick testing to identify children with atopy, and urinary biomarkers of OP exposure were assessed at multiple study intervals.

The AFARE study represents a unique opportunity to study ambient environmental exposures associated with asthma exacerbation in an agricultural community.

The Fresno Asthmatic Children's Environment Study (FACES) study

The Fresno Asthmatic Children's Environment Study (FACES) is a well-characterized, closed longitudinal cohort study of children with asthma in California's Central Valley. The cohort, initiated in 2000, is comprised of 315 children with a physician's diagnosis of asthma recruited between ages 6 – 11 years old [21].

The Fresno and Clovis region in the San Joaquin Valley of California has a population of about 522,053 and is one of the most productive agricultural regions in the U.S. with large territories devoted to agriculture and animal husbandry. The region is also traversed by a major transportation corridor – two major interstate highways, the California State Highway 99 (CA-99) from northwest to southeast and Interstate 41 (I-41) from north to south. Fresno is also to the

east of Interstate 5 (I-5) which is the major north-south interstate in the West Coast of the United States, and as with the CA-99, there is heavy-duty diesel traffic that tends to drifts east to the SJV [112]. Consequently, Fresno currently ranks as one of the most polluted cities in the United States in terms of 24-hour average PM2.5 and 8-hour maximum ozone levels [113]. Fresno also has a relatively high prevalence of asthma and asthma hospitalizations compared to state and national averages [114].

Extensive details on recruitment, health evaluations, environmental measurements and characteristics of the FACES cohort are available in detail [21,115]. Briefly, children were recruited through school nurses, advertisements, physicians' offices, and local media, and eligible children (between 6 and 11 years old, active physician-diagnosed asthma, resided within 20 km of the California Air Resources Board air quality monitoring site in Fresno) were followed from 2000 to 2008 with assessment of several local exposure factors including aeroallergens, secondhand tobacco smoke, particulate matter constituents including PAHs, and criteria gaseous air pollutants (NO_2, SO_2, CO, and O_3). Outcome measures included spirometry, wheeze and markers of asthma severity. Detailed information on children's lifetime residential addresses was also obtained.

Although FACES originally collected data to explore the effects of ambient air pollution on children with asthma, data from this cohort represents a unique opportunity to investigate the independent and joint effects of ambient pollutant exposures in this young, susceptible population. The availability of historical addresses for cohort participants allows for the temporal characterization of ambient air pollutant exposures; similarly, the California Pesticide Use Report (PUR) affords us data on commercial agricultural pesticide use, to advance understanding of the

respiratory health impact of pesticides among these children. The California PUR, one of the most comprehensive databases of its kind, has been used as a surrogate of pesticide exposure in a number of epidemiologic studies [116–118], and has been described extensively elsewhere [119–121]. Briefly, the California PUR has required full reporting of agricultural pesticide use since 1990; PUR documents are completed monthly by growers, including information on where, when, and how agriculture pesticides are applied throughout the state.

As such, the SJV region provides another unique opportunity to explore the pulmonary health effects of multidomain environmental mixtures in a geographically diverse cohort (ranging from rural to urban).

The Front Range Ozone + Environment (FROZ+EN) study

The Northern Front Range Metropolitan Area (NFRMA) of Colorado is a geographical region between the North Front Range and the Denver Metro area, where more than 80% of the state's population reside or work within the cities of Denver, Boulder, Longmont, Greeley, and Fort Collins [122]. The air pollution mixture in the NFRMA is unique due to the assortment of urban sources of air pollutants (i.e. traffic and light industrial), concentrated agriculture feed operations, extensive oil and gas production, and regional meteorological conditions [123,124]; all of which create an ambient gas and aerosol pollutant mix that are believed to adversely impact respiratory health [123]. Ozone is particularly problematic in the region. The entire NFRMA has been an EPA ozone nonattainment area since 2008, as summer ozone levels have consistently exceeded regulatory

standards over the last decade despite efforts to limit emission [123–126]; the most recent period of increase has been attributed to increases in local ozone precursor emissions [127]. Further, the dynamics of this region provide atmospheric conditions suitable for production and accumulation of particulate matter and ammonia [123]. During summer months, in particular, smoke from local wildfires (and fires located hundreds of miles upwind) create ambient air pollution mixtures [128], some of which may linger for weeks [129].

The Colorado Department of Public Health and the Environment (CDPHE) provides respiratory hospitalization data from over 80 Colorado Hospital Association (CHA) affiliated hospitals covering the NFRMA region, including daily counts of unscheduled hospitalization related to COPD, pneumonia, asthma, upper respiratory infections and all/combined respiratory diseases. For the FROZ+EN study, these data were available for a 5-year period from 2010 – 2014.

Over the summer of 2014, two atmospheric chemistry airborne field measurement campaigns, the Front Range Air Pollution and Photochemistry Experiment (FRAPPÉ) and the NASA DISCOVER-AQ project, were carried out to study influence of local sources of summertime atmospheric pollution in the NFRMA [123,130]. These campaigns provided in situ size-resolved measurements of an extensive suite of ambient aerosols, resulting in a comprehensive dataset of highly spatially resolved summertime air pollutants [130]. Along with publicly-available fixed ground-level monitors, the aircraft measurements (though limited to a four-week period, 20 July – 18 August, 2014) served to quantify the spatial distribution of atmospheric pollutants over the five-year study period with high accuracy due to the guided and extensive spatial coverage, and reliable repeated measurements [131,132].

These unique characteristics of the NFRMA provide validation for the need to better understand the health effects of co-exposure to pollutants in a region that typifies the agricultural-urban interface.

Dissertation objective and specific aims

The overall objective of this dissertation was to evaluate the association between exposure to ambient ECP and pediatric asthma morbidity. *We hypothesized that joint exposure to multiple ambient environmental exposures would result in adverse respiratory health outcomes.* In addition to modeling multidomain exposure-outcome relationships using the various statistical approaches, we also explore the use of biomarkers as exposure and outcome measures and extend a method of regression calibration to estimate pollutant exposure.

Three specific aims were proposed to provide insight into a variety of populations across rural, agricultural, and urban regions in the US, with unique exposure (component pollutants and ambient mixtures) and outcome characteristics. In each study region, we explored multipollutant approaches, including some novel component pollutants (across multiple domains of ECP exposure) related to adverse respiratory health.

In Aim 1, we used longitudinal data analysis methods to evaluate the effects of short-term exposure to two criteria air pollutants (ozone and $PM_{2.5}$) on a biomarker of asthma exacerbation, in the context of contemporaneous exposure to organophosphate pesticides. The multipollutant approach in this aim involved creating median-dichotomized joint exposure variables for the

criteria pollutants and pesticides, and then using these aggregated variables as categorical independent variables in generalized estimating equations models.

In Aim 2, we implemented Bayesian kernel machine regression (BKMR) to evaluate the pulmonary health effects of a multidomain, multipollutant mixture for a susceptible group of children in the SJV region.

In Aim 3, we evaluated the association between short-term exposure to ozone and $PM_{2.5}$, and unscheduled asthma-related hospitalizations using Poisson regression models. We also explored the use of regression calibration to generate highly resolved spatial exposure indices.

Specific Aim 1**:** Evaluate the independent and joint effects of short-term exposure to organophosphate pesticides and two criteria air pollutants ($O_3$and $PM_{2.5}$) on leukotriene Ɛ4 (LTE4) levels among a cohort of children with asthma in Yakima Valley, a rural agricultural region of Washington State.

<u>*Hypothesis*</u>: *1) Short-term exposure to organophosphate pesticides (OPs) is independently associated with an increase in levels of leukotriene E4 (LTE4), a marker of pulmonary inflammation. 2) Short-term cumulative exposure to ambient criteria pollutants (ozone and $PM_{2.5}$) and OPs is associated with increases in pulmonary inflammation (LTE4).*

Specific Aim 2**:** Evaluate the joint effects of 3-month exposure to a suite of ambient air pollutants ($PM_{2.5}$, PM_{10}, O_3 and NO_2) and pesticides (organophosphates, carbamates and methyl bromates) on lung function at recruitment (measured by spirometry) among a

cohort of children with asthma in Fresno, California using Bayesian Kernel Machine Regression.

Hypothesis: Among children with asthma, recent cumulative exposure to a combination of pollutants sets (ambient air pollutants and pesticides) is associated with decreased lung function measured at study recruitment.

Specific Aim 3**:** To evaluate the association between independent and joint short-term exposure to O_3, $PM_{2.5}$ and unscheduled respiratory hospitalizations in the Front Range of Colorado using Poisson regression methods, adjusting for potential exposure measurement error using regression calibration.

Hypothesis: 1) Estimates of ozone vary by exposure assessment method (airborne vs. fixed-site monitor assessment), and 2) Short-term exposure to ozone and fine particulate matter is associated with increase in unscheduled asthma-related hospitalizations in the NFRMA.

CHAPTER 2: THE AFARE STUDY

CHAPTER 2A: ASSOCIATION OF ORGANOPHOSPHATE PESTICIDE EXPOSURE AND A MARKER OF ASTHMA MORBIDITY IN AN AGRICULTURAL COMMUNITY

SUMMARY

Objectives We explored the short-term impact of pesticide exposure on asthma exacerbation among children with asthma in an agricultural community.

Methods We obtained repeated urine samples from a subset of 16 school-age children with asthma (n = 139 samples) as part of the Aggravating Factors of Asthma in a Rural Environment (AFARE) study cohort. Biomarkers of organophosphate (OP) pesticide exposure (dialkylphosphates (DAPs)), and asthma exacerbation (leukotriene E4 (uLTE4)) were assessed in urine samples. We used generalized estimating equations to examine the association of summed measures of creatinine-adjusted DAPs (total dimethyl alkylphosphate (EDM), total diethyl alkylphosphate (EDE), and total dialkylphosphate pesticides (EDAP)) and uLTE4 concentration, adjusting for multiple confounders, yielding beta-coefficients with 95% CIs.

Results A total of 139 observations were obtained from the 16 children over the study period, the total number of samples per subject ranged from 1 to 12 (median: 10.5). The geometric mean (GM) of creatinine-adjusted EDE, EDM and EDAP in this population were 81.0, 71.8 and 168.0 nmol/g respectively. Increase in uLTE4 levels was consistently associated with increased exposures to DAPs (interquartile range in μg/g): β_{EDE}: 8.7 (95%CI: 2.8, 14.6); β_{EDM}: 1.1 (0.5, 1.7); β_{EDAP}: 4.1 (0.7, 7.5).

Conclusion This study suggests that short-term OP exposure is associated with a higher risk of asthma morbidity, as indicated by increased uLTE4 levels in this cohort of children with asthma in an agricultural community. Additional studies are required to confirm these adverse effects, and explore the mechanisms underlying this relationship.

INTRODUCTION

Pediatric asthma affects an estimated 8.3% of children in the US and is often complicated by acute exacerbation episodes [9]. These exacerbations account for over 700,000 childhood emergency department (ED) visits annually, with almost 5% of children with asthma requiring hospitalization [9,133].

Environmental pollutants are established risk factors for asthma exacerbation [19,134]; the effects of these exposures have been extensively studied, particularly in urban and inner-city asthma populations [16]. Though asthma prevalence in U.S. rural communities is comparable to urban communities [13], the effects of these exposures on exacerbation among children living in rural and agricultural areas have received less attention because of apparent poorer air quality in urban areas [19]. However, pollutant sources in rural/agricultural communities tend to differ from sources in inner-city/urban communities [13,34]; agricultural communities may have fewer urban pollution sources (e.g. motor vehicle traffic and industrial emissions), but have substantially more local sources like pollen and similar aeroallergens, emissions from industrial-scale agricultural operations including windblown dust, animal agricultural emissions and pesticide drift from large crop-growing operations [35].

Organophosphate pesticides (OPs) are widely used in crop production, and exposure levels among children in agricultural communities exceed their non-agricultural counterparts [135]. OP

exposure levels may reflect off-target drift, parent take-home exposure, and exposure through consumption of local food crops [67]. OPs, particularly at high acute doses, result in acetylcholinesterase (AChE) dysfunction [136], and can affect respiratory function with resulting airway hyperreactivity and inflammation [136,137]. Low level, chronic exposures may also affect respiratory conditions [138,139].

Several studies have explored the relationship between exposure to OPs and adverse respiratory health outcomes including asthma [140–142]; the few studies investigating these relationships among children have been based on self-reported exposure metrics [143,144], and early-life exposures [22,145]. However, characterization of short-term exposures among children with asthma and potential exacerbation has been lacking.

We describe results of a longitudinal study of children with asthma who reside in an agricultural region of Washington State characterized by extensive organophosphate insecticide use. This well-defined cohort of children provides a unique opportunity to characterize longitudinal variation in exposure to OPs using well established metabolite biomarkers alongside an emerging biomarker of asthma exacerbation, urinary leukotriene (uLTE4). Noninvasive measurement of uLTE4 has been validated as a marker of systemic cysteinyl leukotriene activity, and an indirect marker of lung cysteinyl leukotriene activity (a lipid mediator known to play a central pathophysiological role in asthma) [146,147].

The primary objective of our investigation was to describe the association between short-term variations in OP exposure and this measure of asthma exacerbation among children with asthma.

METHODS

Study Population

The Aggravating Factors of Asthma in a Rural Environment (AFARE) project was conducted in the Yakima Valley, an agricultural region of Washington State characterized by a high density of fruit crops, vegetables and large dairy operations. Pesticides commonly used in the region include OPs such as phosmet, diazinon, malathion and chlorpyrifos [148,149].

Details about recruitment and baseline health evaluations for AFARE have been reported previously [28]. Briefly, the study involved collection of longitudinal data to explore and identify environmental factors that exacerbate pediatric asthma in rural settings. The children were of school age (between the ages of 6 and 16 years) at baseline and had no serious illnesses other than asthma. For this analysis, we used the repeated measures made on a subset of children for which urine specimens were collected in the AFARE cohort (n = 16), at six-day intervals over a four-month period (July 2012 to October 2012). These participants were chosen based on residential proximity to home-based air monitors used in the main study, as urine samples were collected during trips to attend to monitors.

Outcome Assessment

Urinary Leukotriene E4 Monitoring

Urinary leukotriene E4 was used to assess acute inflammation among children with asthma. ULTE4 was measured from a spot urine sample, scheduled to be collected from each child every 6 days during the study period. After collection, samples were stored at -20°C. Quantitative analysis of uLTE4 was performed in the University of Washington Department of Environmental and

Occupational Health Studies Functional Genomics Laboratory using the Cayman Human Leukotriene E4 EIA Kit (Cayman, Ann Arbor, MI) according to the manufacturer's instructions. Urinary creatinine concentration was measured for each sample to account for urine dilution. Creatinine analysis was conducted by the Department of Laboratory Medicine at the University of Washington. Creatinine-adjusted concentrations were used for final model analyses.

Assessment of Pesticide Exposure

Organophosphate Pesticide Monitoring

Dialkylphosphates in urine result from the degradation of organophosphate pesticides, and primarily reflect a recent exposure; OP pesticides are quickly metabolized and excreted, with average half-lives of about 48 hours [150]. Spot urine samples were analyzed for organophosphates at the same time uLTE4 was assessed. Six urinary DAP metabolites were measured in participants' urine; dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). Metabolite reporting limits were based on the limit of detection (LOD) for each DAP compound, and masses below the limit of detection were approximated as LOD/√2. Urinary creatinine concentration was measured to account for urine dilution.

To provide an indicator of total exposure to OPs, we used summative measures of DAPs for evaluation of associations with the outcome, rather than measures of individual analytes [75]. Summed measures account for circumstances where individual OP pesticides devolve to more than one DAP metabolite [151]. Summed DAP concentrations (total dimethyl alkylphosphate (EDM), total diethyl alkylphosphate (EDE), and total dialkylphosphate pesticides (EDAP)) were estimated by summing up molar concentrations (DAP concentration divided by their molecular

weights) of metabolites. Creatinine-adjusted concentrations of EDM, EDE and EDAP were used for final model analyses.

Covariates

Covariates in our models included potential confounders that were selected a priori based on existing evidence of relationships between the covariate and both respiratory health and ambient exposure to pesticides. Informed by previous studies, subject-specific characteristics potentially associated with asthma and asthma exacerbation were also considered: sex, age, body mass index-for-age, atopy (skin prick test positive to at least one common aeroallergen), use of inhaled corticosteroids at baseline, a measure of pulmonary inflammation collected at baseline (exhaled nitric oxide (eNO) levels), and the number of individuals in household.

Meteorological conditions have been shown to effect asthma morbidity [152], and influence the potential for exposure to pesticides through various routes including an influence on exposure dose due to behavioral changes or the physiochemical properties of pesticides [121,153,154]. To capture meteorological conditions for the week prior to uLTE4 measurements, we used data downloaded from the countyweather package in R which provides data from NOAA's Global Historical Climatology Network on 24-hour average, maximum and dew-point temperature, precipitation, and wind speed.

We explored both forward addition and backward elimination of the covariates to fit the base models, and covariates were included in final models if they substantively influenced effect estimates (≥ 10% change in beta coefficients).

Statistical Analysis

Descriptive statistics were examined, including proportions and means of sociodemographic characteristics of children in this AFARE sub-cohort, exposure to OP pesticides, and uLTE4 levels.

To understand the potential exposure differential to pesticides in an agricultural population, we conducted a comparison of adjusted geometric mean levels of DAPs between children in the AFARE cohort and children of similar age participating in the National Health and Nutrition Examination Survey (NHANES) 2007/2008 survey cycle (a national population-based comparison group) [155], using the Wilcoxon rank-sum test. The 2007/2008 NHANES sample included 476 children between the age of 9 and 17 years; no sample weights were added to the NHANES data.

We evaluated associations of uLTE4 with OP metabolite concentrations using generalized estimating equations (GEEs) with an exchangeable correlation matrix. The use of GEE accounts for the correlation and lack of independence of observations observed in this panel study by using quasi-likelihood methods and fairly robust variance estimators [156]. Although GEE calculation of standard errors are fairly robust to the choice of correlation matrix, we explored associations using both the exchangeable (assuming all of the correlations are equal) and autoregressive (decreasing correlation for farther time periods) working correlation structures.

We fit three separate regression models for the effect of summed measures of dimethyl alkylphosphate (EDM), diethyl alkylphosphate (EDE), and all dialkylphosphate pesticides (EDAP) on uLTE4, adjusted for the set of selected confounders. In all models, the mean outcome was modeled as the linear response to the primary exposure of interest.

We presented the results in models as effect sizes per interquartile range increases in exposure for comparability among the summed pesticide measures.

RESULTS

Study Population

Baseline demographic and general health characteristics are presented in Table 2.1. Sixteen children were included in our analysis. The average age at baseline was 12 years (SD: 3), 56.3% were male, and nearly all (93.8%) self-identified as Hispanic/Latinx. Most of the children resided in-town (81.3%), 56.3% were from low-income families, and about 87.5% relied on public health insurance/aid. Approximately 69% of the children were taking corticosteroid medication at the time of enrollment, and 12 children (75.0%) were identified to be skin prick positive to at least one aeroallergen. Based on a clinical exam performed at baseline, more than half of the subjects (56.3%) were classified as overweight, defined as body mass index-for-age above the 85th percentile.

We compared the subset of participants selected for our study with the main AFARE cohort (n=58), and found no significant differences in attributes likely to modify the exposure-outcome association (Appendix Table 1). Compliance with collection of urine samples varied: the total number of samples per subject ranged from 1 to 12 (median: 10.5). A total of 139 observations were obtained from the 16 children over the study period. One observation was excluded because of incomplete data, resulting in 138 observations for analyses. The median level of creatinine-

adjusted uLTE4 among participants was 84.8 µg/g creatinine (geometric mean: 84.8 µg/g) over the study period.

TABLE 2.1 AFARE SUB-COHORT CHARACTERISTICS (N = 16)

Variable	Level	n (%)
Sex	Male	9 (56.3)
Birth country	US	11 (68.8)
	Other	5 (31.3)
Ethnicity	Hispanic/ Latinx	15 (93.8)
Income	≤ $15k/year	9 (56.3)
Residence	In Town/Urban	13 (81.3)
	Rural	3 (18.8)
Total number of household members	< 5	5 (31.3)
	≥ 5	11 (68.8)
Insurance	Public Insurance or Aid	14 (87.5)
	Private Insurance/Self	2 (12.5)
Skin prick test positive (atopy)	Yes	12 (75.0)
Inhaled corticosteroid use	Yes	11 (68.8)
BMI for age (85th percentile)	Above	9 (56.3)
Age (years)	Median (IQR)	11.0 (4.0)
Baseline fractional exhaled nitric oxide level (ppb)	Median (IQR)	12.0 (12.0)
Creatinine adjusted Leukotriene E4 level (µg/g creatinine)	Median (IQR)	84.8 (73.3)

Pesticide Exposure Levels

OP metabolite concentrations are summarized in Table 2.2. Percentage above the limit of metabolite detection ranged between < 0.7% and 64.0%; DMTP and DMP were the most prevalent DAP metabolites (detected in approximately 64.0% and 35.3% of participants respectively), whereas DEDTP was the least prevalent metabolite (detected in < 0.7% of participants). The geometric mean (GM) of creatinine-adjusted EDE, EDM and EDAP were 81.0, 71.8 and 168.0 nmol/g respectively, and the two summed measures of DAP (EDE and EDM) were moderately correlated ($\rho = 0.5$; $p = 0.1$, Figure 2.1).

TABLE 2.2 CONCENTRATIONS OF ORGANOPHOSPHATE PESTICIDE URINARY METABOLITES IN STUDY POPULATION

Pesticide Metabolite (ng/mL)			Unadjusted (nmol/L)					Creatinine adjusted (nmol/g creatinine)				
	LOD	Percent > LOD	Mean	GM	Median	IQR	Max	Mean	GM	Median	IQR	Max
DEP	1.0	28.8	6.1	4.9	3.5	2.5	31.0	5.4	3.9	3.5	4.3	57.7
DETP	1.0	28.8	1.6	1.0	0.7	0.3	28.0	1.5	0.8	0.7	0.8	20.5
DEDTP	1.0	0.7	0.7	0.7	0.7	0.0	1.0	0.7	0.6	0.5	0.4	11.5
DMP	5.0	35.3	10.2	5.9	3.5	4.5	177.0	9.5	4.7	3.7	5.3	273.8
DMTP	5.0	64.0	11.1	4.4	4.0	5.3	384.0	10.9	3.5	2.6	4.1	594.0
DMDTP	2.5	18.7	2.4	1.0	0.7	0.0	65.0	2.2	0.8	0.6	0.7	43.2
EDE	-	-	-	-	-	-	-	125.4	81.0	71.2	79.9	1324.9
EDM	-	-	-	-	-	-	-	166.2	71.8	58.3	77.5	6591.1
EDAP	-	-	-	-	-	-	-	291.6	168.0	142.9	197.3	6696.8

• **Abbreviations**: LOD, limit of detection; GM, geometric mean; IQR, interquartile range; DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; DMDTP, dimethyl dithiophosphate; DEP, diethyl phosphate; DETP, diethyl thiophosphate; DEDTP, diethyl dithiophosphate; EDE, diethyl alkylphosphates; EDM, dimethyl alkylphosphate; EDAP, total dialkylphosphate.
• Metabolites were adjusted to creatinine standards of 100 mg/dl.

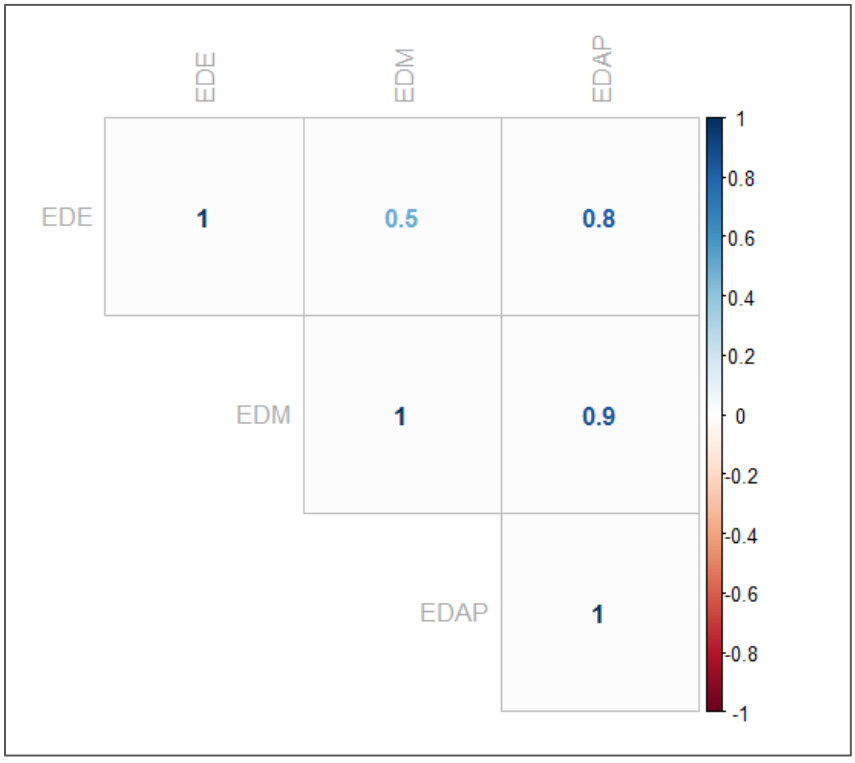

FIGURE 2.1. SPEARMAN'S CORRELATIONS BETWEEN SUMMED ESTIMATES OF DIALKYLPHOSPHATE (DAP) CONCENTRATIONS.
Abbreviations. EDE, diethyl alkylphosphate; EDM, dimethyl alkylphosphate; EDAP, total dialkylphosphate

Comparison with NHANES data

Figure 2.2 presents creatinine-adjusted geometric means and surrounding 95% confidence intervals for the total diethyl and dimethyl phosphate molar concentrations for children from our AFARE study and the NHANES study population. The distribution of summed DAP metabolite levels for children in the AFARE study were significantly higher than NHANES levels, with the Wilcoxon rank-sum test with continuity correction for comparing geometric means for all three measures resulted in p-values less than 0.05. The geometric mean of summed concentration of DAPs in the AFARE cohort were over 1.5 times that of the NHANES population (EDE: 81.0 vs. 53.1 nmol/g, EDM: 71.8 vs. 40.1 nmol/g, and EDAP: 168.0 vs. 110.6 nmol/g; all $p < 0.05$).

