

Industrial Animal Agriculture in the Yakima Valley,
Air Pollution, and Pediatric Asthma Morbidity

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Abstract

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Background: Elevated rates of pediatric asthma morbidity have been observed in some regions of the rural United States; however, the potential links between asthma exacerbations and environmental exposures common to rural settings are largely unexplored in the research literature. The growth of industrial-scale agriculture has degraded outdoor air quality in many rural communities including by emissions of large animal feeding operations (AFOs).

Methods: We conducted a longitudinal, repeated measures study of community-level AFO exposures and pediatric asthma morbidity in the Yakima Valley, an agricultural region of Washington State. Our cohort consisted of school-aged children with preexisting asthma (n=58) participating in the Aggravating Factors of Asthma in a Rural Environment (AFARE) study, a

community-based participatory research project. Over a 26 month period, subjects' respiratory health was assessed on a biweekly basis using the Asthma Control Questionnaire (ACQ), a survey of asthma symptom severity and "rescue" medication use, and daily home spirometry, which provided measurements of forced expiratory volume in one second (FEV1). Three outdoor air exposures related to AFO pollution were investigated in separate analyses: regional particulate matter of 2.5 μm or less in aerodynamic diameter (PM2.5); ammonia, repeatedly measured at 18 residential sites across the study area; and a novel metric of AFO airborne "plume" exposure, calculated using proximity and size of nearby AFOs as well as daily wind conditions.

Results: We found that on average, children in the AFARE cohort experienced decrements in FEV1% one to two days following elevated exposure to PM2.5, ammonia, and AFO plumes. In addition, elevated PM2.5 was associated with increased symptom severity and medication usage as reported on the ACQ. No statistically significant associations between ACQ results and ammonia or AFO plume exposure were observed, however.

Conclusions: Our findings indicate that children with asthma may experience short-term respiratory effects following increased exposure to airborne AFO pollutants, adding to a growing body of research evidence that AFO-related air pollution may cause community-level health effects. To our knowledge, our work represents the first analyses of time-varying changes in respiratory health for children with asthma, a vulnerable subgroup of rural populations. Additional research to confirm these findings in other study populations is warranted.

TABLE OF CONTENTS

LIST OF FIGURES	iii
LIST OF TABLES	iv
ACKNOWLEDGEMENTS	vi
DEDICATION	viii
CHAPTER I: BACKGROUND	1
a. Pediatric asthma and air pollution	2
b. Pediatric asthma in the rural U.S.	4
c. Animal feeding operations (AFOs) and regional air pollution	6
d. AFO exposures and community health: Previous studies	10
e. The AFARE study	13
f. Dissertation specific aims and organization	17
g. References	26
CHAPTER II: REGIONAL PM _{2.5} AND SHORT-TERM EFFECTS ON PEDIATRIC ASTHMA MORBIDITY	34
a. Abstract	35
b. Introduction	37
c. Methods	38
d. Results	46
e. Discussion	51
f. Conclusions	55
g. References	65
CHAPTER III: AMBIENT AMMONIA AND SHORT-TERM EFFECTS ON PEDIATRIC ASTHMA MORBIDITY	68
a. Abstract	69
b. Introduction	71
c. Methods	73
d. Results	79
e. Discussion	81
f. References	87
CHAPTER IV: AFO PLUME EXPOSURES AND SHORT-TERM EFFECTS ON PEDIATRIC ASTHMA HEALTH	98
a. Abstract	99
b. Introduction	101
c. Methods	103
d. Results	111
e. Discussion	115
f. Conclusions	120
g. References	121
CHAPTER V: DISCUSSION	134

a. Overall summary of findings	135
b. Strengths and limitations	139
c. Suggestions for future research	143
d. References	148

LIST OF FIGURES

Figure Number	Page
Figure I.1: Map of the AFARE study region	18
Figure I.2: AFARE data collection timeline	19
Figure II.1: 24-hour-average PM _{2.5} concentrations measured at the Toppenish-Yakama air monitor in the WA State Department of Ecology air monitoring network	63
Figure II.2: Associations between FEV1% and PM _{2.5} measured on multiple lag days for complete case (CC) data	64
Figure III.1: Map of study region, including 18 monitoring locations and 97 regional animal feeding operations (AFOs)	92
Figure III.2: 24-hour average ammonia concentrations (n=814) measured at six-day intervals over a 14 month sampling period	93
Figure III.3: Associations between FEV1% and ammonia (NH ₃) concentrations measured at the nearest neighbor monitors	97
Figure IV.1: Correlation between monitored ammonia concentrations and four versions of E_{plume}	128
Figure IV.2: Associations between FEV1% and estimated plume exposure	130
Figure IV.3: Measured ammonia and $E_{\text{plume},4}$ by monitoring site	131