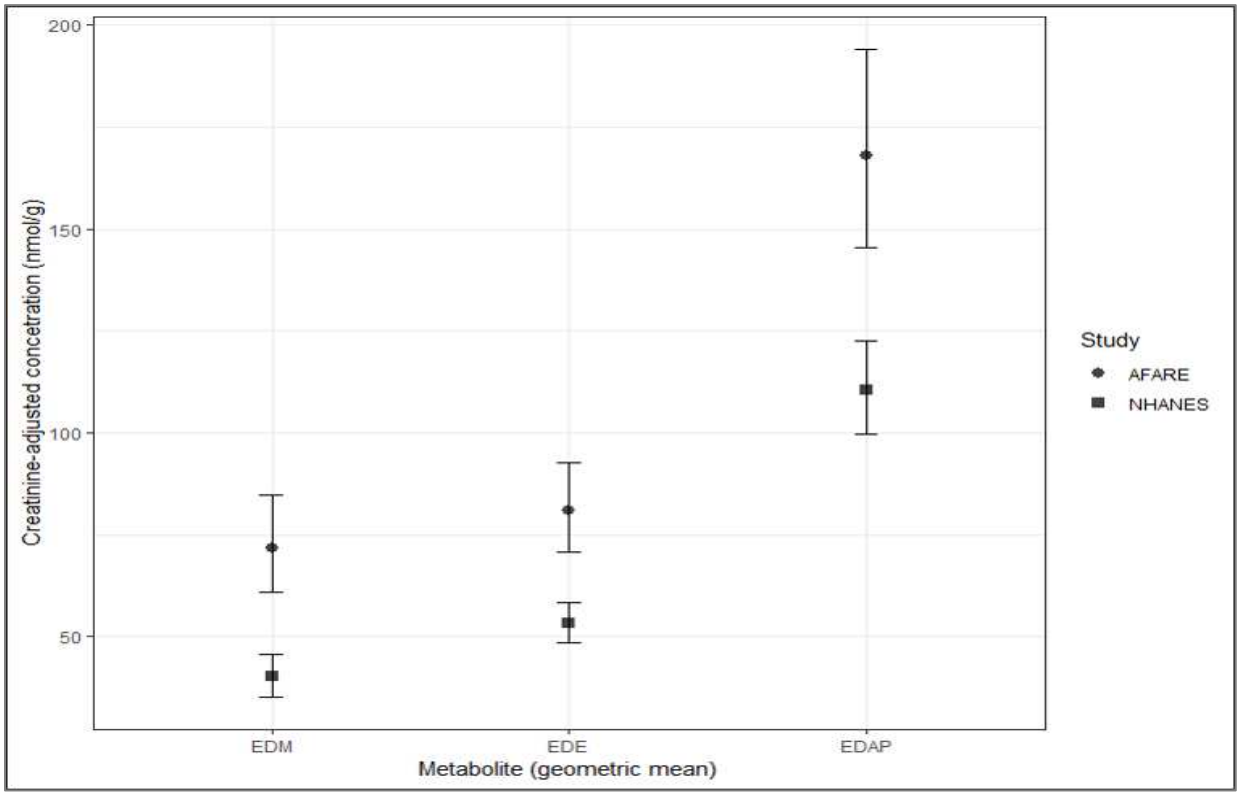

FIGURE 2.2. COMPARISON OF CREATININE-ADJUSTED SUMMED DAP METABOLITE DISTRIBUTIONS FROM AFARE CHILDREN WITH DISTRIBUTIONS OBSERVED IN CHILDREN IN NHANES.
Abbreviations. EDE, diethyl alkylphosphate; EDM, dimethyl alkylphosphate; EDAP, total dialkylphosphate; AFARE, Aggravating Factors of Asthma in a Rural Environment; NHANES, National Health and Nutrition Examination Survey.

Relationships between DAP concentrations and uLTE4

Regression analyses demonstrated that IQR increases in the creatinine-corrected urinary concentration of summed DAPs (EDE: 79.9 nmol/g; EDM: 77.5 nmol/g; and EDAP: 197.3 nmol/g) was associated with increases in urinary LTE concentrations (β_{EDE}: 9.3 (95%CI: 1.7, 16.9); β_{EDM}: 0.9 (0.3, 1.6); β_{EDAP}: 3.9 (-0.1, 7.9) all in µg/g creatinine, Table 2.3). These observed associations persisted after adjusting models for age, use of inhaled corticosteroids, allergy, maximum temperature and wind speed (β_{EDE}: 8.7 (95%CI: 2.8, 14.6); β_{EDM}: 1.1 (0.5, 1.7); β_{EDAP}: 4.1 (0.7, 7.5) all in µg/g creatinine).

TABLE 2.3. OBSERVED INCREASE IN CREATININE-ADJUSTED UTE4 LEVELS PER IQR INCREASE IN PESTICIDE EXPOSURE

Exposure	Urinary leukotriene E4 concentration (µg/g creatinine)	
	unadjusted β (95% CI)	adjusted β (95% CI)
EDE	8.7 (2.8, 14.6)	< 0.05
EDM	1.1 (0.5, 1.7)	< 0.05
EDAP	4.1 (0.7, 7.5)	< 0.05

- Separate models were fit for each exposure measure.
- Adjusted models included age, atopy (skin prick test positive to at least one common aeroallergen), use of control medication, wind speed and temperature.
- Abbreviations. EDE, diethyl alkylphosphates; EDM, dimethyl alkylphosphate; EDAP, total dialkylphosphate.

DISCUSSION

Pesticides may be overlooked contributors to the risk of asthma and asthma exacerbation, especially among children. Our study provides novel evidence on the role of organophosphate pesticides as acute environmental triggers for worsening asthma morbidity among children with asthma.

Our results suggest that uLTE4 was associated with short-term exposure to OPs. The increases in uLTE4 levels were observed across summed measures of methyl DAPs and ethyl DAPs (EDE and EDM respectively), as well as total DAPs (EDAP). These findings extend the results of previous studies of that suggest use of pesticides in homes or early life exposure may be associated with increased risk of asthma or wheezing [22,144,145,157]. More specifically, due to its repeated measure design and biomarker-based exposure assessment and outcome, our study provides unique insight into the adverse association between acute exposure to OPs in an agricultural community and pediatric asthma morbidity.

Cysteinyl leukotrienes are well known to play a major role in airway inflammation and asthma exacerbation [146,158,159]. Urinary LTE4 serves as a stable indicator of cysteinyl leukotrienes pathway activity associated with asthma [158,159], and has been recommended as a valid biomarker to predict risk of asthma exacerbation especially in acute settings [147,159,160]. In recent years researchers have used uLTE4 as a biomarker of adverse respiratory effect in environmental epidemiology. For example, Rabinovitch and colleagues conducted a longitudinal cohort study of asthmatic school children in Denver and found that the morning maximum ambient particulate matter concentrations were significantly associated with uLTE4 [24]. In a subsequent study, they also found that uLTE4 predicted severe asthma exacerbations among children exposed to tobacco smoke [160]. Although the severity of asthma exacerbations are traditionally categorized using patient symptoms/signs and lung function tests [1], several studies have shown positive correlations between inflammatory markers such as uLTE4, and measures of asthma morbidity (using traditional measurements as a proxy) [159–161].

Despite positive methyl DAP- and ethyl DAP-associations with uLTE4 observed among children, the effect estimate for EDE association was stronger than that of EDM. The reasons for this difference are unclear, but this result might suggest that the OP-related adverse respiratory effects identified in this community could stem largely from the OPs that devolve to DE metabolites (e.g., chlorpyrifos, diazinon) [162].

Our results also indicated that the geometric mean concentrations of the DAP metabolites observed in the AFARE cohort were higher than those observed in children of similar age in a representative US population (NHANES). The elevated levels of DAPs in our study, compared to US children, highlights the additional exposure burden (most likely ambient and proximity-based) encountered in populations with significant agricultural activity [67,135].

Several mechanisms underlying OP-associated asthma morbidity have been suggested. OPs primarily inhibit various forms of cholinesterase, leading to excessive cholinergic activity in neuronal junctions of the parasympathetic nervous system responsible for modulating airway control [71,136,139]. In addition, evidence from animal models and human epidemiology and toxicology studies suggest that other non-cholinergic mechanisms may be responsible for respiratory morbidity. While acute OP intoxication may induce a significant systemic inflammatory response with consequent pulmonary inflammation [163], low-level chronic exposures may result in disruption of muscarinic receptors activity in the airways, production of reactive oxygen species and subsequent activation of a series of stress-responsive signaling pathways, or interaction with irritant receptors in the bronchial mucosa causing release of inflammatory mediators [71,163]. However, future studies at the molecular level are needed to clarify the role of OPs in asthma morbidity.

Our study has a number of important limitations, most notably the use of DAPs as biomarkers of OP exposure. Although DAP metabolites in urine are considered an objective measure of OP exposures, pesticide-specific information cannot be derived from the quantitative measurement of these metabolites; consequently, we are unable to provide information on the specific OPs which participants were exposed. Moreover, we are unable to provide information on the origin of the DAPs (environmentally preformed DAPs vs. metabolite DAPs resulting from exposure to the parent compounds). However, DAPs have been employed as a measure of OP exposure in numerous epidemiological studies, and represent a non-invasive validated marker of broad exposure to OPs [22,75,151].

We are limited by a lack of more traditional clinically-defined measures of asthma exacerbation like symptom reports, use of albuterol, and spirometry. Although we use uLTE4 as an inflammatory biomarker as with previous studies, we are unable to directly translate this marker to the clinical level/severity of asthma exacerbation. Nevertheless, a few studies have provided some context for correlation between acute exacerbation events and uLTE4. For example, Rabinovitch et al. showed clinically significant decreases in pulmonary function (percent predicted forced expiratory volume in 1-second, FEV_1 by 4.7%) per IQR increase in uLTE4 among children with asthma [161].

An additional study limitation is with regards to collection of urine samples. First morning void samples have been suggested as a more stable or representative measurement of exposures in urine [164]. We believe that the longitudinal nature of our study, as well as using creatinine-adjusted values minimizes issues of measurement variability in our population, providing more representative exposure information.

Finally, our study was conducted within an agricultural community, and as such levels of OP exposure are bound to be higher than in the general population. This limits our ability to generalize our results to these populations.

CONCLUSIONS

This study highlights the importance of understanding the role of organophosphate exposure as a potential pediatric asthma exacerbator. Increased exposure to organophosphate pesticides was associated with a marker of asthma exacerbation through inflammation in this cohort of children with asthma in an agricultural community in the US.

Although there is a growing body of epidemiological and toxicological research exploring the associations between OP exposure and respiratory symptoms and conditions, there is limited evidence of effects of OPs in children with asthma. Future studies on OP-associated respiratory morbidity in children with asthma should consider including clinically defined measures of asthma exacerbation such as spirometry, medication use, and symptom days.

CHAPTER 2B: CHARACTERIZING THE JOINT EFFECTS OF PESTICIDE EXPOSURE AND CRITERIA AMBIENT AIR POLLUTANTS ON PEDIATRIC ASTHMA MORBIDITY IN AN AGRICULTURAL COMMUNITY

SUMMARY

Background: Environmental contributions to pediatric asthma morbidity have been studied extensively in urban settings; exposures characteristic of agricultural and rural communities have received less attention despite a comparable burden of morbidity.

Methods: We obtained repeated urine samples (n=139) from 16 school-age children with asthma in the Yakima Valley of Washington State between July and October, 2012. Biomarkers of organophosphate pesticide (OP) exposure (dialkylphosphates (DAPs)), and asthma exacerbation (leukotriene E4 (LTE4)) were analyzed in samples. Corresponding 24-hour average particulate matter < 2.5 μg ($PM_{2.5}$) and maximum 8-hour ozone concentration data for the study period were available from local monitoring stations. We evaluated the independent and multi-pollutant associations between LTE4 and exposure to ambient air pollutants and DAPs using generalized estimating equations. For multidomain, multipollutant models, we created categorized pollution combination levels and estimated the relative health impact of exposure to pollutant mixtures.

Results: In single-pollutant models, an interquartile range increase in exposures to DAPs was associated with increase in LTE4 levels (β: 4.1 (0.6, 7.6) μg/g). $PM_{2.5}$ and ozone were also associated with increase in LTE4, though confidence intervals contained the null value. Increase in LTE4 levels was consistently and significantly associated with increase in median-dichotomized multipollutant combination exposures; the highest effect estimates were observed with joint highest (versus the lowest) category of the three-pollutant exposure ($PM_{2.5}$, ozone and OP; β: 53.5, 95% CI: 24.2, 82.8 μg/g).

Conclusion: Concurrent short-term exposure to criteria air pollutants and OPs in an agricultural community was associated with an increase in a marker of asthma morbidity.

INTRODUCTION

Pediatric asthma continues to be a significant public health issue with approximately 8.4% of children in the United States living with the disease [10,165]. Asthma also remains one of the most

frequent causes of pediatric hospitalization [166,167], and costs the United States over $50 billion in health expenditures and lost productivity annually [13,166,168,169].

Children with asthma are disproportionately impacted by environmental agents [170]. The effects of these exposures on pediatric asthma morbidity has been well-studied, particularly in urban settings [16,19]. However, the contribution of environmental agents in rural and agricultural settings remains largely unexplored. As the sources and composition of these environmental pollutants vary between urban and rural settings [13,52], associations widely described in literature (predominantly urban) are unlikely to be generalizable to rural areas. Agricultural communities may have fewer urban ambient air pollution sources (e.g. motor vehicle traffic and industrial emissions), but have substantially more unique local sources like pollen and similar aeroallergens, and emissions from industrial-scale agricultural operations including windblown dust, animal agricultural emissions and pesticide drift from large crop-growing operations [35,44], all of which have been suggested to contribute to respiratory disease [67,142].

Although environmental health research has traditionally focused on estimating the effects of single pollutant exposures, children are invariably exposed to and affected by a mixture of exposures unique to their environment [76,92,171]. Moreso, assessing the health impacts of environmental exposures in the context of the "one atmosphere" approach requires a more robust characterization of exposures to local sources of environmental pollution beyond single-pollutant risk or total exposure within specific pollutant groups [107,172,173].

Exposure to agricultural pesticides, particularly organophosphate (OP) insecticides, has been linked to adverse respiratory outcomes in agricultural settings [141], and children are especially susceptible to the effects of pesticide exposure [65,68]. However, appropriate characterization of

this association in terms of pediatric asthma morbidity is limited [174]. Further, the respiratory health effects of pesticides in the context of other local air pollutants, is not well understood. A *multidomain* approach that considers the joint effect of multiple classes of environmental agents (specifically, ambient air pollutants and agricultural pesticides) may provide a more concrete representation of association between multipollutant exposure to environmental pollutants and respiratory morbidity.

Respiratory health effects for children with asthma in rural agricultural communities may be affected by multiple sources, including biogenic (e.g. dust, pollen) as well as anthropogenic pollutants. We focus specifically on 1) ozone and particulate matter < 2.5 μm in diameter ($PM_{2.5}$), pollutant concentrations regulated by federal law, and among ambient criteria pollutants, are responsible for a majority of human health damages [175], and 2) organophosphate (OP) pesticides, a group of widely used insecticides with potential health hazards, and also subject to federal standards.

We adopted a conceptually simple method to evaluate independent and joint effects of exposure to air pollution ($PM_{2.5}$ and ozone) and organophosphate pesticide (using well established metabolite biomarkers) on a biomarker of pulmonary inflammation and asthma exacerbation.

METHODS

Study Population

The Aggravating Factors of Asthma in a Rural Environment (AFARE) project was conducted in the Yakima Valley of Washington State. This region is characterized by a high density of large-scale agricultural operations including production of fruit crops and vegetables. Details about

recruitment and baseline health evaluations have been reported previously [109]. Briefly, the AFARE study collected longitudinal data to explore and identify ambient environmental factors associated with pediatric asthma exacerbations in an agricultural community. The children were between the ages of 6 and 16 years at baseline and had no serious illnesses other than asthma. For this analysis, we used the repeated measures made on a subset of children for which urine specimens were collected in the AFARE cohort (n = 16), at six-day intervals over a four-month period (July 2012 to October 2012). All study procedures were approved by the Institutional Review Boards of the University of Washington and Colorado State University.

Asthma Morbidity Assessment

Urinary Leukotriene E4 Monitoring

We used urinary leukotriene E4 (LTE4) to assess asthma morbidity (pulmonary inflammation) in this study. LTE4 is a validated marker of systemic cysteinyl leukotriene activity, and an indirect marker of lung cysteinyl leukotriene activity, a lipid mediator known to play a central pathophysiological role in asthma [146,147]. Cysteinyl leukotrienes are eicosanoids produced by a variety of cells associated with inflammation. Measurement of LTE4 represents a noninvasive method to assess acute pulmonary inflammation among children with asthma.

LTE4 was measured from spot urine samples, scheduled to be collected study participants every six days during the study period. Samples were subsequently stored at -20°C before analysis. Quantitative analysis of urine samples for LTE4 was performed in the University of Washington Department of Environmental and Occupational Health Studies Functional Genomics Laboratory using the Cayman Human Leukotriene E4 EIA Kit (Cayman, Ann Arbor, MI) according to the manufacturer's instructions. Creatinine concentration was also measured for each sample to

account for urine dilution; this analysis was conducted by the Department of Laboratory Medicine at the University of Washington. Creatinine-adjusted concentrations were used for all final model analyses.

Environmental Pollutants and Meteorological Data

Organophosphate Pesticide Monitoring

Spot urine samples were analyzed for organophosphate pesticide metabolites at the same time LTE4 was assessed. Six urinary dialkyl phosphate (DAP) metabolites that result from the degradation of different OPs were measured in participants' urine; dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). Metabolite reporting limits were based on the limit of detection (LOD) for each DAP compound, and masses below the limit of detection were approximated as LOD/√2. Creatinine concentration was measured similarly to account for urine dilution.

We used summative measures of DAPs rather than measures of individual analytes, to provide a better indicator of total OP exposure [75], as well as to account for circumstances where individual OP pesticides devolve to more than one DAP metabolite [151]. Summed urine DAP concentrations (total dimethyl alkylphosphate (EDM), total diethyl alkylphosphate (EDE), and total dialkylphosphate pesticides (EDAP)) were estimated by summing molar concentrations (DAP concentration divided by their molecular weights) of metabolites. Creatinine-adjusted total dialkylphosphate pesticides (EDAP) was used for final single- and multi-pollutant model analyses.

Ambient $PM_{2.5}$ and Ozone Measurements

We obtained daily measurements of ambient concentrations of $PM_{2.5}$ from the local US Environmental Protection Agency (EPA) central site air monitor in Toppenish, Washington. Ozone data were obtained from the US EPA Air Quality System Data Mart as an 8-hr daily maximum in parts per billion. Because the ozone monitors (n=8) are sparsely distributed in Central Washington, we used data from all available monitoring sites that had complete pollution data during the study period and averaged measurements from the three closest monitors within 100 miles of participant homes. We explored the effect of $PM_{2.5}$ and ozone measured on multiple lag days; to correspond with the limited exposure window for OP exposure, final analyses were performed with weekly (7-day) average $PM_{2.5}$ levels (as we did not have consecutive daily pollutant measurements), and lag-1 ozone levels.

Meteorology

To capture meteorological conditions for the week prior to LTE4 measurements, we used data downloaded from the *countyweather* package in R which provides data from NOAA's Global Historical Climatology Network on 24-hour average, maximum and dew-point temperature, precipitation, and wind speed.

Statistical Analysis

We evaluated associations of a marker of asthma exacerbation (LTE4) with exposure to ambient pollution (weekly average for $PM_{2.5}$ and day-prior 8-hour maximum for ozone), and organophosphate pesticides (DAP) using generalized estimating equations (GEEs) with an exchangeable correlation matrix. In all our models, the mean outcome was modeled to be linear in response to the primary exposure of interest.

We presented the results in single-pollutant models as effect sizes per interquartile range increases in exposure so as to make the associations comparable between the pollutants. For multipollutant models, we created categorized pollution combination levels and estimated the relative health impact of exposure to pollutant mixtures. This method assumes that there are similar functional characteristics (categorical effects) for individual components of joint exposures. Levels of individual exposure metrics (OP, $PM_{2.5}$ and ozone) were split into dichotomous indicator categories (high and low) based on the median values observed in the cohort. Then we aggregated the high and low pollutant levels to form two- and three-pollutant exposure mixture categories as shown in Table 2.4. For a two-pollutant mixture ("OP + ozone", "OP + $PM_{2.5}$", "$PM_{2.5}$ + ozone"), there would be three categories of pollutant mixture reflecting *high* exposure (both pollutants at high exposure levels), *moderate* exposure (one pollutant at high exposure level), and *low* (both pollutants at low exposure levels); and for the three-pollutant mixture ("OP + ozone + $PM_{2.5}$"), there would be four categories of pollutant mixture reflecting *high* exposure (all pollutants at high exposure levels), *moderate* exposure (two pollutants at high exposure level), *mild* exposure (only one pollutant at high exposure levels), and *low* exposure (no pollutants at high exposure levels). Mixture exposure categories were then included as independent variables in GEE models, using the homogeneous "low" categories as the reference category, and controlling for confounders.

Covariates included in all models as potential confounders were selected *a priori* based on existing evidence of relationships between the covariate and both respiratory health and exposure to air pollution: temperature, wind speed, precipitation and relative humidity (averaged over the week prior to LTE4 measurements), week and month of the year as two possible markers of temporal trends (known high-risk periods for increased exacerbation include the return to school in the fall

and respiratory virus season [176,177]), as well as subject-specific characteristics potentially associated with asthma and asthma exacerbation: sex, age, use of inhaled corticosteroids at baseline, a measure of severity at baseline (exhaled nitric oxide (eNO) levels), and the number of individuals in household.

TABLE 2.4. SUMMARY OF MIXTURE CATEGORIES USED FOR MULTIPOLLUTANT ANALYSIS

Pollutant Levels			Exposure Category			
OP	Ozone	$PM_{2.5}$	3- Category		4-Category	
High	High		2	High		
Low	High		1	Moderate		
High	Low					
Low	Low		0	Low		
High		High	2	High		
Low		High	1	Moderate		
High		Low				
Low		Low	0	Low		
	High	High	2	High		
	Low	High	1	Moderate		
	High	Low				
	Low	Low	0	Low		
Low	Low	Low			0	Low
Low	Low	High			1	Mild
Low	High	Low				
High	Low	Low				
Low	High	High			2	Moderate
High	Low	High				
High	High	Low				
High	High	High			3	High

In sensitivity analyses, we repeated the multipollutant analysis after restriction to exposure days below the US Environmental Protection Agency (EPA) National Ambient Air Quality Standards for ozone and $PM_{2.5}$. Further, to investigate whether the health impact of joint multipollutant categories was sensitive to the choice of thresholds, we assessed multiple combinations for cut-point choices at the 25^{th}, 50^{th} and 75^{th} percentile thresholds: we varied the cutpoints that distinguish between high and low exposure, and then reran models with adjusted joint multipollutant exposure categories created from these new cutpoints.

Model diagnostics were also performed to explore the possibility of influential subjects using the "leave one out" method. These analyses did not indicate the presence of significant impact of a single observation on model fit or estimates. We used the quasi-information criterion (QIC) as an estimator of the relative quality (model fit) of statistical models.

Analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina) for GEE and mixed model analyses, and R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria) for exploratory and descriptive analyses.

RESULTS

Overall, the mean age of the 16 children included in this analysis was 12 years and 56.3% were male (Table 2.5). Nearly all (93.8%) of the children self-identified as Hispanic/Latinx, 56.3% were from low-income families, and 87.5% relied on public health insurance/aid. Approximately 69% of the children were taking corticosteroid medication at the time of enrollment, and 12 children (75.0%) were identified to be skin prick positive to at least one aeroallergen. Based on a clinical

examinations performed at baseline, more than half of the subjects (56.3%) were classified as overweight (body mass index-for-age above the 85th percentile).

Compliance with collection of urine samples varied: the total number of samples per subject ranged from 1 to 12 (median: 10.5). A total of 139 observations were obtained from the 16 children over the study period. One observation was excluded because of incomplete data, resulting in 138 observations for analyses.

Individual-level creatinine-adjusted urinary LTE4 over the study period are illustrated in Figure 2.3. The median level of LTE4 among participants was 84.8 pg/mg creatinine (geometric mean: 84.8 pg/mg) over the study period.

Twenty-four hour weekly average $PM_{2.5}$ concentrations had a median (IQR) of 8.7 (8.2) $\mu g/m^3$ over the study period with the highest values occurring in late September (Figure 2.4A). There were multiple weekly periods with average $PM_{2.5}$ exposure levels above the EPA 24-hr ambient air quality standards (35.0 $\mu g/m^3$), likely coinciding with a wildfire in Washington state during this period [178]. The other predominant sources of $PM_{2.5}$ emissions in the region include fossil fuel combustion, waste disposal and agricultural crop and livestock-related dust [179]. The median (IQR) maximum 8-hour ozone value for individuals over the study period was 43.0 (10.0) ppb (Figure 2.4B). There were also multiple days with observations above EPA 8-hr daily maximum ambient air quality standard (70.0 ppb). The median (IQR) total OP metabolite (EDAP) level was 142.9 (197.3) nmol/g creatinine (Figure 2.4C). Spearman correlations of ambient air pollutants showed very weak positive correlations between EDAP and both ozone and $PM_{2.5}$ (both $\rho < 0.1$). The correlation between ozone and $PM_{2.5}$ was positive and slightly stronger ($\rho = 0.2$).

TABLE 2.5. CHARACTERISTICS OF CHILDREN IN AFARE SUB-COHORT

Variable	Level	%
Sex	Male	56.3
Birth country	US	68.8
	Other	31.3
Ethnicity	Hispanic/ Latino	93.8
	Non-Hispanic	6.3
Income	≤ $15k/year	53.3
	$15k - < 30k/year	26.7
	>30k/year	20.0
Residence	In Town	81.3
	Rural/Farm	18.8
Total number of household members	< 5	31.3
	≥ 5	68.8
Insurance	Public Insurance or Aid	87.5
	Private Insurance/Self	12.5
Skin prick test positive (Atopy)	Yes	75.0
Inhaled corticosteroid use	Yes	68.8
Age	Mean	11.9
	Median	11.0
	Minimum	9.0
	Maximum	17.0
BMI for age (85th Percentile)	Above	56.3
	Below	43.7
Baseline fraction exhaled nitric oxide level (ppb)	Mean	22.1
	Median	12.0
	Minimum	6.0
	Maximum	120.0

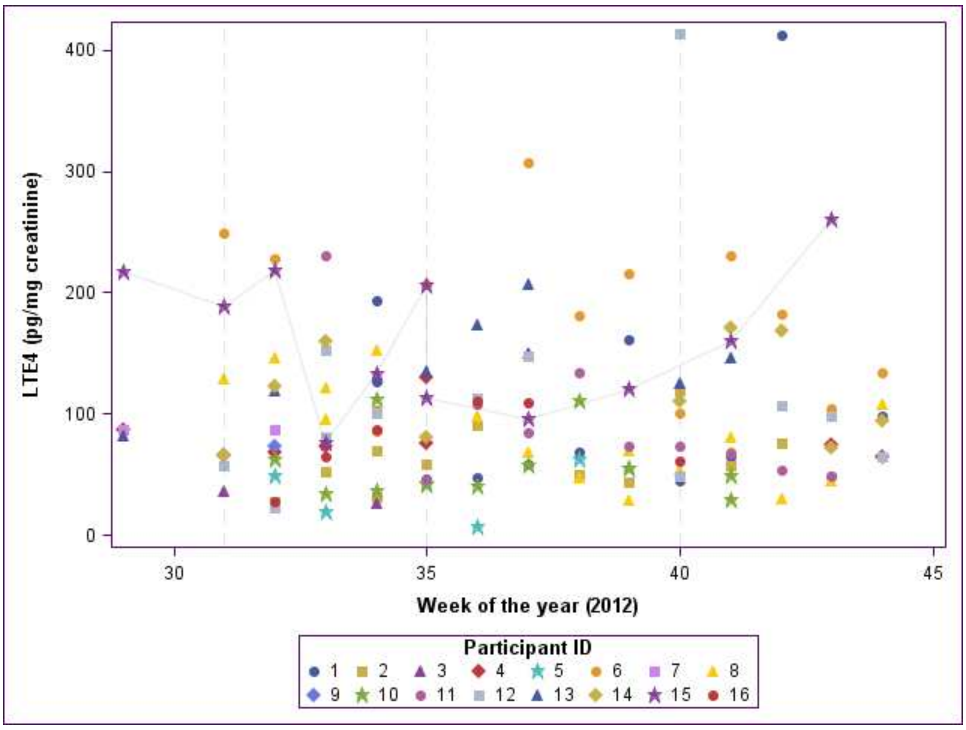

FIGURE 2.3. CREATININE-ADJUSTED URINARY LEUKOTRIENE Ɛ4 (LTE4) LEVELS FOR STUDY PARTICIPANTS OVER THE STUDY PERIOD.
Vertical lines represent the beginning of a new month. Observed LTE4 series for one participant (15) is highlighted.

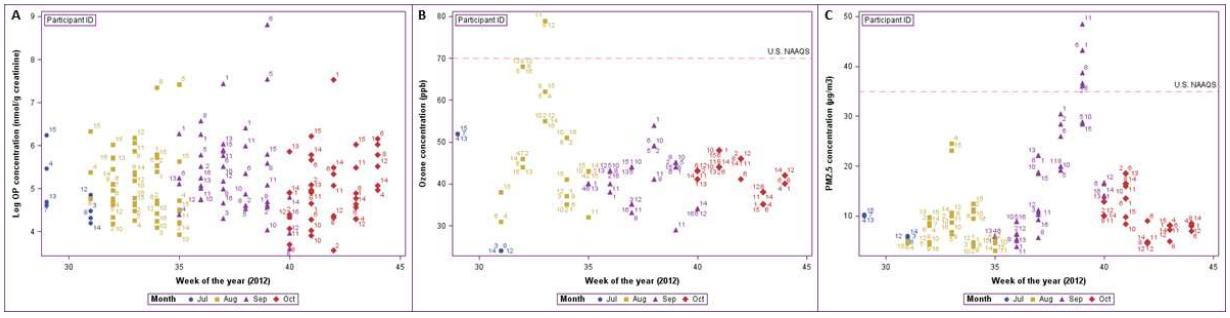

FIGURE 2.4. AIR POLLUTANT AND PESTICIDE EXPOSURE LEVELS FOR STUDY PARTICIPANTS OVER THE STUDY PERIOD.
Numbers in plot area indicate participant IDs. Horizontal line represents U.S. National Ambient Air Quality Standard. **A**. Creatinine-adjusted urinary log organophosphate (dialkylphosphate, OP) levels. **B**. Maximum 8-hour ambient ozone levels. **C**. 24-hr average ambient $PM_{2.5}$ concentrations.

In addition, we observed no evidence of patterns in pollutant concentrations by residence (in-town vs. rural/farm), although less than 20% of participants resided in or next to farms (Appendix Figure 1). However, concentrations of the exposure ($PM_{2.5}$, ozone and OP) and outcome (LTE4) measures exhibited a fair amount of temporal variability over the study period.

The associations of pollutants with LTE4 using single-pollutant (as continuous pollutant exposures), two-pollutant (two of OP, $PM_{2.5}$ and ozone), and three-pollutant models (all three pollutant exposures) are presented in Figure 2.5. In single-pollutant models, an interquartile range (IQR) increase in OP levels was associated with a LTE4 increase of 4.1 pg/mg creatinine (95%CI: 0.6, 7.6). We also observed elevated associations between LTE4 levels and ozone (β: 5.8, 95%CI: -3.3, 14.8) and $PM_{2.5}$ (β: 2.1, 95%CI: -9.2, 13.4), although confidence intervals included the null value.

All the models with median-dichotomized multipollutant combination exposures showed associations with increase in LTE4 levels. We observed the highest change in LTE4 effect estimate for the highest (versus the lowest) category of the three-pollutant exposure (β: 63.6, 95% CI: 32.4, 94.7); mild and moderate exposure categories resulted in approximately 27.5 (95% CI: 3.6, 51.5) and 62.5 (95% CI: 18.1, 107.0) pg/mg creatinine increases in LTE4 respectively, compared to the lowest exposure category. Despite the significant overlap between estimates and confidence intervals, we also observed a form of tiered dose-response pattern across the categories of exposure severity. In addition, effect estimates were similar for models that excluded observation points (n=9) above the EPA standards for ozone and $PM_{2.5}$ (Figure 2.5B).

In subanalyses of multipollutant models, we examined individual pollutant contribution to two-pollutant mixture categories. Within the limitations of overlapping confidence intervals, we observed that for two-pollutant models, either moderate $PM_{2.5}$ or OP, as well as high $PM_{2.5}$ and OP were associated with increased LTE4 compared to the reference homogeneous low mixture category (Figure 2.6).

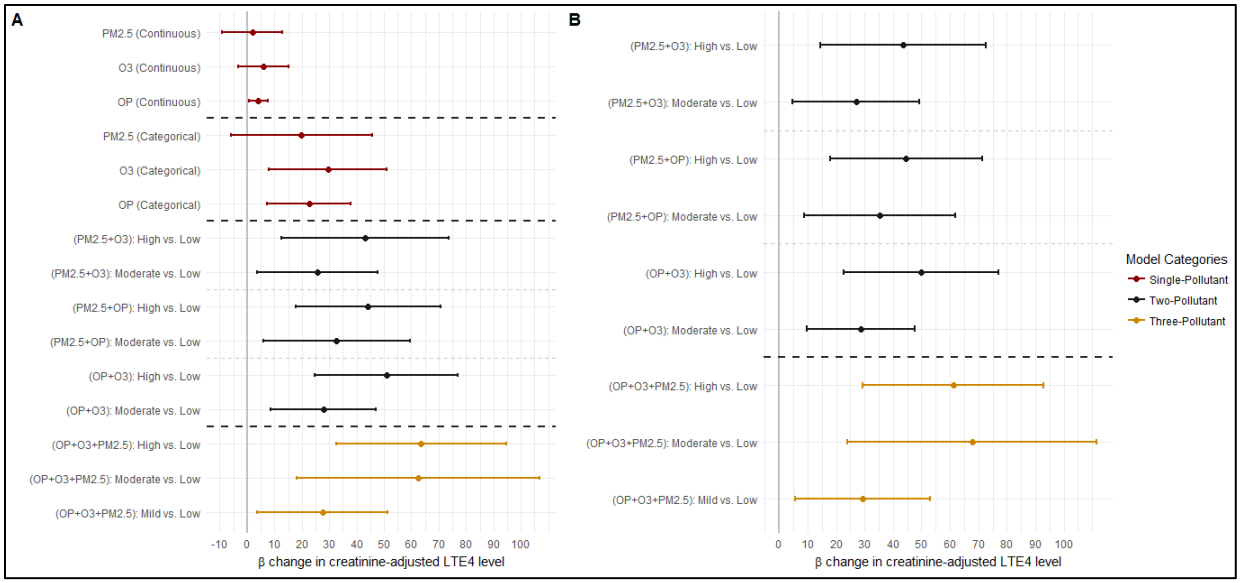

FIGURE 2.5. ESTIMATED EFFECTS (Β CHANGE AND 95% CONFIDENCE INTERVALS) OF POLLUTANTS ON CREATININE-ADJUSTED LTE4 LEVELS (IN PG/MG CREATININE).

A. Single-pollutant and multipollutant models using the median cutoff for categorization. **B.** Multipollutant models using median cutoff for categorization, excluding observations below EPA cutoff values. All models were adjusted for sex, age, use of inhaled corticosteroids at baseline, number of individuals in household, temperature, wind speed, precipitation and relative humidity. $PM_{2.5}$ indicates 24-hour-average exposure to particulate matter < 2.5 μm in diameter; Ozone, 8-hour maximum concentration of ozone; OP, urinary measure of metabolite of organophosphate exposure, total dialkylphosphate.

In contrast, for two-pollutant mixtures with ozone, only the highest mixture categories containing ozone (compared to the reference homogeneous low mixture category) resulted in increased LTE4 effect estimate.

The results from the sensitivity analysis using combinations of the 25^{th}, 50^{th} and 75^{th} percentiles as thresholds are shown in Tables 6.2 and 6.3. We observed that the associations between high, moderate, mild and low exposure groups generally persisted in these models. However, the magnitude of effect estimates (and 95% CI) varied with the differing cutpoints. For example, combination *c4* which developed exposure categories based on the 25^{th} percentile for $PM_{2.5}$ (5.3 $\mu g/m^3$), 50^{th} percentile for ozone (40.0 ppb), and 25^{th} percentile for OP (92.0 ng/mg creatinine),

resulted in an estimated 17, 39 and 52 pg/mg creatinine increase in LTE4 levels among the mild, moderate and highest levels of joint exposures respectively (compared to the lowest category).

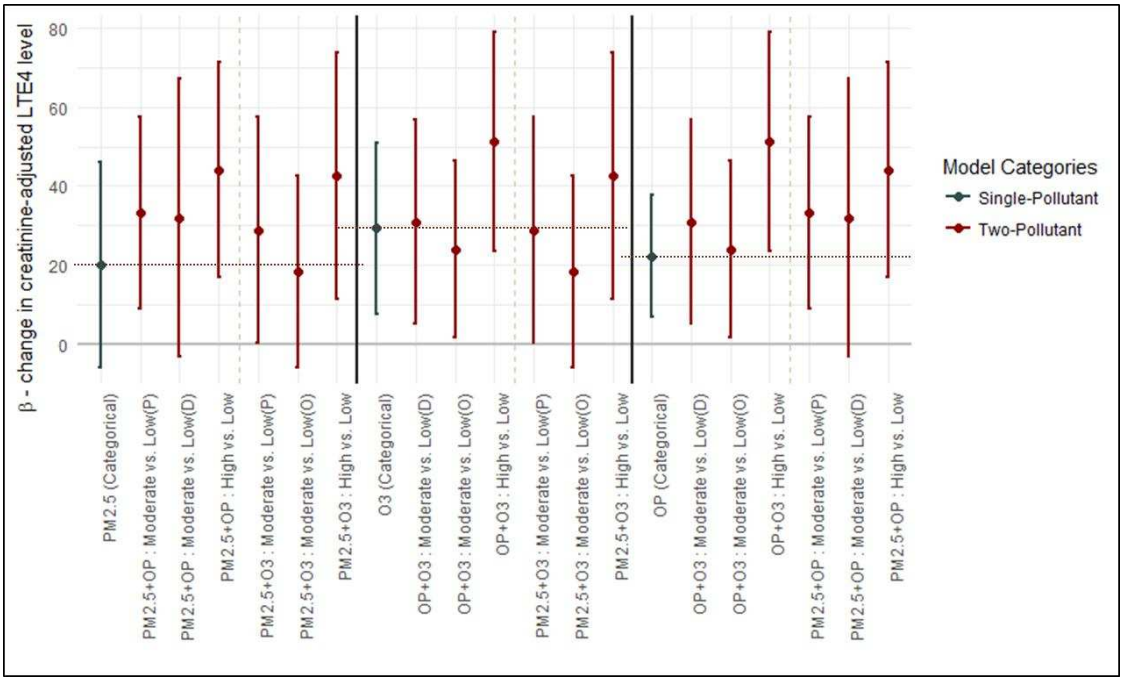

FIGURE 2.6. ESTIMATED EFFECTS (Β CHANGE AND 95% CONFIDENCE INTERVALS) ON CREATININE-ADJUSTED LTE4 LEVELS (IN PG/MG CREATININE) FROM TWO-POLLUTANT MODELS INDICATING SPECIFIC POLLUTANT CONTRIBUTION TO EXPOSURE MIXTURE CATEGORIES.
Letter in parenthesis indicates pollutant with highest exposure level; $PM_{2.5}$: particulate matter < 2.5 µm in diameter, O_3: ozone, OP: total dialkylphosphate (organophosphate). Horizontal lines indicate level of effect estimates of single pollutant in comparison to multipollutants.

Finally, we observed minimal differences between model fit/performance relative to selected cutpoints, based on the quasi-information criterion (QIC) values (Appendix Table 2, Appendix Table 3). The best fit models for the multipollutant association with LTE4 tended to involve lower cut points (at 25%) for ozone and OPs, and the higher cut points for $PM_{2.5}$ (at 75%).

DISCUSSION

Geographical and population-based differences in the prevalence of asthma morbidity necessitate more refined assessment of the environmental exposures experienced by different populations. Our results provide insight into the effects of important criteria ambient pollutants on the

respiratory health of children with asthma in a rural agricultural community, all in the context of contemporaneous exposure to OP pesticides.

To the best of our knowledge, no other study has considered environmental exposure to OP pesticides and criteria pollutant in joint health effects models; our study represents the first longitudinal, repeated measures study of joint assessment of community-level OP exposures, ambient air pollution and a marker of pulmonary inflammation among children in a largely agricultural setting.

Our findings highlight, within the limitations of this study, several important implications. First, single-pollutant models suggest independent positive associations between urinary LTE4 and short-term exposure to $PM_{2.5}$, ozone, and OP pesticides, though only associations with exposure to OPs had a 95% confidence interval that excluded null value. These findings, along with many previous studies using single-pollutant approaches, provide pertinent information about the potential role of the individual exposures, which is required to demonstrate relevance for the combination of these pollutants in multipollutant models [77,88].

Next, the multipollutant models suggest increases in LTE4 was consistently associated with joint exposures combinations of $PM_{2.5}$, ozone and OPs. Our observation of ordered trends across categories of severity in two- and three-pollutant models may indicate increased risk of adverse health effects with increasing total mixture levels, though our small sample size resulted in significant overlap among the categories. We also observed a unique pattern of these trends with specific joint exposures; relative to the lowest categories, joint adverse associations with LTE4 levels observed for all higher levels of $PM_{2.5}$ and OPs, but only mixture categories with the highest levels of ozone showed increased/positive changes in LTE4, compared to the low mixture

categories. This most likely indicates that the relationship between ozone and LTE4 (and by extension inflammation) in mixtures is not a simple linear one, with the worst effects seen at comparatively higher exposure levels, although underlying interaction mechanisms remain unclear.

Finally, the observed associations between exposure mixture categories were present at concentrations below NAAQS standards for $PM_{2.5}$ and ozone. Although these regulatory standards are predicated on single-pollutant research [76], our results reiterate the shortcomings (especially among susceptible individuals) of the standards [180], as well as signify a possible pathway to proffering standards based on multipollutant approaches.

The deleterious relationship between ozone, $PM_{2.5}$ and pediatric asthma morbidity have been thoroughly studied and established by multiple observational and experimental studies.

Single-pollutant studies have found independent associations between short-term ozone and $PM_{2.5}$ exposure, and asthma-related symptoms, hospital visits and clinical measures of exacerbation. For example, Lewis *et al.* found that ambient $PM_{2.5}$, PM_{10}, 8- and 1-hr peak ozone concentrations were associated with increased odds of respiratory symptoms in a set of children with asthma in Detroit [180]. Similarly, Loftus *et al*. showed that $PM_{2.5}$ pollution in this AFARE agricultural setting resulted in increased asthma symptoms and decreased lung function [109].

Conversely, the impact of OPs on pediatric asthma morbidity has rarely been explored. In a previous study among children in the AFARE study, the authors showed that urinary pesticide metabolite levels (indicating short-term OP exposure) were significantly higher among the children in the AFARE cohort compared to children of similar age participating in the National Health and

Nutrition Examination Survey (NHANES), indicating additional exposure burden (most likely ambient and proximity-based) encountered in populations with significant agricultural activity [181]. They also pointed out that this OP exposure was associated with increased urinary LTE4 levels. Two other studies exploring urinary DAP metabolites among children from the CHAMACOS birth cohort found a significant association between early-life exposure to OPs and respiratory symptoms, and lung function in childhood [22,145]. Although these symptoms and signs were consistent with pediatric asthma morbidity, their sample cohort was not focused on children with asthma. Moreso, post-natal short-term exposures to OPs were not explored.

The major research paradigm in environmental epidemiology research is to examine single-pollutant effects on health outcomes. The limited number of multi-pollutant studies often focus on a combination of criteria air pollutants, using either an additive main effects approach (including co-pollutants as co-exposures in regression models), the interaction approach (as described by Dominici et al. [76]), or other semiparametric and parametric approaches [83,182]. Although the effect of multipollutant exposures have been linked to pediatric asthma morbidity [26,101,105,183–185], the difference in mixture components and approaches to quantify these pollutant mixtures limits direct comparability with our study results. Even less common are studies that explore non-criteria environmental pollutants, or multiple exposure domains. Research on multidomain (in addition to multipollutant) exposures are important when considering the health effect of cumulative chemical exposure in communities with a mix of pollutant sources [88]; the individuals in such communities tend to be exposed to multiple diverse chemicals or environmental risk factors simultaneously [107].

The exact mechanisms by which these three pollutants cause respiratory morbidity, individually or as part of a mixture, are poorly understood. However, across epidemiological and toxicological studies, airway inflammation and hyperresponsiveness are two mechanistic features consistently associated with all three pollutants [19,163]. Cysteinyl leukotrienes are a measure of endogenous release of inflammatory mediators, and are recognized as a key mediator of airway inflammation [146,158,159]. Hence, LTE4, the stable end product of cysteine leukotriene metabolism can be considered as a logical marker of the endpoint of this inflammatory process.

Our approach to multipollutant analysis employed median dichotomization splits to generate exposure categories based on distributional properties of the single-pollutant exposure data in the cohort. This conceptually simple method builds on an unsupervised profile generation technique which transforms pollutant mixture concentrations into flexible variables that subsequently represent simple exposure profiles of the pollutant combination. We are able to generate interpretable effect estimates with reasonable inferential properties including better characterizations of the total environment, effect measure modification within the mixtures, as well as identify combinations of pollutants that may be the most harmful.

Other simplified methods for multipollutant analyses have been reported in the literature. For example, Hong *et al*. presented a combined index for combinations of pollutant concentrations, calculated as the sum of mean scaled single pollutant concentrations [186]. Their index method is easy to interpret, but unsuitable for highly skewed data, and is unable to clearly delineate which mixture component, or combination of pollutant levels is relatively more harmful. More recently, a study by Liu and Peng examined the cardiovascular health effects of three-pollutant mixtures (ozone, nitrogen dioxide and fine particulate matter) in 85 US counties, using a method called

PANCAKE (PollutANt CAtegory KnittEd) to categorize pollutant levels [187]. Their categories were based on thresholds of increasing magnitude, and PANCAKE created indicators for different mixture compositions. However, the PANCAKE method is more suited to ecological-level studies and requires large sample sizes to generate enough samples for exposure mixture categories.

There are several limitations in our study and analytic approach. First, using DAPs as a measure of ambient OP exposure is limited by the lack of specificity with respect to the OP from which they were derived, and reliability may be affected by human exposure to preformed DAPs in food or the environment [117,188]. We believe that any related measurement error will most likely be nondifferential, with possible attenuation of effect estimates. Moreover, a substantial body of literature has demonstrated significant temporal and spatial associations between ambient pesticide application/use/measurements and DAP levels [135,189,190]. Our measures of ozone and $PM_{2.5}$ were obtained from central monitors in proximity to children's homes. Such residential exposure assessment fails to account for time-activity patterns. Further, we were limited in this particular study in identifying spatial variation of exposures to environmental agents. Again, any errors resulting from this would most likely be nondifferential, and likely may have masked any true exposure-outcome associations by biasing results toward the null and/or increasing the standard errors association with effect estimates. Future studies should focus on better characterization of spatial exposure patterns in an agricultural community.

Further, to arrive at biologic plausibility for the joint effects of exposures on our outcome, we assume similar pathophysiological pathways for all three component pollutants. It is possible that the effects seen are due to simultaneously present differing mechanisms of action. For example, each pollutant may lead to respiratory morbidity through one or more of: direct insult on lung

tissue receptors; indirectly through effects mediated by oxidative stress or inflammatory mechanisms; simultaneous direct and indirect mechanisms; or with the mechanism and effect of a specific pollutant acting as an adjuvant for another [19,191]. Toxicological data that appropriately quantifies the pathophysiological activity for individual pollutants may be required. However, it is unlikely that differences in individual pollutant pathophysiologic mechanisms explains all of the observed effects.

Another possible source of exposure misclassification may be related to using dichotomized exposure cutpoints. To evaluate exposure cutpoint bias, we manually assessed the joint effects of pollutant exposures at multiple dichotomization splits. The observed results indicate a robustness of our chosen median cut-points in this population.

Data constraints limited us to short-term lag exposure analyses, as well as limited characterization of the influence of seasons with respect to this particular agricultural community. Finally, we had no symptomatic/clinical marker of asthma exacerbation. However, multiple studies have highlighted correlations between acute exacerbation events and LTE4: Green *et al.* showed that urinary LTE4 levels among adults with asthma increased by over 30% during asthma exacerbations, compared with levels at follow up [159]; and a Rabinovitch *et al.* study indicated clinically significant decreases in pulmonary function (percent predicted FEV_1 by 4.7%) per IQR increase in LTE4 among children with asthma [161]. We do recognize that combining measures, such as biomarkers with clinical characteristics, most likely characterizes asthma exacerbation better than a single marker [192].

Several issues need to be considered in interpreting our study results. Our choice of pollutants in single- or multipollutant models do not represent a full suite of possible pollutant exposures, even in this particular agricultural community. Moreso, those selected for our analyses are likely correlated with multiple other key pollutants and may only be acting as surrogates for unmeasured or poorly measured pollutants. We also acknowledge the possible contribution of indoor exposures that may act as allergens (such as mites and cockroaches, pets, gas stoves, and tobacco). However, the children in our study were enrolled in an asthma education program to address these common indoor factors prior to collection of urine samples.

Finally, exposures to the ambient air pollutants and pesticides were limited to a four-month period. Without patterns of variability across multiple time periods, we can only make cautious interpretations of the magnitude or significance of exposure-outcome associations. Again, more detailed studies that include time points from a larger number of seasons are required to provide a better characterization of temporal variations, and to validate our study methods.

The identification and mitigation of environmental triggers in a rural/agricultural setting is one effective component of community- and clinic-based asthma management strategies, the success of which depends on proper characterization of the relevant pollutant species beyond the commonly measured (and more urban) air pollutants. In this panel study, we explore the deleterious associations between a biomarker for pulmonary inflammation, and exposure to agricultural organophosphate pesticides and two important criteria air pollutants among children with asthma. Additionally, we extend a multipollutant statistical framework to examine the joint

effect of this distinct combination of ambient exposures that underscore the experience of simultaneous exposures to environmental triggers in an agricultural community.

More emphasis on region and population-specific analysis of pollutant mixtures and potential health effects is required, including development of tools and approaches for these epidemiologic analyses, with a focus on ultimately refining and enforcing more appropriate environmental standards.

CHAPTER 3: THE FACES STUDY

THE JOINT EFFECT OF AMBIENT AIR POLLUTION AND AGRICULTURAL PESTICIDE EXPOSURES ON LUNG FUNCTION AMONG CHILDREN WITH ASTHMA

SUMMARY

Background. The evaluation of potential health impact of environmental chemical agents among susceptible populations is often limited to understanding the effects of exposure to a single chemical or a group of similar chemicals at one time. As a result, the effects of environmental chemical mixtures are rarely considered. In this study, we apply a multidomain, multipollutant approach to assess the association between pediatric lung function measures and selected ambient air pollutants and pesticides.

Methods. Recent exposure (three months prior) to ambient air pollutants (ozone (O_3), nitrogen dioxide (NO_2), particulate matter with a median aerodynamic diameter < 2.5µm ($PM_{2.5}$) and < 10µm (PM_{10})) and pesticides (organophosphates (OP), carbamates (C) and methyl bromide (MeBr)) was reconstructed for a cohort of children with asthma from the San Joaquin Valley of California, USA. We obtained air pollutant concentrations from the US Environmental Protection Agency (EPA) Air Quality System Data Mart, and pesticide exposure information from the California Pesticide Use Report (PUR) database. We created exposure metrics based on proximity to participants' residential addresses. We implemented Bayesian kernel machine regression (BKMR) models to estimate the association between environmental exposures and lung function measures (forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and forced expiratory flow between 25% and 75% of vital capacity ($FEF_{25–75}$)).