LIST OF TABLES

Table Number	Page
Table I.1: Summary of previous studies of AFO exposure and community health	20
Table I.2: Demographics of AFARE cohort at enrollment	23
Table I.3: Asthma risk factors of AFARE cohort, assessed at enrollment	24
Table I.4: General asthma-related health of AFARE cohort at enrollment	25
Table II.1: Demographics and baseline health description of AFARE cohort by atopy	57
Table II.2: Summary of longitudinal health data collection	59
Table II.3: Association between asthma symptom control index and IQR increase in weekly PM2.5	60
Table II.4: Odds of specific asthma symptoms associated with an IQR increase in weekly PM2.5	61
Table II.5: Association between FEV1% and IQR increase in 24-hour-average PM2.5 measured one day prior	62
Table III.1: Characteristics of AFARE children enrolled during air monitoring (n=51)	91
Table III.2: Ammonia (NH3) concentrations by season	94
Table III.3: Median ammonia (NH3) and metrics of AFO proximity: Correlation	95
Table III.4: ACQ index and average ammonia concentrations	96
Table IV.1: Variations on the calculation of daily AFO plume exposure	126
Table IV.2: Summary of longitudinal health data collection	127
Table IV.3: Associations between asthma symptom control (ACQ index) and estimated AFO plume exposure	129

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DEDICATION

I dedicate this work to my own little animals, Mazama and Ezra.

CHAPTER 1: BACKGROUND

a. Pediatric asthma morbidity and air pollution

An estimated 7 million children in the US have asthma, making it the most common chronic disease of childhood [1]. Asthma is a respiratory disease characterized by chronic airway inflammation, bronchial hyperresponsiveness (an exaggerated response of airways to nonspecific stimuli), and recurrent episodes of reversible airway constriction and obstruction [2]. For a child with asthma, the objectives of asthma therapy include minimizing the impact of asthma symptoms upon daily life and reducing the incidence of recurrent exacerbations (informally referred to as “asthma attacks”), which can range in severity from mild to life-threatening [3]. Pediatric asthma exacerbations are responsible for approximately 700,000 visits to emergency departments (ED) and 200 deaths annually in the US, and substantial disparities in asthma morbidity according to socioeconomic status, race/ethnicity, and geographic location have been described [4].

Research described in this dissertation is focused upon acute exacerbations as well as asthma “worsenings,” short-term changes in respiratory health that may precede an exacerbation for children with pre-existing asthma [5]. Relatively modest worsenings in a child’s asthma health can manifest as a temporary increase in asthma symptom severity, increase in rescue medication use, or a decrease in lung function [6]. These temporary worsenings are significant not only because they may progress to a severe exacerbation but also because worsenings themselves contribute to lowered quality of life for asthma patients independent of more acute episodes [7].

In general, mechanisms of worsenings and exacerbations occur according to pathways of airway inflammation, often caused by environmental factors [3]. Exposure to outdoor air pollution, for example, is believed to induce oxidative stress and airway inflammation through

involvement of both the innate and acquired immune system [8-10]. The oxidative damage to respiratory tract epithelium cells caused by inhaled air pollutants stimulates an influx of inflammatory cells, including eosinophils, neutrophils, mast cells, macrophages and T-cells, and resultant release of cellular mediators. This inflammatory response to air pollution is observed for exposed individuals in the absence of pre-existing asthma [11]; however, this increase in airway inflammation is additionally linked to both airway hyperresponsiveness and airway obstruction for exposed individuals with asthma [12, 13]. Chronic inflammation is further implicated in the process of airway remodeling, another important feature of asthma pathogenesis. During remodeling, the healing of damaged airways results in important structural changes, such as an increase in smooth muscle mass [14, 15]. Airway hyperresponsiveness, obstruction and remodeling are jointly responsible for the classic symptoms of asthma, including shortness of breath, limitations of activity, wheezing, and coughing, all more pronounced upon waking and at night [12].