Results. Our study population was comprised of 153 children, predominantly non-Hispanic White male children with mild intermittent or mild persistent asthma. There were strong correlations between air pollutants (in particular, NO_2 and $PM_{2.5}$) and between air pollutants and pesticides (OPs with NO_2 and O_3). In BKMR analysis, NO_2 was the main driver of joint effects in association models. The overall effect of mixtures was associated with reduced FEV_1 and FVC, particularly when all the environmental exposures were above their 60th percentile, compared to all of them at their 50th percentile. For the FEF_{25-75} model, we observed effects opposite those for the other outcome measures, with higher quantiles appearing less harmful. However, 95% credible intervals around all of the joint effect estimates contained the null value, indicating lack of statistical significance. In addition, we observed possible interaction between component pollutants in all three models.

Conclusion. The role of multipollutant exposures on pediatric asthma morbidity in regions with an agricultural-urban interface is understudied particularly in the context of multidomain exposures. This study contributes to the limited literature and demonstrates the potential strengths of the multidomain approach using a modeling technique (BKMR) that provides feasible solutions to these types of research questions.

INTRODUCTION

The effects of short- and long-term exposure to ambient environmental agents on respiratory health and pulmonary function in children have been widely reported, particularly among children with asthma. These studies have significantly improved the understanding of the deleterious effects of individual pollutants and chemicals such as criteria air pollutants including particulate matter, ozone, and nitrogen dioxide [19,63], and additional ambient pollutants including

polycyclic aromatic hydrocarbons [73,74] and pesticides [22,75]. With regard to methodologic frameworks, these studies have largely employed risk-factor epidemiology, which attempts to isolate the impact of one pollutant (or total effect within one specific chemical group), though the general consensus is that individuals are rarely (if ever) affected by single chemical agents in isolation [88,171]. As the field of environmental epidemiology moves towards more multipollutant approaches, examining the effects of simultaneous exposure to multiple diverse environmental chemicals is increasingly important, especially when these ambient mixtures impact common receptors and pathophysiologic pathways and share similar endpoints [88,193,194]. A multidomain approach considers the joint effect of multiple classes of environmental agents along the theme of the "total environment" paradigm which takes into consideration a more comprehensive range of concurrent exposures experienced by a population [94].

Although chronic diseases of childhood are universally multifactorial and associated with exposure assessment challenges, the national burden of disease for pediatric asthma and the strong environmental antecedents associated with both asthma incidence and asthma-related morbidity result in a particularly salient example to conduct multidomain studies. Children with asthma who have spatially and temporally heterogeneous exposure to multiple domains of environmental chemicals may provide substantial insight into understanding the health effects of co-exposure to pollutants in a multidomain context.

The San Joaquin Valley (SJV) of California represents a unique opportunity to explore the pulmonary health effects of multidomain environmental mixtures, given that the SJV is situated along a major transportation corridor, and its urban areas are surrounded by agricultural operations, creating a distinctive mix of pollutants [112]. Further, the region has a relatively high

prevalence of asthma and asthma hospitalizations compared to state and national averages [55,195]. An understanding of the nature of adverse multipollutant effects in this community may potentially lead to better environmental quality management, as well as a reduction in the pediatric asthma burden.

As with any environmental mixtures analysis approach, the multidomain approach involves many logistical and statistical challenges [88,171]. Two especially important challenges limit analyses. First, in the face of widespread complex and multifactorial human exposures, a major challenge is defining the environmental agents of interest relevant to human health. Identifying appropriate quantitative exposure metrics to represent the relevant exposures is also tasking. Secondly, selection of an appropriate statistical method to estimate the effects of the multipollutant exposure on health given the research questions and available data is difficult. Traditional regression models like generalized multivariable regression (using ordinary least squares) are usually "naive" to the complex structure of mixtures with accompanying multiple highly correlated exposures, sometimes non-linear (and non-additive) exposure-outcome associations, and high-dimensional interactions. Results from such models are prone to unstable parameter estimates with large standard errors and are often difficult to interpret. Given the regional characteristics of the SJV, we address the first challenge in this study by exploring two domains of environmental chemicals that have been highlighted in single- and multipollutant studies as important contributors to adverse respiratory health outcomes among children: recent exposure to ambient air pollutants and pesticides. With regard to the second challenge, several methods have been suggested for multipollutant mixtures analyses. Bobb et al. (2014) recently proposed a Bayesian kernel machine regression (BKMR) method to estimate joint health effects of multiple pollutants

[97]. BKMR was the most suitable mixture method available to meet our study objectives: evaluate the overall health effect of cumulative environmental exposure mixtures. Specifically, BKMR provides 1) an estimation of multipollutant exposure-response (E-R) function that may include nonlinear association and complex interactions and 2) simultaneous (rather than sequential) hierarchical variable selection to identify both important domains and important pollutants within domains that contribute the E-R relation. The simultaneous modeling feature provides a realistic assessment of the uncertainty associated with identifying the important pollutants in the mixture rather than obtaining standard errors conditional on the set of pollutants included in the model [96].

We implemented BKMR to explore the pulmonary health effects of co-occurring multidomain (ambient air pollutants (AAP) and pesticides), multipollutant exposures for a group of children with asthma in the SJV region. In particular we examined a) whether recent exposure (prior 3 months) to the mixture of AAP and pesticides jointly is associated with adverse pulmonary effects; b) the exposure–response relationships between combinations of environmental exposures and lung function; and c) whether the impact of an individual environmental exposure is more pronounced when it occurs as part of a mixture (i.e., whether the components of the mixture interact).

METHODS

Study Population

Data for this study were collected as part of the Fresno Asthmatic Children's Environment Study (FACES), a longitudinal epidemiologic study of children with current asthma in Fresno, California. The details on recruitment and health evaluations have been reported previously [21,115]. Briefly, the FACES study collected data on short- and long-term effects of ambient air pollution on children

with asthma between 2000 and 2008. A total of 315 children between the ages of 6 and 11 years at baseline who had a primary residence within 20 km of the Fresno EPA Supersite Monitor [196] were followed over this period. Study participants provided a detailed account of their general health history and exposures to several factors including secondhand tobacco smoke. Participants also performed scheduled spirometry throughout the study period and answered questions about asthma-related symptoms.

For this study, we conducted analyses of first available health evaluation (typically at the study baseline) for children with complete information on respiratory and overall health, demographic data and house addresses. The Institutional Review Boards of the participating institutions (University of California, Berkeley, and secondarily, Colorado State University) approved the study protocol, and written informed consent/assent was obtained from the parents or legal guardians of all participants.

Pulmonary Function Measures

Spirometry was performed using the EasyOne spirometer (Medical Technologies, Chelmsford, MA, USA). Children were asked to complete three (up to a maximum of eight attempts) acceptable flow-volume loop maneuvers, in accordance with recommendations for spirometry performance provided by the American Thoracic Society/European Respiratory Society. Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC) and forced expiratory flow 25–75% ($FEF_{25–75}$) were primarily assessed. Our study makes use of the first available recorded measures (one observation per participant), typically the spirometry measures collected at baseline. Internal sex-, age-, height- and ethnicity-specific residual values for FEV_1, FVC, $FEF_{25–75}$ were computed for

analysis using spirometric reference equations for the US population [197]; lung function variables were regressed on age and height after controlling for sex and race/ethnicity.

Air Pollution Exposures

Air quality data were obtained from the EPA Air Quality System for individuals based on their geocoded residential address. Pollutant concentrations from the air monitoring stations located closest to the residential location (within 10 km) were obtained for 24-hr measurements of particulate matter with aerodynamic diameter < 2.5 μm ($PM_{2.5}$) and <10 μm (PM_{10}), 8-hour maximum concentration over 24 hours for ozone (O_3), and 1-hour maximum concentration over 24 hours for nitrogen dioxide (NO_2). We summarized concentrations as quarterly averages for exposures 3 months prior to pulmonary function measurements, as simultaneous pesticide exposures are only reported in ≥ 3-month cycles.

Pesticide Exposures

We estimated agricultural pesticide exposure based on residential location and the California Pesticide Use Report (PUR) Data. The PUR, one of the most comprehensive databases of its kind, provides the amount (kg), date, and location (to one-square-mile sections) of specific pesticides (active ingredient) applied in the state quarterly. We characterized potential participant exposures to three classes of pesticides (carbamates (C), methyl bromide (MeBr), and organophosphates (OP)). Organophosphates and carbamates are acetylcholinesterase inhibitor pesticides implicated in exacerbation of respiratory disease and asthma due to their cholinergic action on airway smooth muscle and mucus-secreting epithelial cells [66]. MeBr, a restricted-use fumigant, has also been implicated in adverse respiratory effects; the toxicological mechanism of action of MeBr is poorly understood, but believed to be due to the high reactivity associated with alkyl halides [198]. Using

the purexposure package in R [199] (https://github.com/leighseverson/purexposure), we created pesticide exposure measures corresponding to the 3-month period prior to spirometry, based on residential addresses using algorithms developed by Gunier *et al*. [119]. Pesticide exposure was estimated by calculating the percentage of land area for each section within a 3km buffer (this choice selected based on the spatial scale most strongly correlated with pesticide fate and transport); multiplying kilograms of active ingredient applied to each section (within the 3-month time frame) by the proportion of area within selected buffer, summing the area weighted mass (in kg) for all sections that intersect the buffer, and dividing by the buffer area.

Covariates

We developed a set of plausible confounders *a priori* based on previous studies of lung function and exposure to air pollution or pesticides [22,75,109,184,200–203], as well as directed acyclic graphs (DAGs). As described above, the pre-analysis transformation of outcome variables using residual values included the use of age, height, sex and race/ethnicity as confounders; we also considered meteorological conditions (linear representations of temperature and precipitation averaged over 3 months prior to spirometry), and seasons (fall/winter/spring/summer, based on evaluation of meteorological and air pollution patterns in the Fresno region); as well as subject-specific characteristics potentially associated with asthma and asthma exacerbation: body mass index (linear BMI), maternal education level (≤/> 12th grade education), household income (</≥ $30k), insurance status (yes/no), atopy (yes/no), and a measure of asthma severity (modified Global Initiative for Asthma (GINA) score, </≥ 3); and other competing deleterious exposures: smoker currently in home (yes/no), and self-reported residence proximity to a major roadway (</≥ 1 block away).

Statistical Analysis

We applied BKMR to estimate the association between exposure to a multipollutant mixture of air pollutants and pesticides and each outcome measure of lung function. Extensive details on the statistical approach are described elsewhere [96,97]. BKMR offers two important advantages in the context of characterizing the risk from multiple pollutants in environmental epidemiology studies. First, BKMR is able to estimate the exposure-response surface for health-related effects of multipollutant ambient exposures without prior specification of the E-R function. This means that the models can flexibly estimate potential nonlinear (such as threshold effect or polynomial effect) or non-additive interactive (for example synergism only beyond a particular copollutant exposure threshold) relationships, as are prevalent in environmental epidemiology studies [93,117,204–207]. Second, beyond a component-wise variable selection, the BKMR approach performs a hierarchical variable selection (HVS) that can incorporate prior knowledge on the structure of the environmental mixture. Observed E-R associations in environmental epidemiology studies may depend on only a subset of the mixture components [187,208], the HVS in BKMR uses methods within a Bayesian paradigm to identify important mixture components responsible for the health effects of the mixture whole accounting for the overall structure of the mixture, and possible correlations between exposures [97].

The hierarchical or multistep approach to variable selection first estimates the probability that each pre-specified pollutant group/domain be included in the model, and then assesses for evidence in the data that one of the components in a domain drives the group's effect on the outcome. Variable selection yields posterior inclusion probabilities (PIP) computed as the posterior probability of a *regression coefficient* being assigned a non-zero value, and representative of

relative variable importance by magnitude. PIP values range between 0 and 1 indicating the level of certainty or uncertainty that the component is included in the model and is associated with the outcome; a value of 1 indicates the component was always included into the model and associated with the outcome, and a PIP value of 0 indicates the component was never selected in the model and no association with the outcome.

The primary form of the BKMR model is given as:

$$y_i = h(x_{i1}, \dots, x_{iM}) + z_i^T \beta + \epsilon_i$$

For each subject $i = 1, \dots, n$, BKMR relates the health outcome (y_i) to the M components of the exposure mixture $x_i = (x_{1i}, \dots, x_{Mi})$ through an unknown but smooth function $h(\cdot)$, which represents the exposure-response function that accommodates non-linearity and/or interaction among the mixture components, while controlling for C relevant confounders $z_i = (z_{1i}, \dots, z_{Ci})$.

For our analyses, we implemented BKMR with the Gaussian kernel function, which captures an extensive range of underlying functional forms for $h(\cdot)$. A Gaussian kernel assumes that two subjects with similar exposure profiles would most likely have a similar health outcome profile, intuitively reducing the possible exposure-response surface while accounting for a wide range of realistic scenarios [97].

We ran three primary BKMR models to assess exposure association with regression-adjusted FEV_1, FVC and FEV_1/FVC. For each model, we selected HVS formulation. The HVS specification incorporates our prior knowledge on the structure of the mixture (with respect to domains) and provides pollutant (component-) and domain (group-) specific importance (PIP) ranks/scores. We grouped the exposures into two domains: air pollutants (O_3, NO_2, $PM_{2.5}$, PM_{10}), and pesticides (C,

MeBr, OP). In sensitivity analysis, we created three exposure groups, splitting air pollutants into particles ($PM_{2.5}$, PM_{10}) and gases (O_3, NO_2).

We modeled the effects of multipollutant exposures on lung function, Y_i, as

$$Y_i = h[Group\ 1 = C_i, MeBr_i, OP_i; Group\ 2 = NO_{2i}, O_{3i}, PM_{2.5i}, PM_{10i}] + \beta^T Z_i + e_i$$

Like with most complex Bayesian models, we sampled values from the posterior distributions using a Monte-Carlo Markov Chain a computer-driven sampling method that characterizes the distribution by randomly sampling values out of the distribution without the integration of high-dimensional functions. We ran the default MCMC sampler (described in detail in Bobb *et al*. [97]) for 25,000 iterations after a burn in of 25,000 and every fifth sample was kept for inference.

We used square root and then centering and scaling (subtracting the vector mean and dividing by the standard deviation) transformation for the exposure measures to account for the severe right-skewedness typical of pesticide concentrations [209]. All other continuous variables (outcome and covariates) were centered and scaled to achieve uniformity. Hence, we expressed changes in exposure concentration as z-scores, and health associations as a change in z-score of lung function (FEV_1, FVC or $FEF_{25–75}$) residuals. Convergence of the Markov Chain was monitored by inspecting trace plots of model parameters.

As a follow-up analysis and, in particular, to test for potential interactions among mixture components, we fitted multivariable linear regression models to estimate the effects of specific constituents of interest.

Finally, all statistical model assumptions were inherent in the BKMR model [96,97], and did not require manual assessment or justification of linearity and additivity for the functional form of the E-R relationship as with parametric modeling methods.

We analyzed the data using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina) for data cleaning and linear regression, and R version 3.3.0 (R Foundation for Statistical Computing, Vienna, Austria) for BKMR analysis using the *bkmr* package [210]. We refer to associations as statistically significant if 95% posterior credible intervals exclude the null.

RESULTS

Study Population Characteristics

Of the 315 children recruited for the FACES study, complete exposure, outcome, and covariate data were available for 153 children (48.6%, Table 3.1). There were no significant differences in sociodemographic or exposure characteristics between children included and those excluded from the study (Appendix Table 4).

The majority of children included in our study were non-Hispanic White (46.4%), male (60.1%), and on average 9 years (SD: 1.8); the mean BMI was 18.5 kg/m^2 (SD: 4.6). Over three-quarters of participants were atopic (defined by positive skin reaction to one or more allergens or physician's diagnosis of allergic rhinitis or eczema, 78.4%), and were also more like to have a diagnosis of mild intermittent or mild persistent asthma (GINA category < 3, 82.3%), and be insured (94.8%). Over half (52.3%) of participants had addresses within one block of a freeway, and only 5.2% reported either parent as a current smoker. With regard to pulmonary function, 11.1%, 7.8% and 28.8% of

participants had FEV_1, FVC and FEF_{25-75} values below 80% of the predicted reference value, respectively.

TABLE 3.1. DESCRIPTIVE CHARACTERISTICS OF FACES SUBCOHORT

	n = 153
Age years, mean (SD)	9 (1.8)
Male Gender, %	60.1
Height (in), mean (SD)	52.3 (4.8)
BMI (kg/m^2), mean (SD)	18.5 (4.6)
Ethnicity, %	
Non-Hispanic Black	13.7
Non-Hispanic White	46.4
Hispanic	39.9
Mother > 12th grade education, %	60.8
Insured, %	94.8
Atopy, %	78.4
Father/Mother Smokes (Current), %	5.2
Proximity to Freeway (< 1 block away), %	52.3
Severity (GINA ≥ 3), %	17.7
FEV_1, mean in L (SD)	1.7 (0.4)
FEV_1 < 80% predicted, %	11.1
FVC, mean in L (SD)	2.0 (0.5)
FVC < 80% predicted, %	7.8
FEF_{25-75}, mean in L/s (SD)	1.8 (0.4)
FEF_{25-75} < 80% predicted, %	28.8

Exposure Characteristics

The distributions of pollutant metrics are presented in Table 3.2. The mean (37.9 $\mu g/m^3$, SD: 10.7) concentrations of daily (24-hr) $PM_{2.5}$ exceeded the current National Ambient Air Quality Standard (NAAQS) 24-hr standard of 35 $\mu g/m^3$. All other ambient air pollutants had distributions below NAAQS standards, with average NO_2 and O_3 values well below the current NAAQS annual standard over the entire time period (NO_2 mean: 15.5 ppb, SD: 3.3; O_3 mean: 35.8 ppb, SD: 12.4).

As expected, the distribution of pesticide exposure concentrations was right skewed, with several participants assigned low and zero exposure values based on their residential addresses.

Correlations between pairs of the exposure variables and meteorological conditions are presented in Figure 3.1. In general, the strongest correlations among AAP are seen between NO_2 and $PM_{2.5}$ (ρ = 0.9), which are both negatively correlated with O_3 (ρ = -0.6 and -0.5, respectively). OPs were moderately positively correlated with O_3 (ρ = 0.5) and moderately negatively correlated with NO_2 (ρ = -0.5), while temperature and precipitation were, as expected, correlated with O_3 (ρ = 0.8 and -0.7 respectively).

TABLE 3.2. EXPOSURE DATA SUMMARY STATISTICS

Pollutant/Pesticide	Minimum	Mean	SD	Median	Interquartile Range	Maximum
Nitrogen Dioxide (ppb)	9.5	15.5	3.3	14.4	5.3	23.1
Ozone (ppb)	12.3	35.8	12.4	38.0	16.4	58.2
Particulate Matter < 2.5 µm ($PM_{2.5}$) (µg/m^3)	6.7	16.3	9.8	11.2	8.1	40.2
Particulate Matter < 10 µm (PM_{10}) (µg/m^3)	19.5	37.9	10.7	32.5	16.9	65.9
Carbamates x 10^6 (kg/3km^2)	0.0	0.1	0.3	0.0	0.1	2.4
Methyl Bromides x 10^6 (kg/3km^2)	0.0	3.9	9.9	0.0	0.0	48.9
Organophosphates x 10^6 (kg/3km^2)	0.0	0.9	1.1	1.1	1.2	5.4

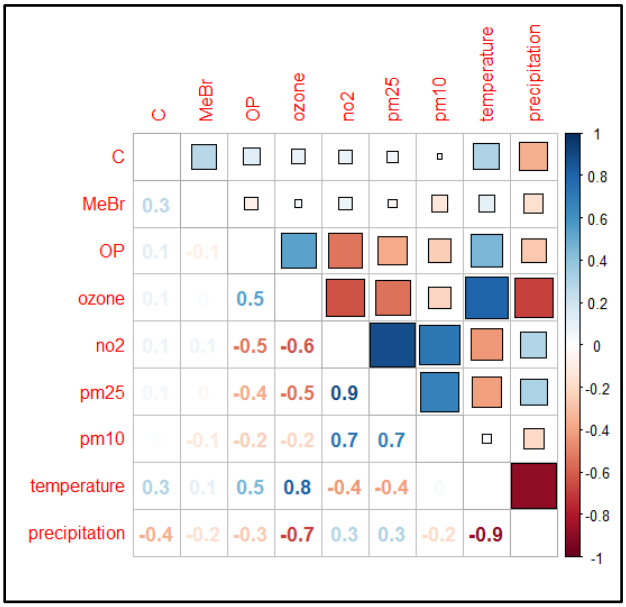

FIGURE 3.1. PAIRWISE PEARSON CORRELATION AMONG THE SEVEN ENVIRONMENTAL EXPOSURE MEASURES AND METEOROLOGICAL PARAMETERS.
Abbreviations. NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with a median aerodynamic diameter < 2.5µm; PM_{10}, particulate matter with a median aerodynamic diameter < 10µm; OP, organophosphates; C, carbamates and MeBr, methyl bromide.

BKMR Analyses

The PIP from the BKMR models for the two exposure domains (group PIP) and each exposure (conditional PIP) are presented in Table 3.3. PIP values represent the probability that certain components or component groups/domains are responsible for the health effects of the mixture, following the variable selection process. We used a commonly implemented PIP threshold for "variable importance" of PIP > 0.5 [96,211,212]; values above 0.5 indicated the importance of both individual components and domains in model inclusion, and association with the outcomes. The PIPs in each model indicated a clear separation between the domains, with AAPs driving the mixture in the FEV_1 and FVC models (PIP: 0.75 and 0.78 respectively), and pesticides driving the mixture in the FEF_{25-75} model (PIP: 0.77). Within the exposure domains, NO_2 and OP were the most important drivers for both FEV_1 and FVC models in our study (PIP: 0.59 and 0.55 respectively). In the FEF_{25-75} model, MeBr was the most important pesticide and PM_{10} was the most important AAP, although both PIP values fell below the 0.5 threshold for variable importance (PIP: 0.46 and 0.28 respectively).

TABLE 3.3. GROUP AND CONDITIONAL POSTERIOR INCLUSION PROBABILITIES (PIPS) FROM BKMR USING EXPOSURE DOMAIN GROUPS FOR HIERARCHICAL VARIABLE SELECTION.

Exposure	PIPs (by PFT)					
	FEV_1		FVC		FEF_{25-75}	
	Group	Conditional	Group	Conditional	Group	Conditional
O_3	**0.75**	0.10	**0.78**	0.10	0.51	0.27
NO_2	**0.75**	**0.59**	**0.78**	**0.55**	0.51	0.19
$PM_{2.5}$	**0.75**	0.25	**0.78**	0.29	0.51	0.25
PM_{10}	**0.75**	0.06	**0.78**	0.06	0.51	**0.28**
C	0.67	0.12	0.55	0.16	**0.77**	0.41
MeBr	0.67	0.11	0.55	0.15	**0.77**	**0.46**
OP	0.67	**0.77**	0.55	**0.69**	**0.77**	0.13

Group PIPs indicate the posterior probability of an exposure domain being included in the model; Conditional PIPs indicate the posterior probability of a single exposure within the domain to be included in the model. Both provide an illustration of the relative ranking of variable importance for each exposure domain as well as each exposure within a particular domain. Cell colors indicate different exposure domains: grey, ambient air pollutants; white, pesticides. Bold font indicates highest PIP in column.

In the HVS models fit with three groups in sensitivity analysis, results from the main analysis persisted. The gases (driven by the E-R relationship NO_2) were the main drivers of the FEV_1 and FVC models, while pesticides (driven by the E-R relationship with MeBr) remained the main drivers of the FEF_{25-75} model (Appendix Table 5).

In Figure 3.2 we graphically present the shape of the exposure-response function for each component of the pollutant mixture while accounting for all the other components (held at their median). Overall, we observed mostly small changes in lung function per unit increase in exposure, with considerable variability especially at higher concentrations. For FEV_1 and FVC models, we observed approximately linear associations with lung function for NO_2, O_3 and PM_{10}; a near-linear trend was also observed for OP at higher concentration; and largely null trends for the other exposures. For the FEF_{25-75} model, we observed approximately linear effects for MeBr, O_3, $PM_{2.5}$ and PM_{10}, with a linear trend observed for C at higher concentrations.

Figure 3.3 displays summaries of the overall effect of the mixture on the lung function measures. Each panel represents a numeric summary of the change in lung function associated with a simultaneous change in each of the seven exposures from the 25th percentile to 75th percentile, as compared to their median value (50^{th} percentile). Panels A and B showed a consistent decrease in FEV_1 and FVC with increased exposure to the AAP and pesticide mixture. The joint effects were particularly deleterious when all exposures were above their 60^{th} percentile, however the credible intervals around the effect estimates contained the null value, indicating lack of statistical significance. For example, the effect of the overall mixture at the 30^{th} percentile (compared to the median) was a 0.2L (0.3 SD, 95% CI: -0.3, 0.9) change in the FVC, but a -0.1L (-0.2 SD, 95% CI: -0.5, 0.4) change in FVC at the 70^{th} percentile (compared to the median). For the FEF_{25-75} model, we see

ordered effects opposite those observed for the other outcome measures, with higher quantiles appearing less harmful. The effect estimates are, however, relatively smaller (within ± 0.1 SD of the median value), and credible intervals largely overlap the null across all quantiles.