Previous epidemiologic studies have implicated several specific outdoor air pollutants in short-term increases in asthma morbidity, assessed as asthma hospitalizations, increases in asthma symptoms and medication use, or decrements in lung function [16-22]. Nearly all research supporting these relationships, however, has been conducted in urban areas, and the role of outdoor air pollution in exacerbating asthma in rural remains largely unexplored. Because the sources and composition of air pollution are expected to vary significantly between settings, the relationships between pediatric asthma exacerbations and air pollution described in previous studies may not be generalizable to public health practice in rural settings.

b. Pediatric asthma in the rural US

Fewer studies of air pollution and pediatric asthma are conducted in rural areas because urban areas tend to have poorer air quality, often due to significant motor vehicle traffic as well as more industrial pollution sources overall [23]. In addition, asthma is traditionally considered to be a disease of developed, industrialized countries and urban communities with modern lifestyles [24-26]. Less attention is given to pediatric asthma in rural areas, in part due to the “hygiene hypothesis” which postulates that children raised in rural farming environments are less predisposed to asthma and other allergic diseases on account of early-life farm exposures associated with growing up on farm [27]. Most research supporting this theory has been conducted in rural communities of European countries, distinct in many ways from lifestyles in modern agricultural regions of the United States [28-32].

Recent surveys across the US indicate that the prevalence of rural childhood asthma may be comparable to -- and in some cases, higher than-- that of urban and suburban neighborhoods [33-35]. Not only may rural asthma prevalence be higher than commonly believed, but there are also several reasons why children with existing asthma in rural communities may be more susceptible to the effects of asthma and may experience more exacerbations than children with asthma in urban areas. Families in rural communities often face barriers to attaining effective asthma therapy because of limited access to healthcare resources, lower quality of available asthma care, and poorer insurance coverage in comparison to non-rural areas [36]. On average, parents in the rural US are more likely to live in poverty and attain lower educational levels, both risk factors for poor asthma management and higher frequency of exacerbations [37-38]. Also, pediatric asthma in rural populations often is under-diagnosed [39], and undiagnosed cases are more likely to be uncontrolled [40]. Overall, pediatric asthma morbidity poses a significant public health

burden for rural communities across the US.

While many of the important sources of urban air pollution are less prevalent in rural areas (e.g. motor vehicle traffic), there are several sources of air pollution unique to rural environments that may contribute to pediatric asthma severity. For example, concentrations of pollen and other common aeroallergens generally are higher in rural environments, and exposure to aeroallergens is linked to asthma exacerbations [41]. Residents of rural counties in the United States are more likely to rely upon biomass combustion for cooking and heating, a practice that affects indoor air as well as regional outdoor air quality, and which has demonstrated links to the respiratory health in the community [42]. Also, some outdoor air pollution found in the rural US is attributed to emissions from industrial-scale agricultural operations, such as wind blown dust and pesticide drift from large crop-growing operations and several types of air pollution released from animal feeding operations [43].

Overall, the relationship between air pollution and respiratory health for children in rural settings poses a public health problem distinct in several ways from the urban context. Characterization of environmental triggers of asthma that may be unique to a rural, agricultural context could inform community- and clinic-based asthma management. The identification and mitigation of environmental triggers is one effective component of asthma management strategies, but the success of this intervention depends on proper characterization of the relevant environmental factors [44].

c. Animal feeding operations (AFOs) and regional air pollution

One source of air pollution more relevant in a rural than urban setting is industrial agricultural. The evolution of modern agriculture in the United States has been characterized by a steady shift away from smaller, family-owned agricultural operations towards large, consolidated operations [45]. This movement has included the rise of large animal feeding operations (AFOs), facilities where up to hundreds of thousands of livestock or poultry are confined for the production of meat or dairy products [46]. Solid and liquid animal wastes are generated at AFOs in considerable amounts. One head of cattle, for example, produces 50 to 60 pounds of manure a day, and a dairy AFO housing 2500 cows produces more wastes than humans in a city of 400,000 [47]. The wastes are commonly stored onsite as a “slurry” of solids and liquids in open or covered waste lagoons and may be applied to adjacent fields as fertilizer or distributed to other off-site growing operations [48]. When manure is generated, stored on site and to applied to farmlands, numerous chemical and biological pollutants are emitted to regional air sheds, and subsequent effects on regional air quality have been documented [43]. Specific components of AFO emissions most likely to cause respiratory system toxicity are described below.