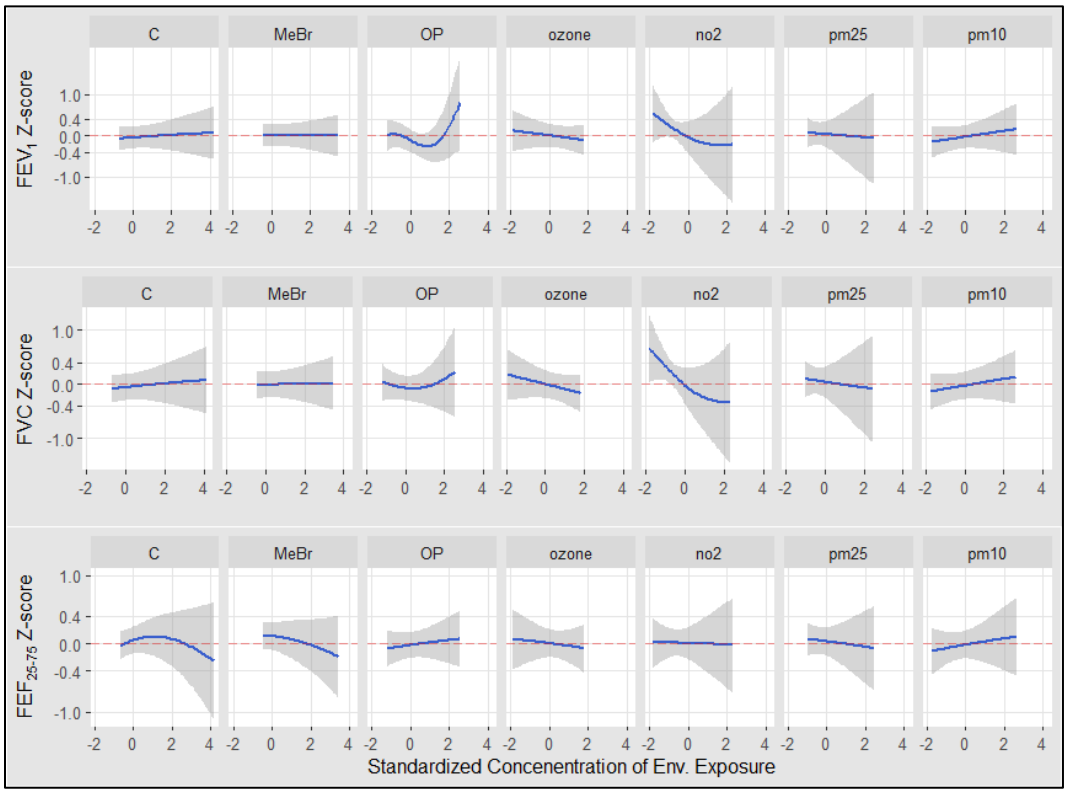

FIGURE 3.2. THE EXPOSURE-RESPONSE RELATIONSHIP BETWEEN EXPOSURE TO ENVIRONMENTAL AGENTS AND LUNG FUNCTION.
Figure shows univariate relation between each exposures and outcomes, with other exposures fixed at their median value. The results were assessed by the BKMR model adjusted for age, height, sex, race/ethnicity, temperature, precipitation, season, BMI, maternal education level, household income, insurance status, atopy, a measure of asthma severity (modified Global Initiative for Asthma (GINA) score), smoker currently in home, and proximity to a major roadway.
Abbreviations. NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with a median aerodynamic diameter < 2.5µm; PM_{10}, particulate matter with a median aerodynamic diameter < 10µm; OP, organophosphates; C, carbamates and MeBr, methyl bromide.

We also estimated univariate summaries of the change in lung function values associated with a change in each single exposure from its 25th percentile to the 75th percentile, with all of the other exposures fixed at three preselected thresholds (25th, 50th, or 75th percentiles). These

illustrations (Figure 3.4) provide further indication of the main driver pollutants within the overall mixture.

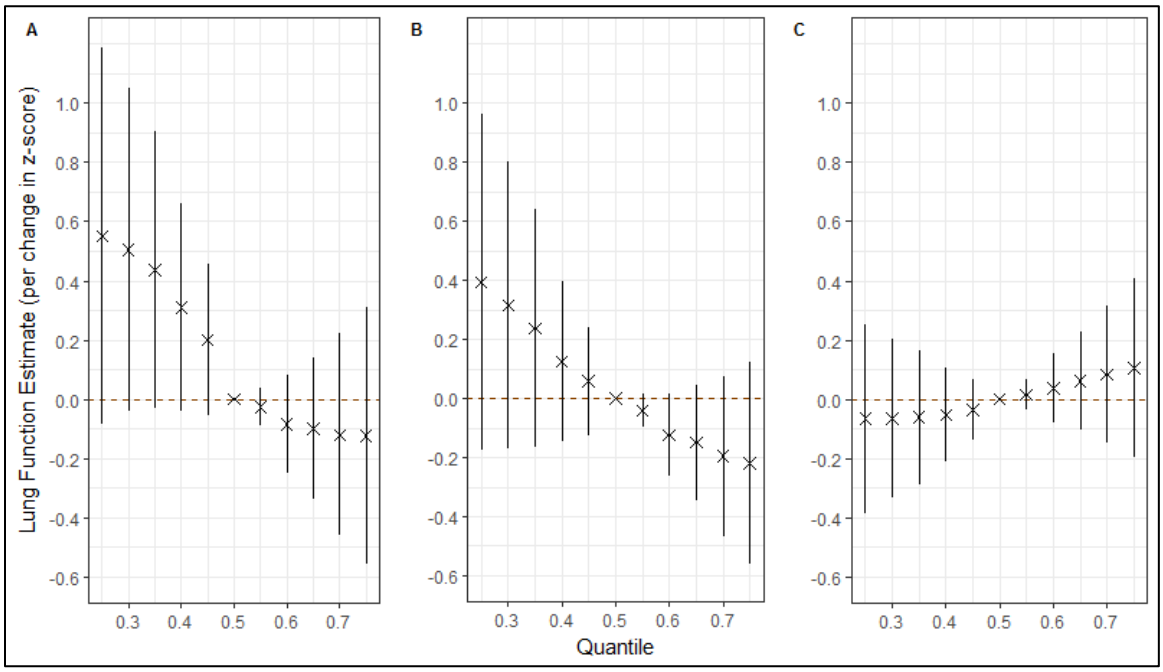

FIGURE 3.3. OVERALL EFFECT (95% CI) OF MIXTURES ON THE THREE LUNG FUNCTION MEASURES.
Figures depict the effect on lung function outcomes (FEV_1, FVC, and FEF_{25-75}) when all the environmental exposures (ozone, nitrogen dioxide, $PM_{2.5}$, PM_{10}, organophosphates, carbamates and methyl bromide) at particular percentiles were compared to all the exposures at their 50th percentile. The models were adjusted for age, height, sex, race/ethnicity, temperature, precipitation, season, BMI, maternal education level, household income, insurance status, atopy, a measure of asthma severity (modified Global Initiative for Asthma (GINA) score), smoker currently in home, and proximity to a major roadway.

The single pollutant associations (estimates and 95% credible intervals) with lung function are characterized by the distance from the horizontal dotted lines. In general, AAPs had relatively stronger effects on lung function measures than pesticides, although the positive effect of carbamate pesticides appeared to be an important driver of the exposure association in the FEF_{25-75} model. We also noted that the estimates in Figure 3.4 suggested possible interaction (nonparallel estimates within some single pollutant associations), particularly for NO_2 and $PM_{2.5}$ in panels A and B.

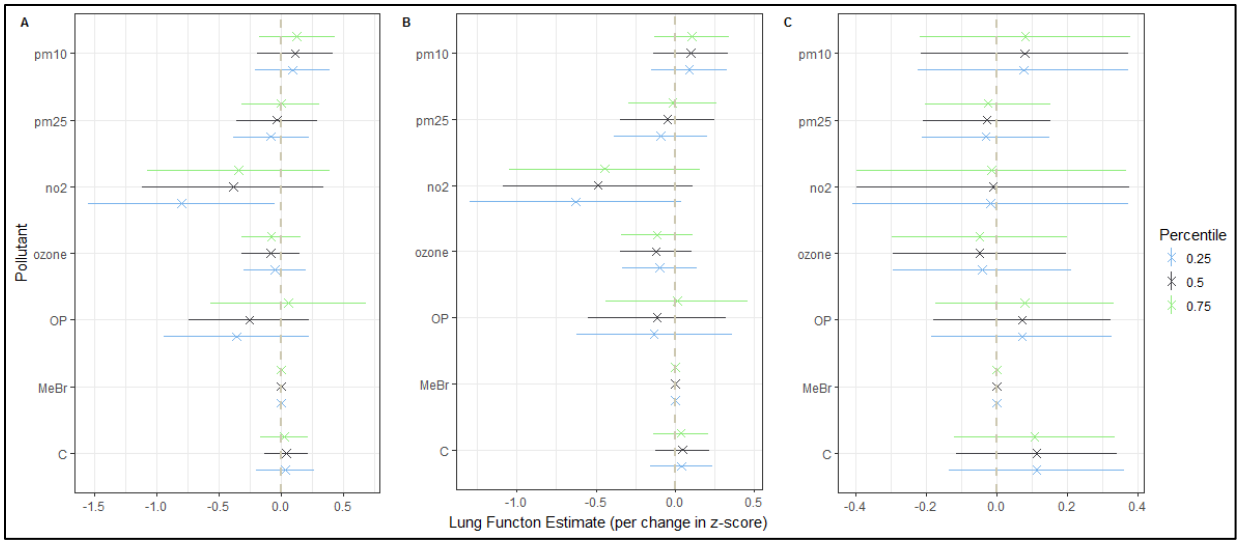

FIGURE 3. 4. SUMMARY OF THE CONTRIBUTION OF INDIVIDUAL EXPOSURES ON THE OUTCOME (ESTIMATES AND 95% CREDIBLE INTERVALS).

This plot compares the lung function when a single exposure is increased from the 25th to the 75th percentile, while all the other exposures are fixed at either the 25th, 50th, or 75th percentile. A. FEV_1; B. FVC; and C. FEF_{25-75}. Grey vertical dashed line represents the null. Abbreviations. NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with a median aerodynamic diameter < 2.5µm; PM_{10}, particulate matter with a median aerodynamic diameter < 10µm; OP, organophosphates; C, carbamates and MeBr, methyl bromide.

To assess possible two-way interactions, we plotted bivariate cross-sections of the exposure–response function (Appendix Figure 2). Although we observed mostly parallel patterns (parallel lines indicate no-interaction), Appendix Figure 2 highlights nonparallel patterns (non-parallel lines indicate possible interaction) for 'NO_2 + $PM_{2.5}$' and 'OP + $PM_{2.5}$' in both FEV_1 and FVC models, and for 'C + MeBr' and 'MeBr + ozone' in the FEF_{25-75} model, indicating possible interaction between these exposures. At higher NO_2 exposure levels, the negative effects of $PM_{2.5}$ on FEV_1 and FVC appear to be diminished. Similarly, at higher levels of OPs, the $PM_{2.5}$ curves on FEV_1 and FVC appear to be less steep. The negative effects on FEF_{25-75} observed for MeBr were greatest at the highest levels of C, and less harmful at higher levels of ozone.

Summary of linear regression (no-interaction) models assessing the associations between exposure to environmental agents and lung function measures are displayed in Table 3.4. When examined one exposure at a time, most agents were negatively associated with FEV_1 and FVC; NO_2

and OP had statistically significant associations in opposite directions (β_{NO2} (95% CI): -0.3 (-0.6, -0.1); β_{OP} (95% CI): 0.2 (0.0, 0.4)). When mutually adjusting for other agents, only NO_2 remained statistically significant. In FEF_{25-75} models, only MeBr had statistically significant associations with lung function in the single exposure model (β_{MeBr} (95% CI): -0.2 (-0.3, -0.0)). In multivariable linear regression (interaction) models including all the main exposure effects and all interactions highlighted by BKMR (as above), we observed statically significant interaction terms for only 'NO_2 + $PM_{2.5}$' in FEV_1 ($p = 0.02$) and FVC ($p = 0.01$) models, and 'C + MeBr' in the FEF_{25-75} model ($p < 0.05$).

The linear regression models provide quantification of the association between individual exposures and lung function in single exposure, copollutant and interaction models. In general, most of the results mirror the E-R associations observed in BKMR models (see Figure 3.2). However linear regression models were unable to capture some of the non-linear associations, for example OP on FEV_1 and FVC or carbamates on FEF_{25-75}. Further, the linear models were unable to quantitatively identify which pollutants drive the mixture, and the estimates obtained in multipollutant and interaction models may be unstable/unreliable in the presence of high correlation between exposure components, as observed in this study.

TABLE 3.4. ASSOCIATION BETWEEN ENVIRONMENTAL AGENTS AND LUNG FUNCTION BASED ON LINEAR REGRESSION MODELS

	FEV_1		FVC		FEF_{25-75}	
Environmental Agent	Single exposure	Multiple exposures	Single exposure	Multiple exposures	Single exposure	Multiple exposures
	β (95% CI)[a]	β (95% CI)[a,b]	β (95% CI)[a]	β (95% CI)[a,b]	β (95% CI)[a]	β (95% CI)[a,b]
NO_2	**-0.31 (-0.55, -0.07)**	**-0.72 (-1.22, -0.22)**	**-0.35 (-0.59, -0.11)**	**-0.79 (-1.29, -0.29)**	-0.02 (-0.28, 0.24)	-0.06 (-0.61, 0.49)
O_3	-0.06 (-0.36, 0.25)	-0.19 (-0.50, 0.11)	-0.09 (-0.39, 0.22)	-0.23 (-0.53, 0.07)	0.11 (-0.21, 0.42)	0.03 (0.31, 0.36)
$PM_{2.5}$	-0.16 (-0.42, 0.11)	-0.01 (-0.47, 0.44)	-0.18 (-0.44, 0.08)	-0.01 (-0.45, 0.44)	0.02 (-0.26, 0.30)	-0.04 (-0.53, 0.46)
PM_{10}	-0.03 (-0.23, 0.18)	**0.42 (0.02, 0.82)**	-0.05 (-0.26, 0.15)	**0.44 (0.05, 0.84)**	0.11 (-0.10, 0.32)	0.10 (-0.34, 0.53)
C	0.01 (-0.14, 0.16)	0.09 (-0.07, 0.25)	0.02 (-0.13, 0.17)	0.10 (-0.06, 0.26)	-0.06 (-0.22, 0.10)	-0.03 (-0.21, 0.15)
MeBr	-0.06 (-0.20, 0.08)	-0.01 (-0.16, 0.14)	-0.04 (-0.18, 0.10)	0.01 (-0.14, 0.16)	**-0.15 (-0.29, -0.00)**	-0.13 (-0.29, 0.04)
OP	**0.21 (0.03, 0.39)**	0.12 (-0.09, 0.32)	**0.21 (0.03, 0.39)**	0.11 (-0.09, 0.32)	0.11 (-0.08, 0.30)	0.10 (-0.12, 0.32)

CI = confidence interval, β estimates represent the mean change in the lung function measure per unit (z-score) increase in the exposure agent. Bold font represents statistical significance.

DISCUSSION

Short and long-term exposure to environmental chemicals including ambient air pollutants and pesticides have been shown to have negative respiratory health effects, particularly among people with asthma. We present a study examining the joint effects of AAPs and pesticides, as multipollutant exposures, on lung function among children with asthma in the San Joaquin Valley, a region with unique and diverse environmental exposure characteristics.

In this cross-sectional study, we used BKMR to explore the effects of a 3-month aggregate exposure of a mixture of AAPs and pesticides as factors that influenced the lung function of children with asthma who live in an urban area surrounded by large areas of agricultural activity and dense vehicular traffic.

The most important drivers of joint effects in models assessing FEV_1 and FVC were the AAPs; NO_2 in particular was identified as the most important contributor (highest conditional PIP).

Although not statistically significant using two-sided 95% credible intervals, the estimated effect of the overall mixture in these models driven by NO_2 followed a negative exposure-response pattern. Higher percentiles of joint exposure to all pollutants resulted in increasingly worse lung function among the study population, particularly when all exposures were above the 60^{th} percentile. The main driver of the FEF_{25-75} model was pesticide exposure. Contrary to the other models, the relationship between the overall mixture and FEF_{25-75} was positive, although these effect estimates were relatively smaller, less precise, and not statistically significant.

We recognize that our non-significant findings may be in part due to small sample sizes, unmeasured or/and residual confounding. Alternatively, the observation of inconsistent effects of cumulative exposures on measures of lung volumes (FEV_1, FVC) versus airway obstruction (FEF_{25-75}) may be due to other factors. A few studies with both volume and flow measures have observed differing associations [213]. In our study, these findings may indicate differences in exposure window sensitivity (the effects of 3-month exposures may be different from acute or chronic exposures) [213,214]. Raanan et al. found reduced lung volumes but no evidence of airway obstruction in association with early life exposures to organophosphate pesticide in 7-year-old children in the CHAMACOS study [22]. Differential findings may also be a function of asthma severity, as FEF_{25-75} in the setting of a normal FEV_1 has been linked to more severe asthma [215]; a large proportion of our population had mild or moderate asthma at the time of outcome assessment. Finally, this difference in model results may be due to a difference in key exposures (pesticides vs. AAP vs. joint exposures). We observed that the main drivers for the FEF_{25-75} models differed from those for the FEV_1 and FVC models. More refined epidemiological and toxicological

analyses are required to conclusively relate effects of these kinds of exposures on specific spirometric measures.

This study contributes to the literature focused on exploring the association between multidomain exposures and health outcomes [107,108]. In particular, it adds to our work on health effects of multidomain exposures as part of the Aggravating Factors of Asthma in a Rural Environment (AFARE) study. We previously observed significant associations between joint exposure to ozone, $PM_{2.5}$ and OPs, and a biomarker of lung inflammation (leukotriene E4) among children with asthma [174]. We build on the multidomain approach by including more plausible exposures and a clinical marker of respiratory health, while accounting for limitations associated with multipollutant analysis.

Overall, our results are difficult to contextualize given the paucity of studies on ambient exposure mixtures and pediatric respiratory health in the multidomain context. However, there is some consistency between our results and several "multipollutant" air pollution health effect studies. For example, Ierodiakonou et al., recently reported negative associations between exposure to air pollution (ozone, carbon monoxide, NO_2, and sulfur dioxide concentrations) and lung function (including FEV_1 and FVC) among children with asthma in a longitudinal study [184]. These effects were observed with multiple short-term exposure windows including 4-month averages. Similarly, in a longitudinal study of school children, Barraza-Villarreal and colleagues showed an inverse relationship between pulmonary function (FEV_1 and FVC) and exposures to ozone and $PM_{2.5}$ among children with asthma[216]. Both multipollutant approaches used linear mixed models which tested the health effect of one pollutant (as the main predictor), while adjusting for exposure to the other pollutants, and fail to account for the nature of correlation between

environmental exposures and possible nonlinear effects on the health outcome [77,96,97]. By using BKMR, we evaluated for a potentially nonlinear and/or non-additive function of pollutant concentrations that effectively reflects the joint association of the exposure mixture with pediatric asthma outcomes.

The few pesticide-respiratory health effect studies among children adopt the single-pollutant approach, and have shown both negative and null effects of pesticide exposure on lung function [22,201,217]. For example, the study by Ranaan et al. provides evidence of significant FEV_1 and FVC decreases with exposure to organophosphate pesticides among children enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study [22]. However, the authors explored early life exposure windows of children with no asthma, only in single-pollutant analyses. More recently, another study on 7-year old children from the same CHAMACOS cohort showed that exposure to agricultural fumigants including MeBr resulted in no adverse respiratory effects [201]. The authors again only conducted single-pollutant analyses. The studies fail to explore complex and nonlinear synergistic effects that likely exists between other environmental exposures within and outside the domain of pesticides.

Several methods have recently been suggested to examine the associations between multipollutant mixtures and health effects in environmental epidemiology. For example, Davalos et al. recently reviewed statistical methods that investigate the association between short-term exposures to multipollutant mixtures, highlighting several broad classes of methods [83]. Billionnet et al. also reviewed current approaches to studying mixtures, and concluded that researchers should base method choices on the specific study characteristics [99]. As a way of examining harmful health effects based on multidomain, multipollutant approach, BKMR provides

tools to disentangle 1) the effects of nonlinearity and collinearity; 2) exposure effects which may be more complex than simple, additive interaction structures; and 3) overall joint effects using Bayesian methods. Notably, the flexible Gaussian kernel function tool in BKMR models have been demonstrated to capture an extensive range of real-world E-R functional forms, and the hierarchical variable selection tool represents a multistep approach to variable selection which accommodates the partitioning of correlated component exposures into groups based on prior external knowledge.

It is important to note that we highlighted high correlation between components of the mixture and nonlinear independent (main effects) associations with lung function parameters. In addition, there was evidence of complex and possibly non-synergistic interactions within the overall mixture, such that the potentiating effects of an exposure on another may be nullified by antagonizing effects from a third exposure (as evidenced by the relationship between MeBr, carbamates and ozone on FEF_{25-75}). These are common types of complexities in the environmental epidemiology field, and while traditional models like generalized multivariable linear regression (using ordinary least squares) are easy to implement and interpret, these models have shortcomings in some regards. Our results indicated that these linear regression may be appropriate for estimating the exposure-response functions when the underlying function is linear or has mild nonlinearities. However, unlike BKMR, the linear models were unable to ascertain which pollutants driving the mixture, and the high correlation between exposure components in the study limited the reliability of results in copollutant and interaction models. Linear regression models were also limited beyond quantifying the association between lung function and individual

exposures; BKMR suitably provided answers to one of our main study objectives, evaluating the "overall" mixture effect on lung function.

The mechanisms for individual or joint effects of environmental exposures continue to be a subject of deliberation and research. Previously published studies of exposure to pesticides/AAPs and lung function have generally suggested that airway inflammation and hyperresponsiveness are important mechanistic features [19,136,138]. However, toxicological mechanisms remain poorly understood. Given the limited understanding of the mechanistic pathways for these exposures, it may be that any joint effects may be possibly due to simultaneously present but differing mechanisms of action. Nevertheless, joint exposure to AAPs and pesticides is not uncommon, especially in regions such as the SJV where there is a high prevalence of children with asthma [55,112], and thus is relevant to consider in multipollutant frameworks [88].

There are some limitations to the current study. First, we rely on area measurements of exposures from central ambient air quality monitors and the California Pesticide Use Report (PUR) data which fail to account for individual time-activity patterns and may result in exposure measurement error. This error would be expected to be non-differential with respect to the study outcome, and independent of any other biases, would likely drive effects toward null values. However, the use of residential addresses to determine exposures is a strength compared with studies using community-level data. We acknowledge that missing lung function data could result in bias. However, the missing data on the outcome was likely random, and demographic characteristics of participants included in the study and those excluded were not appreciably different.

Although our study typifies the agricultural-urban interface, recruitment into the FACES cohort was geared towards capturing more urban exposures (within distance of the Fresno Super Site

monitor) than agricultural pesticide exposures. Hence, it is likely that the effects of pesticide exposure in this cohort are less than representative.

Lastly, as we are limited by PUR data to only 3-month exposures, we were unable to assess the effect of joint exposures at other short-term lags.

Our study addresses the respiratory health effects of exposure to a suite of important ambient air pollutants in the context of simultaneous exposure to regional agricultural pesticides, exploring multiple pollutant exposures in multiple exposure domains. Understanding how environmental exposures, particularly in the context of multidomain, multipollutant mixtures, influence pediatric asthma morbidity in uniquely exposed populations may ultimately inform regulatory policies to reduce these modifiable factors that contribute to disease burden.

CHAPTER 4: THE FROZ+EN STUDY

ESTIMATING EFFECTS OF ACUTE AMBIENT OZONE AND $PM_{2.5}$ EXPOSURE ON UNSCHEDULED ASTHMA HOSPITALIZATIONS IN THE COLORADO FRONT RANGE: THE FROZ+EN STUDY

SUMMARY

Acute exposure to ambient ozone (O_3) and particulate matter with diameter < 2.5 μg/m^3 ($PM_{2.5}$) has been linked to asthma-related morbidity including increased hospitalization. We assessed this association in the Northern Front Range Metropolitan Area (NFRMA) of Colorado, a unique region characterized by urban and agricultural influences on ambient air pollution. We also explored the use of a regression calibration approach to adjust for exposure measurement error affecting these associations.

We obtained measurements for O_3 and $PM_{2.5}$ from fixed-site monitoring networks and estimated individual-level exposure measurements by using an inverse distance weighted (IDW) methods. In addition, we obtained highly-resolved airborne measurements of summertime O_3 for a one-month period in 2014. We linked this data to hospital admissions data for asthma-related diagnoses for the study period (2010-2014). We estimated age- and sex-stratified associations between O_3, $PM_{2.5}$ (using multiple exposure lags) and asthma-related hospital admissions, adjusting for spatial and temporal covariates. We also evaluated regression-calibrated O_3 association with asthma hospital admissions based on the airborne measurements.

Across multiple lags, we observed an increase in relative risk (RR) for asthma-related hospital admission with exposure to year-round and winter $PM_{2.5}$ and summertime O_3, although confidence intervals around the RR contained the null value. For example, an interquartile range

(IQR) increase in $PM_{2.5}$ was associated with about a 1.5% increase in asthma-related admissions ($RR_{1\text{-day lag}}$ (95%CI): 1.1051 (0.9994, 1.0311)), while an IQR increase in O_3 was associated with a 0.9% increase in asthma-related admissions ($RR_{1\text{-day lag}}$ (95%CI): 1.0087 (0.9536, 1.0669)). Effects were consistent across lags for both $PM_{2.5}$ and O_3 models, although modest negative lagged effects were observed with O_3 models. Associations stratified by age, sex and season also revealed differences within categories. For example, exposure to $PM_{2.5}$ (one-day lag, IQR) was associated with a significant 2.9% increase in admissions among adults 65 years and older (RR: 1.0288, 95%CI: 1.0001, 1.0583), compared to a 0.7% decrease in admissions among the 15 – 64 year age group (RR: 0.9931, 95%CI: 0.9708, 1.0160). Antagonistic multiplicative interaction was also observed between $PM_{2.5}$ and O_3 ($p = 0.047$), although the difference was minimal after accounting for outlier observations.

In summertime O_3 models adjusted with regression calibration, positive association with asthma-related hospitalization persisted; the slightly larger effect estimates were surrounded by wider confidence intervals that contained the null values.

Ambient exposure to O_3 and $PM_{2.5}$ was associated with increased risk of unscheduled asthma-related hospital admissions in the NFRMA. Our results suggest the presence of concentration-dependent measurement error between fixed-site monitoring and airborne monitoring of ambient O_3, leading to possible attenuation of risk when using fixed-site O_3 concentrations as a surrogate for personal exposure measurements. Regression calibration provides a way to obtain unbiased estimators of effects in regression models when one or more predictors are measured with error.

INTRODUCTION

Ambient air pollution exposures have been associated with respiratory morbidity in numerous observational studies in the US [218–221]. For conditions like asthma, unscheduled hospitalization has been used as a measure of morbidity, with higher rates of asthma-related hospitalization indicative of the burden of disease in a particular region [29,205,222,223]. Exposure to ozone (O_3) and particulate matter with diameter < 2.5 $\mu g/m^3$ ($PM_{2.5}$) have been widely studied in the context of asthma-related hospitalizations [26,81,224–228], as these pollutants are responsible for a majority of air-pollution related health hazards [175].

Most of these types of studies have relied on pollutant concentrations measured at one or more fixed-site ambient monitor as a surrogate for personal or proximal exposure [229–231], particularly for exposures measured over a long term. While ambient monitoring network data ostensibly characterizes the temporal patterns in air pollution concentrations, data from fixed-site monitoring networks typically lack information on the spatial heterogeneity of pollutant species, and hence limits how associated study results can be interpreted [229,232]. These implications are especially important for secondary pollutants like O_3 which are formed at some distance from the primary source of its precursors [233,234]; fixed monitors located in in urban core areas with dense traffic (such as highways and city centers) are more likely to capture long range transport of O_3 and less local sources, given that local O_3 would be formed downwind of where monitors are placed [233,235].

To minimize exposure measurement error, several alternatives have been considered in addition to surrogates to personal exposure measures. Dispersion and land-use regression models are popular choices in environmental health studies, but may be limited by their emphasis on long-

term average concentrations and relatively crude characterization of temporal variability in air pollution concentrations [229]. Further, these models may demonstrate considerably high levels of uncertainty in areas where limited information is available [236]. Regression calibration is an alternative methodology to obtain estimates of effect of direct pollution exposure on health, while still using surrogates such as fixed-site monitors or spatial model derivatives, with relatively little or no personal monitor data [237,238]. The regression calibration approach provides a way to obtain less biased estimated parameters in regression models when one or several exposure variables are believed to be measured with error [230,237,239,240].

In the Front Range Ozone and Environment (FROZ+EN) study, we examined the association between unscheduled asthma-related hospital admissions and short-term exposure to $PM_{2.5}$ and O_3 in the Northern Front Range Metropolitan Area (NFRMA) of Colorado. We assessed the role of these pollutants on hospitalization in this region, as an independent main exposure effects, and together as part of a multipollutant model. We also evaluated age-, sex- and season-stratified associations between these ambient pollutant exposures and asthma-related hospital admissions.

Finally, we explored the effect of adjusting for exposure measurement error in ambient levels of summertime O_3 (measured at fixed-site monitors) in our association models, using highly-resolved airborne-derived remote-sensing measurements. Although there are no true gold standards in air pollution exposure assessment [237], remote-sensing measurements are considered to be spatially more accurate than fixed monitor measurements, even with supplementary spatial interpolation [131,236].

METHODS

Study Domain

The study's spatial domain included the NFRMA of Colorado, a geographical region extending between the North Front Range and the Denver Metro area. More than 80% of the state's population reside or work in the NFRMA, within the cities of Denver, Boulder, Longmont, Greeley, and Fort Collins [122]. As of 2016, the total population in the eight counties in the region was greater than 3.5 million, with almost 20% of people less than 18 years [110]. The air pollution mixture in the NFRMA is unique due to the combination of urban sources of air pollutants (i.e. traffic and light industrial), concentrated agriculture feed operations, extensive oil and gas production, and regional meteorological conditions [123,124]; all of which create an ambient gas and aerosol pollutant mix that are believed to adversely impact respiratory health. Ozone is particularly problematic in the region. Since 2008, the entire NFRMA is an EPA ozone nonattainment area, as summer ozone levels have consistently exceeded regulatory standards despite efforts to limit emissions [123–126]. These unique characteristics of the NFRMA provide validation for the need to better understand the health effects of co-exposure to pollutants.

Hospital Admissions for Asthma

Hospital admissions data for asthma were obtained from the by the Colorado Department of Public Health and the Environment (CDPHE), obtained from over 80 Colorado Hospital Association (CHA) affiliated hospitals covering the NFRMA region for study years 2010 to 2014. Information on date of the hospital admission, primary International Classification of Diseases (ICD-9) code, demographic characteristics (patient's sex, age, race/ethnicity and health insurance provider), county of residence, and length of admission were included in this database. We considered

hospital admissions with a primary ICD-9 code classified for all forms of asthma (493.x) for our analysis, including observations for primary and secondary diagnoses for each admission. Only hospital admissions that were classified as ‘‘emergency’’ or ‘‘urgent’’ were included in our analyses; other admissions types (e.g., elective procedure) were excluded from the analysis. The CDPHE assigned cases to 3km x 3km grids based on billing addresses.

Ozone

Fixed-site Monitoring Data. Daily maximum 8-hour ozone concentrations were obtained from the United States Environmental Protection Agency (EPA) Air Quality System Data Mart for fixed-site air monitors across the NFRMA. To be included in our analyses, monitors were required to have at least 75% of the days in a month with available data. Ozone was monitored hourly all year round, and measurements were available over the study period (2010 – 2014). Exposures were approximated for each day based on development of a daily ozone surface using standard inverse distance weighted (IDW) methods [241].

Airborne Data. We obtained ozone data from the joint FRAPPÉ/DISCOVER-AQ field campaigns conducted over the Colorado Front Range during the summer of 2014. A detailed description of the technical details of these projects are presented elsewhere [130,242]. Briefly, during July–August 2014 a multi-institution campaign was carried out to investigate possible contributors to the consistently high summertime O_3 episodes in the NFRMA region. Two major components of the campaign were the Front Range Air Pollution and Photochemistry Experiment (FRAPPE), led by the Colorado Department of Public Health and Environment (CDPHE) and the National Center for Atmospheric Research (NCAR), and a Deriving Information on Surface Conditions from Column and Vertically Resolved Observations Relevant to Air Quality (DISCOVER-AQ) deployment led by

NASA. An array of measurement platforms was deployed during the study period and included in situ size-resolved measurements of an extensive suite of ambient aerosols aboard a C-130 aircraft equipped with four-channel chemiluminescence instrument to measure O_3 levels based on the UV absorption. On average, aircrafts measured pollutant components at a height of 300 m [243].

The aircraft measurements were limited to a four-week period (20 July – 18 August, 2014).

$PM_{2.5}$

Daily average concentrations of fine particulate matter ($PM_{2.5}$) were collected from the United States Environmental Protection Agency (EPA) Air Quality System Data Mart for the entire duration of the study period. $PM_{2.5}$ exposures for each day were determined based on standard inverse distance weighted (IDW) methods [241]. Only monitors with data for over 75% of the study period were included in the study.

Meteorological Data

Daily maximum temperature, dew point temperature and precipitation data for the study period were obtained from the National Climatic Data Center network using the *countyweather* package in R. FIPS codes were used to match observations to the closest weather station.

Statistical Analysis

Daily admission counts were matched with our exposure estimates using 3km x 3km grid IDs for the NFRMA region. We performed time-series analyses using the linked exposure and hospital admission data for the years 2010 to 2014. From these data, we performed single-pollutant quasi-Poisson regression models to evaluate the relation between O_3 and $PM_{2.5}$ exposure and daily counts of unscheduled asthma-related hospital admissions. We chose quasi-Poisson regression

model to account for overdispersed count data, with each pollutant metric entered independently into models as continuous variables.

To account for temporal trends, we included day of the week (categorical indicator), daily maximum temperature (spline with 2 degrees of freedom), and a function of time (spline with 12 degrees of freedom per year) in all models.

The basic model had the following form:

$$\log\big(E(Y)\big) = \alpha + \beta_{pollutant} + \Sigma_k \lambda_k DOW_k + ns\,(time, 12 * 5) + ns(temperature, 2)$$

Where Y indicated the count of asthma-related hospital admissions for a given day during the study period.

Results were expressed as relative risks (RR) for every interquartile range (IQR) increase in overall exposure. Models investigated lagged exposures: exposure measured on the day of admission (Lag0), exposure measured 1 day prior to the admission (Lag1), 2 days prior (Lag2), 3 days prior (Lag3), and an average exposure 1–3 days before the outcome (Lag3-day moving average).

To examine susceptibility by certain subpopulations, we stratified the associations between pollutant exposures (3-day moving average) and asthma-related hospital admissions by sex and age (≤ 15, 16 – 64 and ≥ 65 years). We also assessed for possible effect of season on the exposure-outcome associations by stratifying by a dichotomous variable for season (warm [April-September] and cold [October-March]).

Given the nature of ozone pollution in the region [125,243], we examined the summertime (June - August) association between O_3 and $PM_{2.5}$ exposure and daily counts of asthma-related hospital admissions in secondary analysis.

Finally, to assess for possible effect measure modification between exposure to O_3 and $PM_{2.5}$, we developed multipollutant models that included both pollutants and an interaction term (multiplicative interaction assessment), and computed the relative excess risk due to interaction (RERI) [244,245] parameter (additive interaction assessment).

Regression calibration model for ozone

We performed regression calibration using a validated approach proposed by Strand *et al*. [230]. By employing airborne measurements of ozone as "unbiased" surrogate variables of fixed-site ground-level measurements, we computed model estimate coefficients to characterize the effect of ozone on asthma-related hospital admissions.

The method of regression calibration employed in our study uses a 3-step procedure to adjust point and interval estimates for bias due to exposure measurement error. First, we linked the fixed-site O_3 (FSO) measurements and airborne O_3 (ABO) measurements for the period between July and August 2014 using the common 3km x 3km grid ID number. Next, calibration function was estimated as the fixed regression coefficient (θ_1) from linear regression model of the "true" surrogate variable, ABO, on the measured-with-error pollutant exposure variable, FSO, and controlling for the same covariates as in the main model above.

Calibration coefficients equal to 1 suggest no bias, while coefficients < 1 suggest an attenuated effect estimate [231].

Finally, we applied the calibration function to standard regression coefficient estimates fit in main model using quasi-Poisson regression:

$$\beta_1^{adj} = \beta_1/\theta_1$$

We calculated confidence intervals around new estimate using restricted maximum likelihood (REML) estimators of regression coefficients in the calibration and main health effect models [230].

RESULTS

Hospital Admissions for Respiratory Conditions

A total of 16,530 asthma-related hospital admissions were obtained for the study period (2010 – 2014), with most of the visits classified as emergency or urgent (91.29%). Of these unscheduled visits (emergency or urgent), a majority of admissions were for females (59.21%), between 16 and 64 years old (47.45%), and race/ethnicity designation of Non-Hispanic White (58.16%, Table 4.1). The average length of stay in the hospital was typically less than 4 days (72.81%). Admission characteristics during the summertime mirrored the full data set (Table 4.1).

TABLE 4.1. CHARACTERISTICS OF ASTHMA-RELATED HOSPITAL ADMISSIONS IN THE NORTHERN FRONT RANGE METROPOLITAN AREAS OF COLORADO

	Level	Total Asthma-Related Admissions (N = 16,530)	Summertime Asthma-Related Admissions (N = 2,947)
		n (%)	n (%)
Sex	Male	6,743 (40.79)	1,201 (40.75)
Age (years)	≤ 15	5,558 (33.62)	942 (31.96)
	16 - 64	7,843 (47.45)	1,451 (49.24)
	≥ 65	3,129 (18.93)	554 (18.80)
Race/Ethnicity	NHW	9,613 (58.16)	302 (10.25)
	NHB	1,594 (9.64)	293 (9.94)
	Hispanic	1,947 (11.78)	1,739 (59.01)
	Other	1,691 (10.23)	288 (9.77)
	Unknown	1,684 (10.19)	325 (11.03)
Type of admission	Emergency	14,508 (87.70)	2,573 (87.31)
	Urgent	3,423 (18.90)	374 (12.69)
Length of stay (days)	< 4	12,035 (72.81)	2,207 (74.89)
	≥ 4	4,495 (27.19)	740 (25.11)

Summertime refers to admissions from June through August. NHW, Non-Hispanic Whites; NHB, Non-Hispanic Blacks

Exposure Measures

The extent of correlation among the pollutants and meteorological factors followed expected patterns. O_3 concentration levels were negatively correlated with $PM_{2.5}$ levels (r = -0.07), and strongly positive correlated with temperature (r = 0.73, Figure 4.1).

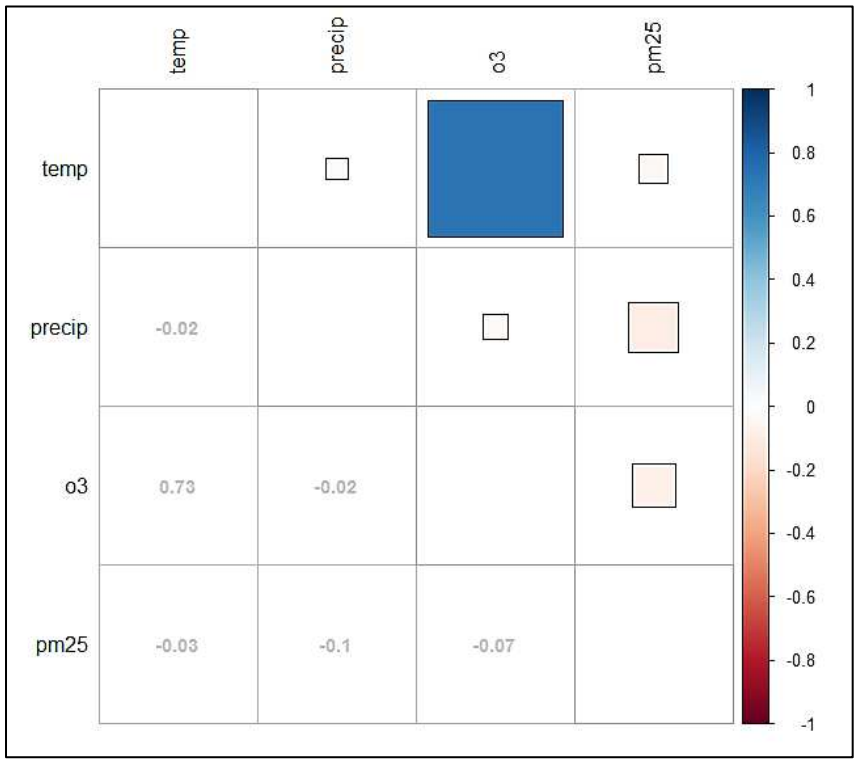

FIGURE 4.1. PAIRWISE PEARSON CORRELATION BETWEEN ENVIRONMENTAL EXPOSURE MEASURES AND METEOROLOGICAL PARAMETERS.
$PM_{2.5}$, particulate matter with a median aerodynamic diameter < 2.5µm; O_3, ozone; temp, maximum temperature; precip, precipitation.

$PM_{2.5}$. An average of 7 (SD: 3.9) monitoring devices were available for daily IDW estimation of $PM_{2.5}$ levels over the study period. The mean twenty-four hour daily average $PM_{2.5}$ concentration for the study period was 7.1 µg/m^3 (SD: 3.6, Figure 4.2A). Temporal patterns were also observed with $PM_{2.5}$ levels over the study period; the higher levels of $PM_{2.5}$ were observed in the colder months (Figure 4.2A). Several observations (n=697, 16 days) in early 2014 were above the current U.S. National Ambient Air Quality Standards (NAAQS) yearly average $PM_{2.5}$ levels (15µg/m^3). Figure

4.2C shows the levels of $PM_{2.5}$ in summer months over the study period. Notably, majority of the $PM_{2.5}$ levels above the U.S. National Ambient Air Quality Standards (NAAQS) occurred in the summer months of 2012, likely coinciding with 2012 Colorado wildfires [246].

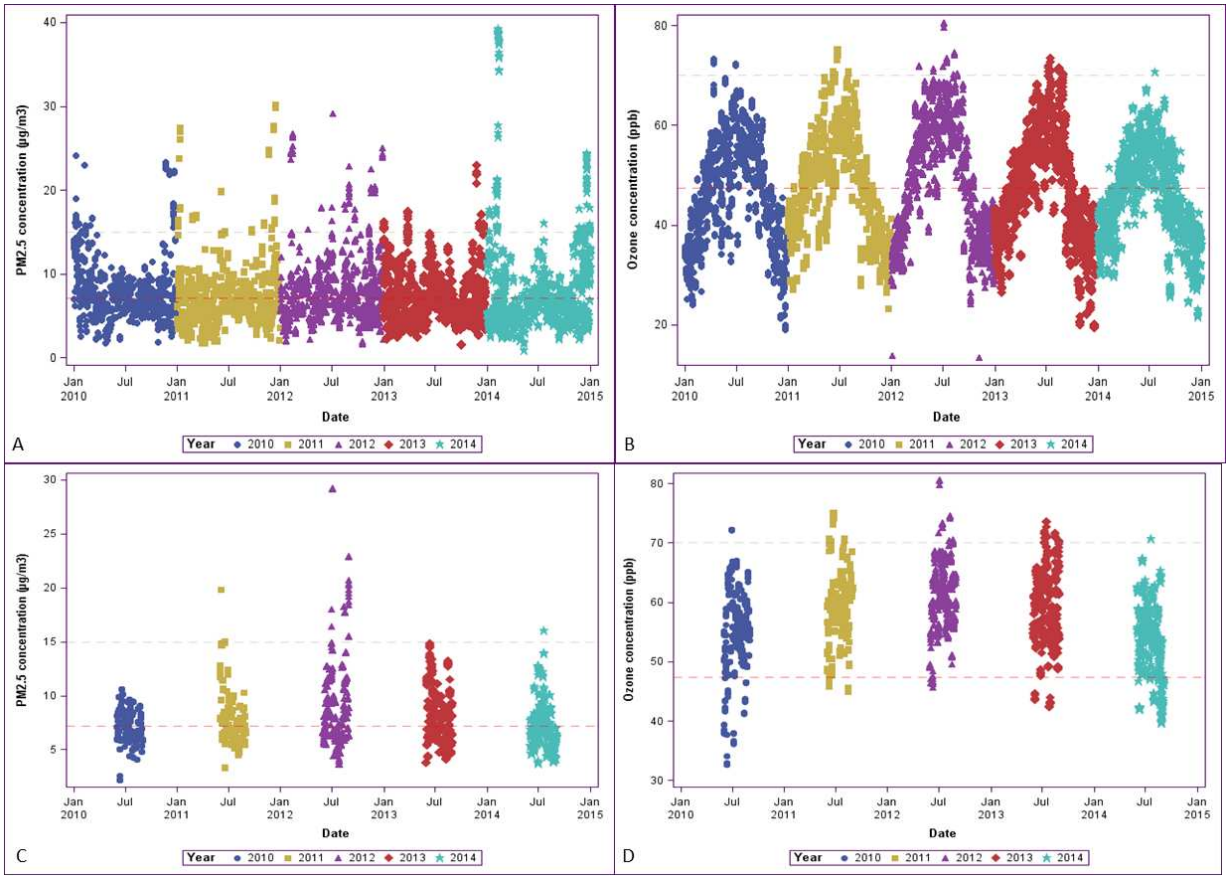

FIGURE 4.2. AIR POLLUTANT EXPOSURE LEVELS OVER THE STUDY PERIOD (2010 - 2014).
Red horizontal line indicates overall mean; grey horizontal line represents U.S. National Ambient Air Quality Standard. **A**. 24-hr average $PM_{2.5}$ concentrations ($\mu g/m^3$) **B.** Eight-hour Daily maximum ozone values (ppb) **C**. 24-hr summertime (June-August) average $PM_{2.5}$ concentrations ($\mu g/m^3$) **D.** Eight-hour daily maximum summertime ozone values (ppb). $PM_{2.5}$, particulate matter with diameter < 2.5 $\mu g/m^3$

Ozone. An average of 35 (SD: 4.4) monitoring devices were available for daily IDW estimation of O_3 levels over the study period. The mean 8-hour daily maximum O_3 (FSO) for the study period was 46.5 ppb (SD: 10.0, Figure 4.2B). Expected temporal patterns were apparent, with higher levels of ozone generally observed in the warmer months. Mean FSO for the summer months was 57.1 ppb (SD: 6.9). We also observed several records (n=121 observations, 14 days) of O_3 above

the EPA 8-hr daily maximum ambient air quality standard (70.0 ppb) in the NFRMA region, most in the summer months (Figure 4.2D).

Main Effects Analyses

Results from the single-pollutant models examining asthma-related hospital admissions for multiple lag day pollutant exposure levels are shown in Table 4.2.

In year-round analyses, the effects of $PM_{2.5}$ on asthma-related hospital admissions was generally consistent across lag days. An IQR increase (3.6 $\mu g/m^3$) in short-term $PM_{2.5}$ exposure was associated with about a 1.5% increase in asthma-related admissions across measured lags, although all the confidence intervals included the null value. On the other hand, the effects of O_3 (from FSO monitoring) on asthma-related hospital admissions was associated with a small but consistent decrease in admissions across all lags. An IQR increase (15.4 ppb) in O_3 exposure was associated with a 1.3% decrease in asthma-related admissions over the study period, though the null value was contained in confidence intervals. Although effect estimates were relatively small, we also observed decreasing effects on asthma-related admissions with longer O_3 lags (Table 4.2). No trends were observed with $PM_{2.5}$ lags.

Summertime Analyses

The effects of $PM_{2.5}$ on summertime asthma-related admissions were tempered compared to year-round levels. For an IQR increase (2.7 $\mu g/m^3$) in short-term $PM_{2.5}$ exposure, we observed about a 0.3% decrease in asthma-related admissions across measured lags (Table 4.2). Interestingly, the relatively wider confidence intervals contained estimates (and width of the 95%CI) observed for year-round analyses.

Conversely, summertime ozone exposures resulted in increase in asthma-related admissions across measured lags. For example, an IQR increase (8.8 ppb) in O_3 exposure (1-day lag) was associated with a 0.9% increase in asthma-related admissions (RR: 1.0087, 95%CI: 0.9536, 1.0669). Again, confidence intervals included the null, and estimates observed for year-round analyses.

TABLE 4.2. ADJUSTED RELATIVE RISKS AND 95% CONFIDENCE INTERVALS FOR ASTHMA-RELATED HOSPITAL ADMISSIONS BY POLLUTANTS

	Year-round					
	$PM_{2.5}$			Ozone		
Lag Days	RR	95% CI		RR	95% CI	
Day 0	1.01516	0.99944	1.03113	0.98719	0.94532	1.03092
Day 1	1.01513	0.99942	1.03109	0.98714	0.94530	1.03084
Day 2	1.01516	0.99946	1.03110	0.98709	0.94528	1.03075
Day 3	1.01514	0.99945	1.03109	0.98702	0.94515	1.03074
3-day moving average	1.01514	0.99945	1.03109	0.98706	0.94516	1.03083
	Summertime					
Lag Days	$PM_{2.5}$			Ozone		
Day 0	0.99742	0.95350	1.04336	1.00867	0.95367	1.06685
Day 1	0.99737	0.95335	1.04342	1.00867	0.95361	1.06691
Day 2	0.99731	0.95334	1.04331	1.00858	0.95389	1.06640
Day 3	0.99736	0.95333	1.04342	1.00855	0.95353	1.06673
3-day moving average	0.99735	0.95338	1.04335	1.00856	0.95388	1.06638

Estimates of relative risks are per interquartile range (IQR) increases. All models adjusted for time trend, day of week and maximum temperature. Summer months (June through August); RR, relative risk; CI, confidence interval; $PM_{2.5}$, particulate matter with diameter < 2.5 $\mu g/m^3$.

Stratified Analyses

The results of stratified analyses demonstrated some differences in associations within age, sex and seasonal categories (Figure 4.3), indicating possible statistical interaction between exposure measures and demographic indicators.

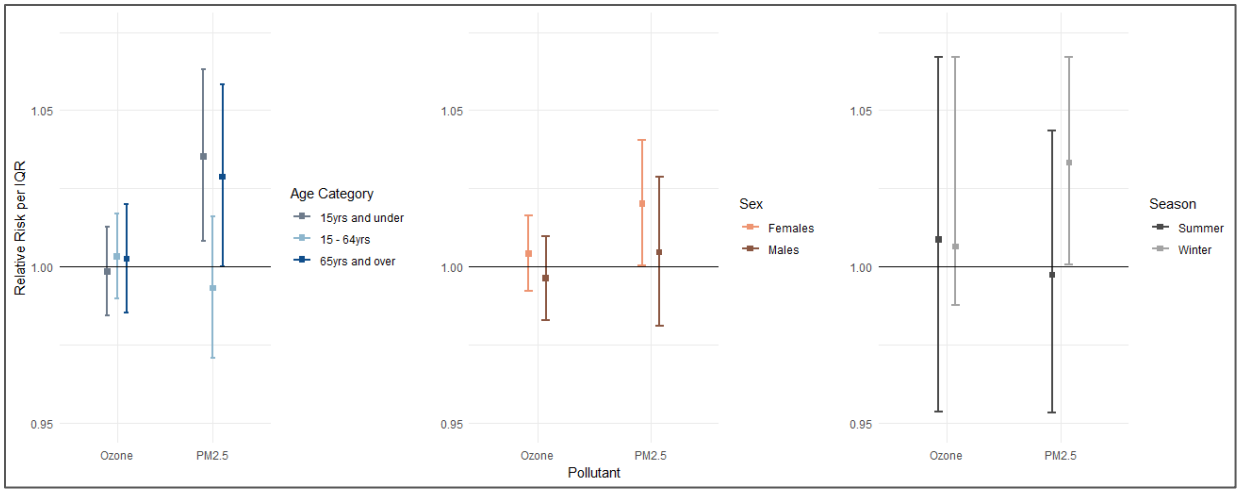

FIGURE 4.3. ADJUSTED RELATIVE RISKS AND 95% CONFIDENCE INTERVALS FOR ASTHMA-RELATED HOSPITAL ADMISSIONS BY POLLUTANTS (ONE-DAY LAG), STRATIFIED BY SEX, AGE AND SEASON.

Estimates of relative risks are per interquartile range (IQR) increases. Bold indicates statistical significance. All models adjusted for time trend, day of week and maximum temperature. Summer months (June through August); winter months (December through February); $PM_{2.5}$, particulate matter with diameter < 2.5 μg/m^3

In stratified age categories, asthma-related admissions following exposure to $PM_{2.5}$ were higher among children under 15 years and adults 65 years and older. Exposure to $PM_{2.5}$ (one-day lag, IQR) was associated with a 3.5% increase in admissions among children 15 years and younger (RR: 1.0352, 95%CI: 1.0081, 1.0630), a 2.9% increase in admissions among adults 65 years and older (RR: 1.0288, 95%CI: 1.0001, 1.0583), and a 0.7% decrease in admissions among people between 16 and 64 years old (RR: 0.9931, 95%CI: 0.9708, 1.0160).

The relative risk for asthma-related hospital admission following exposure to both pollutants was higher for females compared to males. For example, exposure to O_3 (one-day lag, IQR) was associated with 0.4% increase in admissions among females (RR: 1.0042, 95%CI: 0.9923, 1.0163) compared to a 0.4% decrease in admissions among males (RR: 0.9963, 95%CI: 0.9828, 1.0099), though both confidence intervals included the null value.

Finally, the effects of pollutants on asthma-related admissions differed by season. While $PM_{2.5}$ had stronger effects in the winter months (December through February, RR: 1.0033, 95%CI: 1.0006,

1.0671) compared to summertime (RR: 0.9974, 95%CI: 0.9534, 1.0434), the reverse was the case for the effect of O_3 exposure (RR_{winter}: 1.0064, 95%CI: 0.9877, 1.0671; RR: 1.0087, 95%CI: 0.9536, 1.0669).

Overlapping confidence intervals across most categories indicates that statistical interaction between pollutants and assessed covariates was unlikely.

Regression Calibration for Ozone

Linked FSO and ABO data resulted in a total of 2,406 observations over 21 days. Figure 4.4 shows a time-series graph for 8-hour daily maximum O_3 for FSO and ABO monitoring during the one-month observation period. Ozone measurements from the airborne campaigns were moderately correlated with measurements from the fixed-site monitors ($r = 0.50$, $p < 0.05$), the graph shows that ABO measures were generally higher than FSO measures. It is also important to note that the absolute difference between ABO and FSO measures increased as O_3 concentration increased.

The estimated calibration function (θ_1) for the ABO measure was 0.59 (SE: 0.03), indicating likely bias (concentration-dependent measurement error between ABO and FSO concentrations) in O_3 from FSO monitoring.

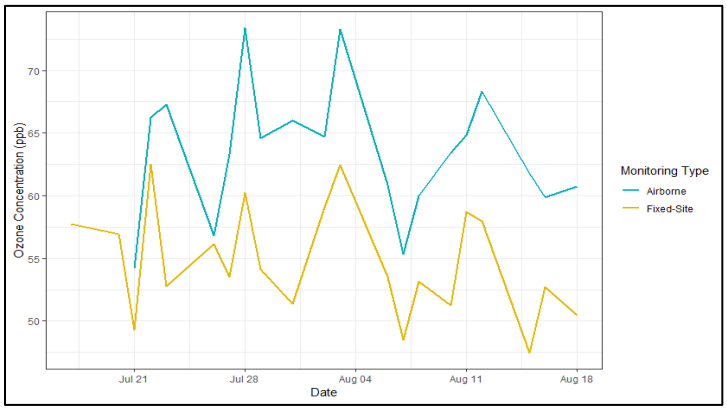

FIGURE 4.4. AIRBORNE AND FIXED-SITE MONITOR 8-HOUR DAILY MAXIMUM OZONE LEVELS MEASURED BETWEEN JULY 20 AND AUGUST 18, 2014.

To account for the lack of seasonal variability in ABO measurements, we limited regression calibration-main effects analyses to summertime observations. By applying this estimated function, θ_1 to the coefficient derived from the summertime main effects model, the adjusted estimate corresponded to an increased relative risk (1.5%, compared to 0.9% in unadjusted models) of asthma-related admission per IQR increase in exposure to ambient O_3 (Figure 4.5). This increase was consistent across exposure lag days, with relatively wider 95% confidence intervals. As in unadjusted (uncalibrated exposure) main effects models, the confidence intervals all contained the null value.

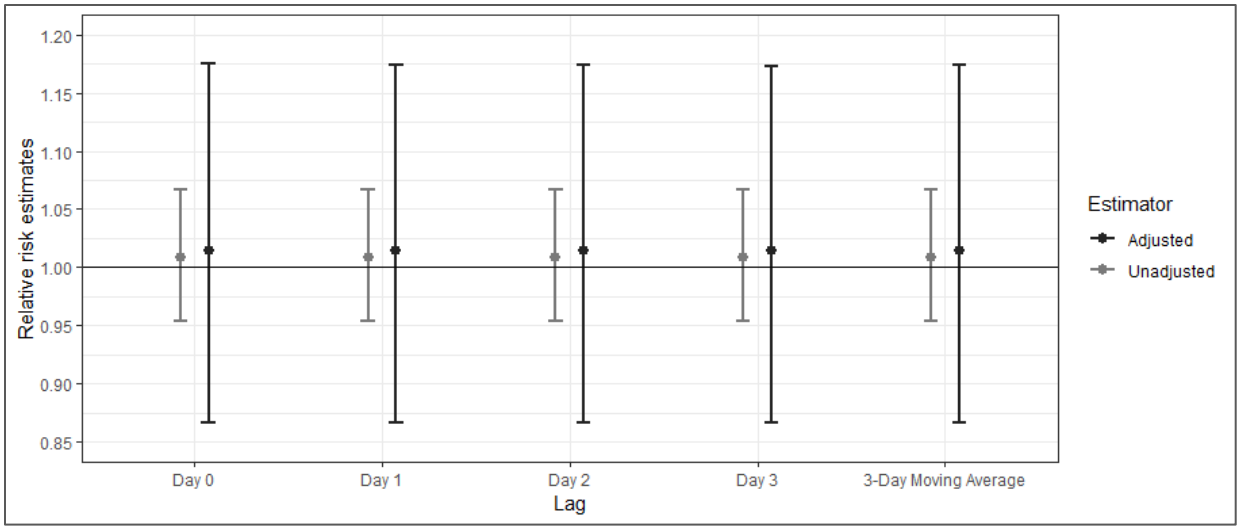

FIGURE 4.5. ADJUSTED RELATIVE RISKS AND 95% CONFIDENCE INTERVALS OF ASTHMA-RELATED ADMISSIONS FROM POISSON MODELS USING FIXED-SITE MONITORING OZONE EXPOSURES (UNADJUSTED), AND AIRBORNE-CALIBRATED OZONE EXPOSURES.
The relative risks were calculated for interquartile range increment in ozone levels.

Multipollutant model

In our multipollutant model assessment for multiplicative interaction, we observed a statistically significant p-value (p = 0.049) for the interaction term containing both pollutants. Figure 4.6 examined effect modification presented as relative risks (95%CI) between the 10^{th} and 90^{th} percentiles for each variable ($PM_{2.5}$ and O_3). Although we observed antagonistic trends (increased

ozone exposure appeared to reduce the marginal effect of $PM_{2.5}$ exposure on asthma hospitalization, and $PM_{2.5}$ reduced the marginal effect of O_3 on asthma hospitalization), the differences were small with overlapping confidence intervals, likely indicating influence of outliers outside the 10th and 90th percentile.

Finally, the computed RERI of -0.0021 (95%CI: -2.0369, 2.0327) indicated no substantial evidence of interaction on the additive scale.

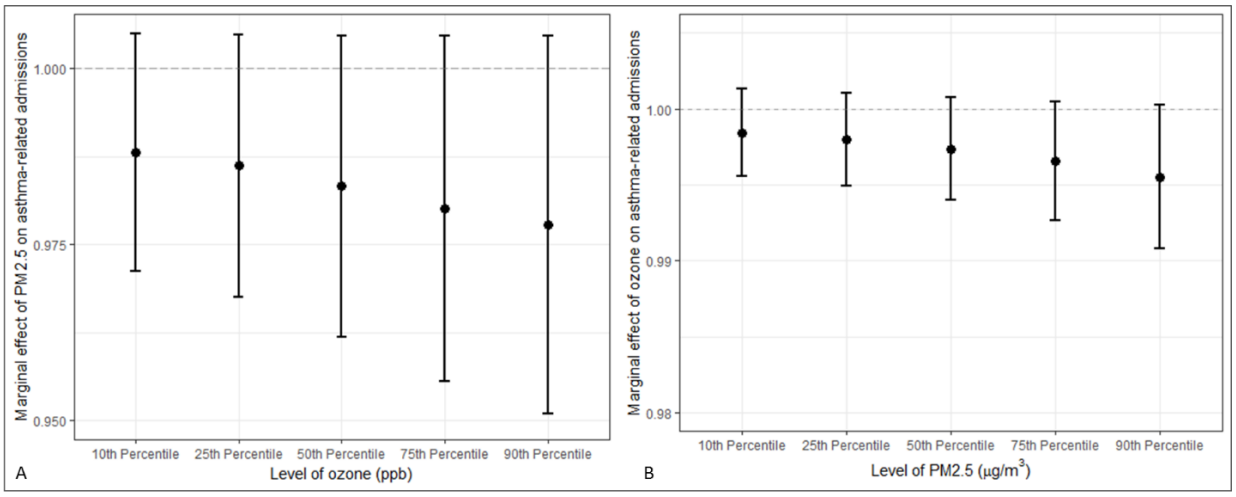

FIGURE 4.6. EFFECT MODIFICATION ON THE $PM_{2.5}$– AND OZONE–HOSPITALIZATION ASSOCIATIONS, PRESENTED AS RRS (95%CI) AT THE 10TH – 90TH PERCENTILES OF EACH MODIFYING POLLUTANT.
A. effect of ozone on the marginal effect of $PM_{2.5}$ on asthma-related hospitalizations. **B.** effect of $PM_{2.5}$ on the marginal effect of ozone on asthma-related hospitalizations. $PM_{2.5}$, particulate matter with diameter < 2.5 µg/m^3; ppb, parts per billion.

DISCUSSION

A continuously growing body of literature supports associations between short-term exposure to ambient air pollutant concentrations and respiratory hospitalization, particularly due to complications of asthma [205,222,224,247,248]. Our time-series analysis sought to reinforce this hypothesis for $PM_{2.5}$ and O_3 exposure in a region with unique pollution sources, meteorology and

topography. In addition, we employed the use of a regression calibration approach to reduce exposure misclassification that exists in, for example time series studies that use central (urban background) ambient exposure data, typically assuming averaged measurements for an entire population.

In this study, we found evidence of associations between asthma-related hospital admissions and short-term exposure to $PM_{2.5}$ and O_3, across multiple lag days, within the limitations of confidence intervals that contained the null value. While year-round and winter $PM_{2.5}$ exposures were associated with increase in relative risk of admissions, O_3 exposure in the summertime was associated with increase in relative risk of admissions. Although these findings provide replicable evidence of the effect of $PM_{2.5}$ and O_3 on asthma-related emergency department visits and hospital admissions in metropolitan areas [26,58,60,226,249,250], our results provide fairly novel information of these associations in the metropolitan area in Colorado.

Lag models indicated that the effect of summertime ozone was both immediate and delayed, with the effect magnitude highest the within the first few days (lag-0 and lag-1) of the admission. No perceivable lag effects were observed with $PM_{2.5}$ effect estimates. Although lag structure has been associated with negative health effects [223,248,250], results have varied depending on characteristics of the population and the health outcome being measured. Findings similar to our results are well documented in the literature [26,109,185,249,251]. For example, a study of air pollution and emergency department pediatric asthma visits showed that associations of highest magnitude tended to occur on the day of the visit [26]. Similarly, Loftus et al. demonstrated that the strongest associations between $PM_{2.5}$ and lung function among children with asthma in an

agricultural community, were observed for measurements on the same day or one-day prior [109]. However, another study exploring temporal lag patterns of the effects of $PM_{2.5}$ on cardiorespiratory hospital admissions in the Denver metropolitan area concluded that stronger respiratory admissions tended to be associated with $PM_{2.5}$ constituent concentrations following longer lag periods [252].

In stratified analysis, we observed interesting differences in associations by sex, age and season. The relative risk of hospitalization for asthma due to exposure to pollutants was higher for females, compared to their male counterparts, in both $PM_{2.5}$ and O_3 models. Several studies have reported different associations between air pollution and morbidity for women and men [223,227,253–256], although results have been inconsistent in these types of studies [254,256]. For example, Luginaah and colleagues reported a relatively stronger effect of air pollution on risk of respiratory hospitalization among adult women compared to men in Canada [255]. Conversely, Delfino et al. reported a stronger reduction in FEV_1 among boys with asthma (compared to girls) following short-term exposure to $PM_{2.5}$ [27].

Possible explanations for differences by sex include biologic differences (including lung physiology and associated pollutant deposition or gas absorption), occupational or activity pattern exposure differences, and differences in overall healthcare [227,256]. More studies are required to elucidate this interaction and identify underlying key mechanisms.

The risk of asthma-related hospitalization secondary to exposure to $PM_{2.5}$ was relatively higher for both adults over 65 years old and children 15 years and under, compared to the 15 – 64 year age group. This finding was unsurprising, as children with asthma and adults over 65 years are typically

sensitive to the effects of air pollution [226]. However, overlapping confidence intervals meant our study was limited in statistical power to detect interaction by age.

Finally, our stratified analyses revealed that the adverse effects of exposure to $PM_{2.5}$ was more pronounced in the winter months, and O_3 in the summertime. This finding was also predictable given that $PM_{2.5}$ levels have been shown to peak in colder weather months [249,257], as in our study. Ozone levels also tend to be highest in the warmer months [19,53,226]; conversely, asthma-related admissions tend to be low in the summer [258], meaning that the observed associations (as in our study) indicate that summertime asthma-related admissions are likely due to air pollution [249,251].

There was evidence of effect modification between $PM_{2.5}$ and O_3 on asthma-related admissions in our study. We observed a reduced relative risk of admissions with increasing levels of both pollutants. Closer examination revealed that the interaction was most likely generated by extreme outliers, for example, unusually high peaks driven by 2012 levels (smoke from both local and long-distance wildfires impacted air quality in the NFRMA in punctuated events in 2012 [246]) of summertime PM2.5. Statistically significant interaction between $PM_{2.5}$ and O_3 was absent when we focused on observations between the 10^{th} and 90^{th} percentiles; excluding either or both unusual periods (2010 and 2012) from analyses also eliminated significant interaction, although sample size was significantly reduced.

In general, high $PM_{2.5}$ levels are limited to the winter when local sources are at their peak, regional sources like wildfires are an exception [246,259,260]. Conversely, O_3 patterns are typically the reverse, as the warmer months see the highest levels of ambient O_3 in the Colorado Front Range

[243,261]. There is also a tendency for a degree of spatial misalignment of the two pollutants (as evidenced by the negative correlation in our study), especially since local $PM_{2.5}$ sources like traffic contain the precursors for O_3 [19,53]. Future studies should be aimed at investigating multipollutant effects of $PM_{2.5}$ and O_3 over extended time periods accounting for longer-term temporal variation.

Our study explored the use of a regression calibration approach to explore the impact of adjustment for errors in the measurement of outdoor air pollution, when using outdoor O_3 measurements as a surrogate for personal exposure. This type of exposure misclassification is common in air pollution epidemiology, largely due to the use of fixed-site monitoring data [73,76,262,263]. Our calibration coefficient suggested that bias was in fact likely, validating our use of regression calibration in this study. In calibrated models, the relative risk of hospitalization due to asthma was higher than in uncalibrated models. However, the confidence intervals around the estimates were wider (the adjusted estimator (calibrator function) has an additional component of variation, owing to the uncertainty in the values of its model parameters [264]) and included the null value. Though the measurement error associated with FSO monitoring O_3 likely contained elements of both classical and Berkson type error [265], the overall impact was most likely due to classic error with bias of effect estimates towards the null, as we observed.

Regression calibration estimation procedures using instrumental variables and unbiased surrogate variables are well documented in the literature [238,266] and have been extended into environmental epidemiology studies to reduce concentration-dependent measurement error [231,239,264,265,267]. In their analysis of home endotoxin exposure and wheeze in infants, Horick et al. applied regression calibration methods to measures of airborne and house-dust

endotoxin, and showed a substantial increase in adjusted effect estimates (compared to unadjusted estimates) [264]. Similarly, Van Roosbroeck and colleagues demonstrated the impact of adjustment for exposure measurement error in a study estimating the effects of outdoor air pollution on respiratory and other health effects [265]. The authors showed that adjusted estimates related to nitrogen dioxide and soot were several times higher than unadjusted estimates from their original analyses. A common thread with these studies was the widening confidence interval, as present in our study.

The regression calibration approach to exposure measurement error in environmental epidemiology studies has several attractive features. As personal monitoring is typically not feasible except for short-term panel studies (financial and logistical constraints are highlighted drawbacks), use of regression calibration methods may assist in reducing uncertainty inherent in estimating health effects from error-prone exposure data. It is also feasible for past studies to calibrate their model estimates based on new surrogate data, given that researchers can obtain adequate new data still relevant to their main study models [230,267].

Although our exposure analysis was limited to $PM_{2.5}$ and O_3, we recognize that we may not be capturing all the (potentially) important ambient exposures in the NFRMA. Concentrated agriculture feed operations are co-located with areas of active oil and gas production in this region, and these emissions contribute to the ambient gas and aerosol air pollutant loads [130,268], some of which have been strongly linked to asthma morbidity [28].

Our study had several limitations. First, due to the nature of hospital admissions, we were unable to evaluate confounding due to other important factors related to the exposure to $PM_{2.5}$ and O_3,

and asthma morbidity such as markers of socioeconomic status (e.g. education level and family income), features of the indoor and personal environments (including ventilation in indoor environment and exposure to tobacco smoke), and occupational exposures (e.g. dust, and chemicals). As with most observational studies, our study was also subject to bias due to unmeasured confounding. In addition, we acknowledge the possibility of diagnosis coding errors. This type of error would most likely be non-differential with respect to exposure to pollutants, attenuating the observed associations.

By utilizing airborne measurements of pollutants as a surrogate for spatiotemporal model calibration, we will be making an assumption that these measurements are less biased than fixed site monitor measurements; airborne measurements are also open to measurement errors [229,241].

In summary, we observed positive and harmful associations between ambient air pollution ($PM_{2.5}$ and O_3) and asthma morbidity characterized by unscheduled hospitalizations in the NFRMA of Colorado. The effects of these pollutants were dependent on demographic (age and sex) and seasonal characteristics. We also observed concentration-dependent measurement error [230] between fixed-site monitoring and airborne monitoring of ambient O_3, and corrected for this measurement error using regression calibration. This suggests that despite the usefulness/effectiveness of fixed-site monitoring of ambient air pollutants in health risk-based studies, the magnitude of effects may be underestimated. Future studies are needed in larger and more diverse populations with longer temporal assessments of multiple pollutant measures to further explore the relationship with respiratory and asthma morbidity.

CHAPTER 5: CONCLUSION

Asthma is one of the most common chronic childhood conditions in the United States responsible for considerable morbidity and related health-care costs. Although this refrain has become increasingly familiar, the complexity of asthma etiology remains poorly understood. Pediatric asthma morbidity is also complex, the epidemiological characteristics have been difficult to explain. For example, after appearing to plateau briefly in the beginning of the last decade, asthma prevalence increased between 2001 and 2009, before plateauing, decreasing and stabilizing at current levels (8.3%, SE: 0.3); adverse asthma outcomes also decreased [10,269]. However, sociodemographic discrepancies in asthma morbidity indices (e.g., asthma exacerbation, hospitalizations, hospital outpatient department and emergency department visits) widened during this period, and even remain in the face of decreasing prevalence [9,10].

The developing understanding of the heterogeneity of pediatric asthma has characterized morbidity as attributable to an individual's genetic and epigenetic variability, mediated by environmental exposures [1,7]. Since genetic properties tend to be stable temporally, the consensus among researchers has been that the aforementioned changes in prevalence suggest environmental rather than genetic factors have been the major drivers [44,270–274]. In particular, several studies have identified associations between pediatric asthma morbidity and exposure to ambient environmental chemicals and pollutants, dependent on regional characteristics [19,20].

The overall goal of this dissertation was to improve scientific knowledge of the role of ambient environmental chemicals and pollutants on asthma morbidity by addressing the following gaps identified in the environmental epidemiology literature: (i) a need to characterize region-specific associations in the context of geography, climatic conditions and population distributions; (ii) a

need to evaluate the health-related impact of multipollutant ambient exposures, especially encompassing multiple domains of exposure along the theme of the total environment paradigm; (iii) a need to identify and utilize appropriate statistical methods for the multidomain, multipollutant approach, depending on study questions and characteristics; and (iv) a need to identify and utilize the best available exposure and outcome measures, particularly in the context of reducing measurement error.

We conducted investigations of ECP and pediatric asthma morbidity in three case studies reflecting signals of multidomain, multipollutant exposures along a rural/urban gradient:

- The Yakima Valley region of central Washington State, a largely rural area characterized by a high density of agricultural operations including farmlands for crop production as well as animal confinement facilities. We used longitudinal data analysis methods to evaluate the effects of short-term exposure to two criteria air pollutants (ozone and $PM_{2.5}$) on a biomarker of asthma exacerbation, in the context of contemporaneous exposure to organophosphate pesticides.

- The San Joaquin Valley near the southern end of the Central Valley of California, a richly productive agricultural region notorious for poor air quality, and high levels of urban-generated ozone and particulate pollution. For this study, we implemented a Bayesian regression method (exposure–response surface estimation) to evaluate the pulmonary health effects of a multidomain (ambient air pollutants and pesticides), multipollutant mixture for children with asthma in the region.

- The Colorado Front Range is a largely urban, densely populated area with a unique mixture of urban sources of air pollutants (i.e. traffic and light industrial), extensive oil and gas production and concentrated agriculture feed operations. Here, we evaluated the association between short-term exposure to ozone and $PM_{2.5}$, and unscheduled respiratory hospitalizations using overdispersed Poisson regression models. We also explored the use of regression calibration to generate highly resolved spatial exposure measures for ozone, a secondary pollutant known for substantial spatial heterogeneity.

RESULTS SUMMARY

Ambient ECP play a role in asthma morbidity

Across all aims, we observed fairly consistent increases in measures of asthma morbidity following exposure to ambient pollutants. In Aim 1, our results show that in independent single-pollutant main-effects models, short-term exposure to organophosphate pesticides, $PM_{2.5}$ and O_3 were associated with an increase in urinary LTE4 levels, a validated marker of pulmonary inflammation and asthma exacerbation. Specifically, an IQR increase in OP levels was associated with a LTE4 increase of 4.1 pg/mg creatinine (95%CI: 0.6, 7.6). This result, along with significant exposure-response associations between LTE4 levels and summed measures of methyl and ethyl dialkylphosphates provided novel insight into the possible role of pesticides role of organophosphate exposure as a potential pediatric asthma exacerbator in rural agricultural communities.

In the FACES study, we observed changes in lung function measures per unit increase in 3-month prior exposure to NO_2, PM_{10}, O_3 (FEV_1 and FVC), and carbamate pesticides (FEF_{25-75}); the shape of exposure-response function curves indicated negative linear trends for each of these components

while accounting for all the other components of the ambient pollution and pesticide mixture (Figure 3.2). Although associations between asthma morbidity and these pollutants have been reported individually in epidemiological studies [19], our methods provided insight into whether the impact of an individual ECP exposures was more pronounced when it occurred as part of a mixture. We were also able to highlight NO_2 as a main driver of the effects of the mixture.

In the NFRMA of Colorado, we observed that exposure to $PM_{2.5}$ and O_3 were associated with increase in the relative risk of asthma-related hospitalization, though associations were only statistically significant among certain age and sex strata. Additionally, O_3 models calibrated with airborne, remote-sensing O_3 measurements indicated the presence of measurement error with attenuation of risk estimates. Ozone is a critical ambient pollutant in this area, as the region has consistently been in non-attainment according to EPA standards. Importantly, these results highlight limitations in utilizing fixed-site monitoring of spatially heterogeneous ambient air pollutants like O_3 in health risk-based studies.

Multipollutant approaches advance our understanding of the exposure-response relationship

Although single pollutant models have contributed significantly to quantification of inherent biological and pathophysiological mechanisms associated with ECP-asthma relationships, much of the environmental epidemiology research community have recommended moving to multipollutant approaches to achieve better characterization of health effects of pollutants.

We used a multidomain approach to answer three distinct multipollutant research questions. In our first aim, we evaluated the effect of regionally-important criteria ambient pollutants on the pediatric asthma morbidity in a rural agricultural community, all in the context of contemporaneous exposure to agricultural pesticides. Our method questioned if the effects of

environmental agents from two domains (ambient air pollutants and pesticides) on an asthma morbidity outcome were interdependent. Our findings of consistently increased LTE4 level associated with joint exposures combinations of $PM_{2.5}$, O_3 and OPs (Figure 2.5) provides a valuable contribution to the very limited literature about the effects of environmental factors on health outcomes in rural and agricultural settings. This study also highlights the use of median dichotomization splits to generate exposure categories based on distributional properties of the single-pollutant exposure data in the cohort; a simple method that provides feasible solutions to our research question.

In the NFRMA study, our multipollutant research question was similar to that in Aim 1. We sought to explore if the effects of $PM_{2.5}$ and O_3 on asthma-related hospital admissions in this region were interdependent. The characteristics of the outcome measures (count data) limited our choice of statistical methods – the use of interaction (product) terms in the Poisson model. We observed statistically significant antagonism between $PM_{2.5}$ and O_3 on the multiplicative scale; although statistical significance was offset by accounting for outliers. With the exception of regional sources like wildfires, $PM_{2.5}$ patterns typically mirror the opposite of O_3 patterns, as local sources in the Colorado Front Range are characterized by high seasonal variability [260]. Additionally, there tends to be a degree of spatial misalignment of the two pollutants (as evinced by the negative correlation in our study), especially since local $PM_{2.5}$ sources like traffic contain the precursors for O_3. Hence, the synergistic effect on asthma morbidity observed with O_3 and $PM_{2.5}$ in the rural region of Yakima is unlikely in the NFRMA.

Finally, in the FACES study, we were able to explore the health effects of multiple domains of ambient ECP exposure in a geographically diverse cohort. In this region characterized by urban

and rural/agricultural contribution to ECP exposure, we were interested in examining whether the joint effect of recent exposure (prior 3 months) to a mixture of ambient air pollutants and pesticides impacted lung function among children with asthma. The overall effect of mixtures was associated with reduced FEV_1 and FVC, particularly when all the exposures were above their 60th percentile. However, we unexpectedly observed a slight improvement in FEF_{25-75} with exposure to the ECP mixture. Although these associations were not statistically significant, we offered several explanations for the observation of inconsistent effects of cumulative exposures on measures of lung volumes. These findings may indicate differences in exposure window sensitivity (the effects of short-term exposures may be different from acute or chronic exposures) for effects on FEV_1 and FVC, compared to FEF_{25-75} [213,214]. Differential findings may also be a function of asthma severity, as FEF_{25-75} in the setting of a normal FEV_1 has been linked to more severe asthma [215]; considering a large proportion of our FACES cohort had mild or moderate asthma at the time of outcome assessment. We believe that these results provide a unique insight into multipollutant research, and intricacies of ECP-lung function associations. As a way of examining harmful health effects based on multidomain, multipollutant approach, BKMR provided us an appropriate tool to answer our research question, while disentangling possible effects of nonlinearity and collinearity; and exposure effects which may be more complex than simple, additive interaction structures.

In general, this dissertation provided significant contribution to the filling the gaps on studies exploring the association between multiple pollutant exposures (and within multiple domains) and health outcomes, as prioritized by the environmental epidemiology community.

Exposure and Outcome Measurement, Biomarkers and Asthma Morbidity

Other notable findings of this dissertation research pertain to the exploration of different exposure and outcome measures dependent on feasibility and study research questions.

In Aim 1, we examined asthma morbidity using urinary LTE4, a validated marker of airway inflammation. Although the role of noninvasive biomarkers of airway inflammation have been limited to adjunctive purposes, several studies have shown good correlation of LTE4 levels with traditional measures of clinical significance [159,161]. In this study, we also used a validated marker of recent exposure to OP pesticides. Dialkylphosphates are urinary metabolites that devolve from the metabolism of OP pesticides [275]. Although, urinary DAP in the general population is believed to primarily result from exposures to non-neurotoxic OP metabolites from an ingestion pathway (i.e. food consumption), we were able to show, as with other studies [75,146], that elevated levels of DAP in our study (compared to US children) highlights the additional exposure burden (most likely ambient and proximity-based) encountered in populations with significant agricultural activity [67,135].

In Aim 3, we used a regression calibration approach [230] to adjust for possible exposure measurement error associated with fixed-site O_3 monitoring. Ambient ozone is a secondary pollutant usually formed at some distance from the primary source. Fixed-site monitoring provides good surrogate measure of the spatial heterogeneity of ozone exposure as well as usually refined temporal data. However, this information is notably of ozone levels downwind from the primary source of pollution in such areas, particularly when monitors are located close to highways. We used airborne remote-sensing measures from a joint NASA/FRAPPÉ project [130] which provided a highly spatially resolved measure of ozone during the summer of 2014 [123,130]. Our results

indicated that some concentration-dependent measurement error between fixed-site monitoring and airborne monitoring of ambient O_3 was present, with attenuation of risk coefficients. Regression calibration provided a method to obtain unbiased estimators of effects in our regression models.

STUDY LIMITATIONS

Our dissertation employs unique exposure and outcome measures, and methods in three distinct studies to evaluate the association between exposure to ambient ECP and pediatric asthma morbidity, and so each study expectedly presented unique limitations.

Aim 1: The Yakima Valley study was limited by a relatively small sample size (n = 139). Small sample size tends to limit a study's power, and ability to detect significant associations especially for interaction analysis. Data constraints limited us to short-term lag exposure analyses, with regard to pesticide: DAP only provide a measure of 24 – 72 hour exposure to OP pesticides, this short exposure window may not reflect the appropriate critical window for an exposure-response association. Data constraints also restricted our ability to adequately characterize the possible influence of seasons with respect to this particular agricultural community. Further research is required to explore these important facets.

As mentioned previously, the use of biomarkers may have resulted in measurement error in our study. First, using DAPs as a measure of ambient OP exposure is limited by the lack of specificity with respect to the OP from which they were derived, and reliability may be affected by human exposure to preformed DAPs in food or the environment [117,188]. We believe that any related measurement error would most likely be nondifferential (with possible attenuation of effect estimates); moreover, a substantial body of literature has demonstrated significant temporal and

spatial associations between ambient pesticide application/use/measurements and DAP levels [135,189,190]. Similarly, the use of urinary LTE4 as a measure of exacerbation may have led to outcome misclassification, particularly in the absence of clinical symptoms or measures. Multiple studies have highlighted the appropriateness of the use of LTE4 as an independent measure of pulmonary inflammation [24,159,160], and ultimately, we do not believe that any errors resulting from this will be differentially associated with exposure levels. We were also unable to properly characterize spatial variation of exposure to environmental agents. Our measures of O_3 and $PM_{2.5}$ were obtained from fixed-site monitors in proximity to children's homes. Such residential exposure assessment failed to account for time-activity patterns. Again, any errors resulting from this would most likely be nondifferential, and likely masked the true exposure-outcome associations by biasing results toward the null and/or increasing the standard errors association with effect estimates.

We assumed similar pathophysiological pathways for all three component pollutants. It is possible that the effect observed were due to simultaneously present differing mechanisms of action. However, it is unlikely that this explained all of the observed effects.

Finally, our study was conducted within an agricultural community, and as mentioned above, levels of OP exposure are bound to be higher than in the general population. This limits our ability to generalize our results to other non-agricultural populations.

Aim 2: The FACES study relied on area measurements of exposures from centrally located, fixed-site ambient air quality monitors and the California Pesticide Use Report (PUR) data, both of which fail to account for individual time-activity patterns and may have resulted in exposure measurement error. This error would be expected to be non-differential with respect to the

study outcome and would likely drive effects toward null values. Besides the use of residential addresses to determine exposures is a strength compared to studies using community-level data. Further, PUR data is limited to only 3-month exposure windows, we were unable to assess the effect of joint exposures at other short-term lags. It is likely that this affected our results, in terms of capturing the critical window for effects on lung function.

We acknowledge that missing lung function data could have resulted in bias. However, missing outcome data was likely random, and demographic characteristics of participants included in the study and those excluded were not appreciably different.

Although our study typifies the agricultural-urban interface, recruitment into the FACES cohort may have been geared towards capturing more urban exposures (within distance of the Fresno Super Site monitor) than agricultural pesticide exposures. Hence, it is likely that the effects of pesticide exposure in this cohort are less than representative.

Lastly, as we are limited by PUR data to only three-month exposures, we were unable to assess the effect of joint exposures at other short-term lags.

Aim 3: In the FROZ+EN study, one limitation is with regard to the nature of hospital admissions data: we were unable to evaluate confounding due to other important factors related to the exposure to $PM_{2.5}$ and O_3, and asthma morbidity such as markers of socioeconomic status (like education level and family income), features of the indoor and personal environments (including ventilation in indoor environment and exposure to tobacco smoke), and occupational exposures (like dust, and chemicals). This may have resulted in residual confounding and biased model estimates. Future studies should be adequately constructed to address these limitations. We

also acknowledge the possibility of diagnosis coding errors. However, we believe that this type of measurement error would most likely be non-differential with respect to exposure to pollutants, attenuating the observed associations.

Fixed-site monitoring of pollutants ($PM_{2.5}$ and O_3) fail to account for individual time-activity patterns and may have resulted in exposure measurement error. This error would be expected to be non-differential with respect to the study outcome and would likely drive effects toward null values.

Finally, by utilizing airborne measurements of O_3 as a surrogate for our regression calibration, we made the assumption that the FRAPPÉ/DISCOVER-AQ measurements are less biased than fixed site monitor measurements and measured with little error. Although unlikely, these measurements are not immune to device or reporting errors. Again, any bias from these would most likely be non-differential with respect to health outcome measures with small magnitude.

As with most observational studies, all three studies were subject to bias due to unmeasured confounding.

FUTURE DIRECTIONS

Overall, the studies described in this dissertation support the hypothesis that joint exposure to multiple ambient environmental exposures increased risk of adverse respiratory health outcomes. Depending on the research questions, our multidomain, multipollutant approach was able to assess the potential respiratory health impacts of individual exposure agents, determine the level and types of interaction among component agents of a mixture, and estimate the pulmonary health effects of cumulative exposure to the mixture.

Our work represents the first such set of investigations in the environmental epidemiology field and therefore warrants additional and confirmatory research in alternative settings. Future work could include improved exposure and outcome assessment, and more incorporative methods for spatio-temporal multipollutant modeling.

We note that air quality standards such as the National Ambient Air Quality Standards (NAAQS) were developed from available scientific studies (largely predicated on single-pollutant research), and have been associated with significant positive effects on human health [57,76]. Such regulatory standards are limited for agricultural pesticides and joint ambient exposures; one of the main challenges with establishing such policies has been the paucity of scientific evidence, especially among vulnerable populations. As unique contributions from urban/rural/agricultural regions continue to compromise outdoor air quality in the US, as well as similar communities worldwide, the health of children with asthma in surrounding communities should be evaluated in the context of multipollutant exposures.

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APPENDIX

TABLE 1. COMPARISON OF SELECTED CHARACTERISTICS BETWEEN FULL AFARE DATASET AND AFARE LEUKOTRIENE E4 SUBCOHORT

	All participants (N=58)	LTE4 subcohort (N=16)	p - value[a]
Child demographics	n (%)	n (%)	
Age at baseline (years), mean (SD)	10.4 (2.7)	9.7 (2.5)	0.35
Female	29 (50.0)	7 (43.8)	0.66
Hispanic/Latino ethnicity	54 (93.1)	15 (93.8)	0.93
Household characteristics			
Household income <$15k/year	24 (41.4)	8 (50.0)	0.54
Baseline asthma health			
Reported controller medication (inhaled corticosteroids) use	41 (70.7)	11 (68.8)	0.88
Atopic asthma[b]	42 (72.4)	12 (75.0)	0.84

[a] calculated using Chi-squared test for comparison of proportions, and Student's t-test for comparison of means

[b] Indicated by positive skin prick test to at least one of 22 common inhalant allergens.

TABLE 2. PERCENTILE VALUES FOR POLLUTANT CUTOFFS FOR AFARE STUDY

Pollutant	Percentile	Value
$PM_{2.5}$ ($\mu g/m^3$)	25	5.30
	50	8.73
	75	13.50
O_3 (ppb)	25	38.00
	50	43.00
	75	48.00
OP (ng/mg creatinine)	25	91.98
	50	142.85
	75	289.25

TABLE 3. ANALYSIS VARYING HIGH-LOW CUTPOINTS FOR JOINT EXPOSURE CATEGORY CREATION

Combinations	PM	Ozone	OP	Category (Reference: Low)	Estimate	U95	L95	QIC
	Percentiles							
c1	25	25	25	Mild	39.38	14.13	64.63	**142.29**
	25	25	25	Moderate	69.17	46.91	91.43	
	25	25	25	High	56.59	35.90	77.28	
c2	25	25	50	Mild	-1.31	-60.82	58.20	**144.08**
	25	25	50	Moderate	23.51	-31.35	78.37	
	25	25	50	High	30.54	-15.83	76.91	
c3	25	25	75	Mild	17.89	-6.88	42.67	**142.98**
	25	25	75	Moderate	39.33	20.27	58.39	
	25	25	75	High	51.97	14.95	88.99	
c4	25	50	25	Mild	39.73	17.64	61.82	**140.93**
	25	50	25	Moderate	68.11	45.07	91.15	
	25	50	25	High	68.01	48.05	87.97	
c5	25	50	50	Mild	4.72	-48.55	58.00	**144.20**
	25	50	50	Moderate	36.81	-12.82	86.43	
	25	50	50	High	49.01	8.11	89.91	
c6	25	50	75	Mild	15.84	-5.74	37.42	**142.41**
	25	50	75	Moderate	49.78	23.56	76.00	
	25	50	75	High	64.16	39.20	89.12	
c7	25	75	25	Mild	39.17	15.16	63.18	**142.48**
	25	75	25	Moderate	26.85	6.53	47.16	
	25	75	25	High	26.85	0.41	53.30	
c8	25	75	50	Mild	20.39	-12.68	53.46	**142.18**
	25	75	50	Moderate	24.12	0.62	47.62	
	25	75	50	High	38.01	-13.81	89.83	
c9	25	75	75	Mild	20.50	2.17	38.82	**142.98**
	25	75	75	Moderate	26.96	9.27	44.64	
	25	75	75	High	45.48	-19.49	110.45	
c10	50	25	25	Mild	18.22	-21.04	57.48	**143.62**
	50	25	25	Moderate	48.98	19.43	78.53	
	50	25	25	High	40.57	2.71	78.44	
c11	50	25	50	Mild	21.71	-10.24	53.67	**143.21**
	50	25	50	Moderate	41.87	9.17	74.56	
	50	25	50	High	54.47	23.63	85.30	
c12	50	25	75	Mild	34.64	18.11	51.18	**142.16**
	50	25	75	Moderate	45.59	18.56	72.62	
	50	25	75	High	67.62	37.61	97.63	
c13	50	50	25	Mild	16.96	-9.96	43.88	**144.36**
	50	50	25	Moderate	61.91	28.04	95.78	
	50	50	25	High	47.29	17.60	76.99	
c14	50	50	50	Mild	27.51	3.55	51.46	**145.24**
	50	50	50	Moderate	62.54	18.09	106.99	
	50	50	50	High	63.58	32.43	94.72	

Combinations	PM	Ozone	OP	Category (Reference: Low)	Estimate	U95	L95	QIC
c15	50	50	75	Mild	33.46	12.59	54.32	**144.90**
	50	50	75	Moderate	53.96	16.06	91.85	
	50	50	75	High	68.20	40.19	96.21	
c16	50	75	25	Mild	32.70	15.09	50.31	**141.73**
	50	75	25	Moderate	32.72	12.63	52.81	
	50	75	25	High	26.63	-0.36	53.61	
c17	50	75	50	Mild	36.57	13.72	59.42	**142.98**
	50	75	50	Moderate	35.04	14.47	55.62	
	50	75	50	High	46.64	4.49	88.80	
c18	50	75	75	Mild	31.68	12.77	50.59	**143.29**
	50	75	75	Moderate	28.02	13.16	42.88	
	50	75	75	High	46.85	-19.19	112.90	
c19	75	25	25	Mild	11.65	-14.05	37.35	**141.46**
	75	25	25	Moderate	21.08	-7.60	49.75	
	75	25	25	High	17.57	-23.14	58.28	
c20	75	25	50	Mild	12.81	-15.37	40.98	**142.11**
	75	25	50	Moderate	28.69	3.37	54.01	
	75	25	50	High	33.79	9.78	57.79	
c21	75	25	75	Mild	23.26	7.53	38.99	**141.60**
	75	25	75	Moderate	37.29	12.82	61.76	
	75	25	75	High	45.44	11.91	78.98	
c22	75	50	25	Mild	8.68	-16.19	33.55	**143.75**
	75	50	25	Moderate	35.72	7.37	64.08	
	75	50	25	High	21.63	-12.82	56.08	
c23	75	50	50	Mild	25.05	1.42	48.68	**143.30**
	75	50	50	Moderate	51.28	14.50	88.06	
	75	50	50	High	42.47	18.73	66.21	
c24	75	50	75	Mild	29.02	8.54	49.50	**143.63**
	75	50	75	Moderate	52.65	19.42	85.89	
	75	50	75	High	45.54	17.52	73.55	
c25	75	75	25	Mild	0.56	-42.15	43.26	**144.67**
	75	75	25	Moderate	6.97	-26.21	40.15	
	75	75	25	High	-25.08	-73.74	23.58	
c26	75	75	50	Mild	14.95	-16.08	45.97	**142.84**
	75	75	50	Moderate	24.32	-0.43	49.08	
	75	75	50	High	-19.71	-49.42	10.00	
c27	75	75	75	Mild	9.49	-18.84	37.82	**143.29**
	75	75	75	Moderate	34.15	8.98	59.32	
	75	75	75	High	-57.90	-99.03	-16.77	

- $PM_{2.5}$ indicates 24-hour-average exposure to particulate matter < 2.5 µm in diameter; Ozone, 8-hour maximum concentration of ozone; OP, urinary measure of metabolite of organophosphate exposure, total dialkylphosphate; L95, upper 95% confidence interval limit; U95, upper 95% confidence interval limit; QIC, quasi-information criterion, a measure of model performance.

TABLE 4. COMPARISON OF SELECTED CHARACTERISTICS BETWEEN FULL FACES COHORT DATASET AND STUDY SUBSET

	full	subset	p-value[a]
	n = 315	n = 153	
Male Gender, **%**	56.5	60.1	**0.460**
Ethnicity, %			
Non-Hispanic Black	15.6	13.7	**0.923**
Non-Hispanic White	44.7	46.4	
Hispanic	39.7	39.9	
Age (years), **mean (SD)**	8 (1.7)	8 (1.7)	**0.789**
Height (in), **mean (SD)**	51.9 (4.8)	52.0 (4.7)	**0.831**
BMI (kg/m^2), **mean (SD)**	17.8(4.4)	18.5 (4.6)	**0.112**
FEV_1 (L/s), **mean (SD)**	1.6 (0.5)	1.6 (0.5)	**0.734**
[a] calculated using chi-squared test for comparison of proportions, and student's t-test for comparison of means			

TABLE 5. GROUP AND CONDITIONAL POSTERIOR INCLUSION PROBABILITIES (PIPS) FROM BKMR USING EXPOSURE DOMAIN GROUPS FOR HIERARCHICAL VARIABLE SELECTION.

Exposure	PIPs (by PFT)					
	FEV_1		FVC		FEV_1/FVC	
	Group	Conditional	Group	Conditional	Group	Conditional
C	0.59	0.17	0.48	0.22	**0.69**	0.39
MeBr	0.59	0.13	0.48	0.18	**0.69**	**0.50**
OP	0.59	**0.71**	0.48	**0.60**	**0.69**	0.11
Ozone	**0.78**	0.15	**0.78**	0.20	0.45	**0.52**
NO_2	**0.78**	**0.85**	**0.78**	**0.80**	0.45	0.48
$PM_{2.5}$	0.63	0.48	0.62	0.50	0.47	0.41
PM_{10}	0.63	**0.52**	0.62	0.50	0.47	**0.59**

Group PIPs indicate the posterior probability of an exposure domain being included in the model; Conditional PIPs indicate the posterior probability of a single exposure within the domain to be included in the model. Both provide an illustration of the relative ranking of variable importance for each exposure domain as well as each exposure within a particular domain.

Text colors indicate different exposure domains: black, ambient air particles; blue, ambient air gases; red, pesticides. Bold font indicates highest PIP in column.

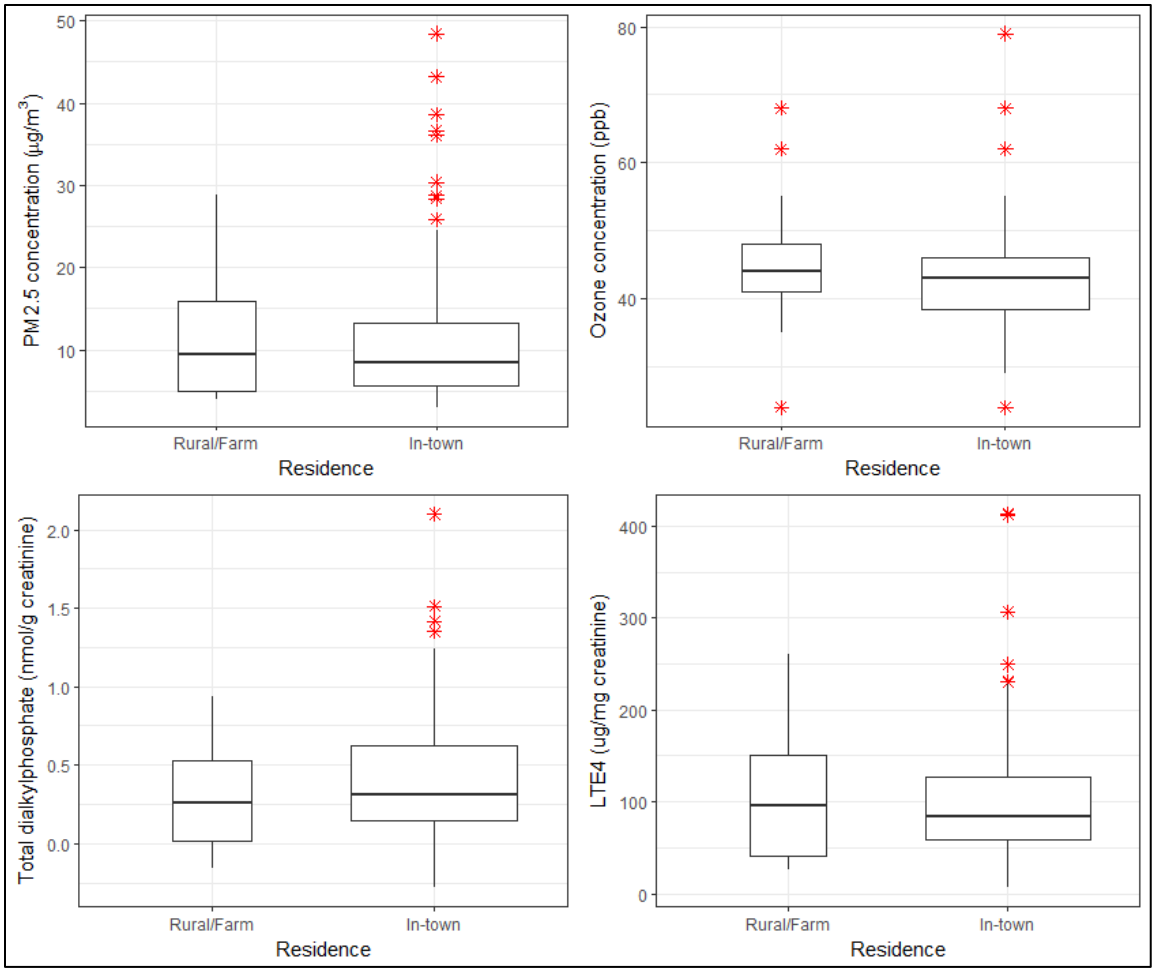

FIGURE 1. DISTRIBUTION OF EXPOSURE AND OUTCOME DATA BY STUDY PARTICIPANTS' RESIDENCE.

Distribution of exposure and outcome data by study participants' residence. Residence classification was based on self-reported proximity to farms.

$PM_{2.5}$ indicates 24-hour-average exposure to particulate matter < 2.5 µm in diameter. LTE4 represents creatinine-adjusted urinary leukotriene E4 concentration.

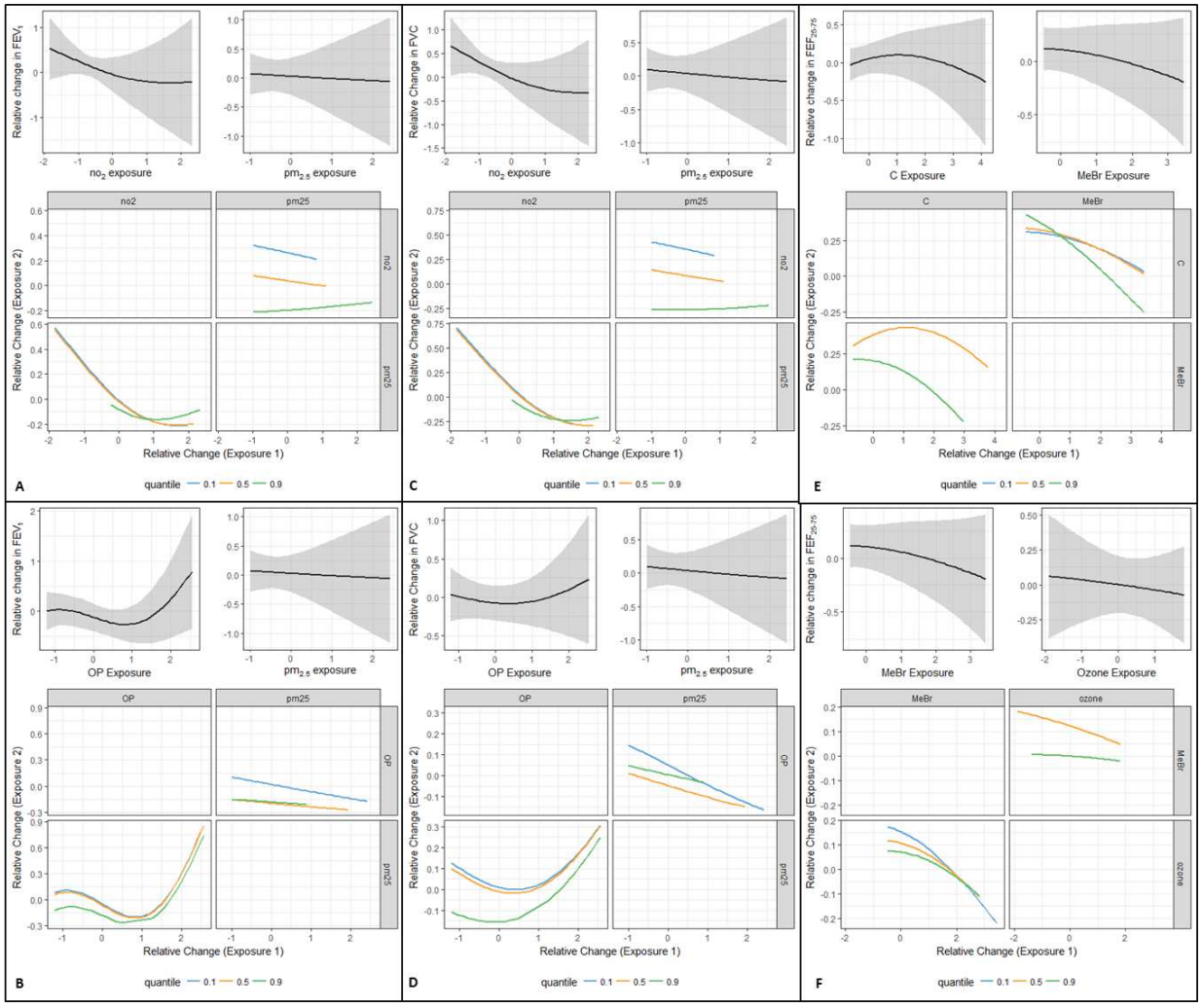

FIGURE 2. SELECTED BIVARIATE ANALYSES LOOKING AT 2-WAY INTERACTION IN FACES COHORT.

Selected bivariate analyses looking at 2-way interaction in FACES cohort. In the top panes, we show exposure-response plots (with 95%CI) between univariate components of the bivariate analyses, and lung function. In the bottom panes, we view the exposure-response function for a given pollutant exposure while a second exposure is fixed at three quantiles (0.1, 0.5, 0.9), and accounting for the remaining exposures (all set at their median). **A**. Bivariate exposure-response relationship for NO_2 and $PM_{2.5}$ on FEV_1. **B**. Bivariate exposure-response relationship for OP and $PM_{2.5}$ on FEV_1. **C**. Bivariate exposure-response relationship for NO_2 and $PM_{2.5}$ on FVC. **D**. Bivariate exposure-response relationship for OP and $PM_{2.5}$ on FVC. **E**. Bivariate exposure-response relationship for C and MeBr on FEF_{25-75}. **F**. Bivariate exposure-response relationship for MeBr and ozone on FEF_{25-75}.

Abbreviations. NO_2, nitrogen dioxide; $PM_{2.5}$, particulate matter with a median aerodynamic diameter < 2.5µm; OP, organophosphates; C, carbamates and MeBr, methyl bromide