i. Ammonia

AFO airborne emissions include ammonia gas, created when urea, present in urine, is broken down by urease, an enzyme found in feces. Ammonia volatilizes out of the slurry mixture while being stored on-site or during application to fields, at a rate determined by the temperature and pH of the medium [49]. Gaseous ammonia has a half-life on the order of days in the atmosphere,

as it is depleted relatively quickly by deposition or following reaction with acid aerosols in the environment, such as nitric or sulfide oxides [50]. Reaction with acid aerosols creates ammonium salt particles, a major component of particulate matter of 2.5 microns or less in aerodynamic diameter (PM_{2.5}). Industrial animal production is the leading source of atmospheric ammonia, and global emissions are on the rise [51].

Ammonia is water soluble and dissolves in the mucosa of the upper respiratory system upon inhalation, causing eye, nose and throat irritation [52]. Irritation of the upper respiratory tract is a suggested trigger for lower respiratory tract dysfunction, including bronchospasm [53]. In addition, ammonia may penetrate to the lower respiratory tract following absorption to particulate matter [54]. Animal studies show that ammonia causes epithelial damage and impairment of the lung's mucociliary clearance mechanism, potentially leading to increased susceptibility to inhaled particles and infectious organisms [52].

ii. PM_{2.5}

AFOs generate considerable amounts of airborne dust consisting of particles of animal feed, manure, microbial by-products, and the animals themselves (e.g. hair and skin) [55]. Most of this particulate matter has limited impact on respiratory health because large particles become trapped in the upper respiratory system, for example in the nose, upon inhalation. In contrast, dust of smaller particle sizes, like PM_{2.5}, can penetrate to the lower respiratory system and stimulate inflammatory responses [56]. The associations between PM_{2.5} and respiratory illness, including exacerbation of pediatric asthma, has been well-established, though most research has been set in urban areas where traffic is the major source of PM_{2.5} [57-60]. AFOs contribute to outdoor PM_{2.5} pollution predominantly through the release of ammonia and the subsequent

transformation to ammonium aerosols, as mentioned above. In the Yakima Valley, where this dissertation research is set, rising ammonia emissions are believed to contribute to elevated PM2.5 concentrations that are threatening the region's attainment of National Ambient Air Quality Standards [60].

iii. Endotoxins and other microbial products

The AFO environment -- in particular any areas where manure is present -- is enriched with a wide diversity of microbial organisms, including bacteria, viruses, protozoa, and fungi. Highly-elevated levels of bioaerosols, consisting of airborne pathogens and their by-products, have been measured in the indoor and outdoor air of AFOs [61], and subsequent impacts on the health of AFO workers and surrounding communities has been suggested [62]. Microbial by-products include endotoxin and peptido-glycans, components of bacterial cell walls that result when bacteria die and lyse. Endotoxin and other forms of microbial debris are potent inflammatory agents. Inhalation of endotoxin is followed by binding to CD-14 and Toll-like receptors (TLR4) receptors on membranes of macrophages and dendritic cells, stimulating the production of pro-inflammatory cytokines in airways [63].

Endotoxins are common in indoor household environments as well, with increasing concentrations associated with pets, lack of air conditioning, and pest infestation [64]. Even though early life exposure to endotoxin is associated with reduced risk of asthma development later in life [65], some evidence indicates that exposure to endotoxin is a risk factor for acute exacerbations for individuals with pre-existing asthma [66-68].

iv. Hydrogen sulfide

Hydrogen sulfide is a toxic gas characterized by its intensely unpleasant odor and is produced by the anaerobic microbial decomposition of animal manure. Adverse effects of acute hydrogen sulfide poisoning, including death, are described in the occupational health literature [69]; however, poisoning occurs at levels far higher than those found in the ambient environment. A small number of epidemiologic studies have been conducted to assess relationships between childhood asthma exacerbation and ambient hydrogen sulfide, including a time series study that detected an increased incidence of childhood hospitalizations for asthma following days of elevated hydrogen sulfide [70]. One group of investigators described evidence for a protective effect of low-level hydrogen sulfide on asthma, however, and suggested plausible mechanisms by which hydrogen sulfide may act to reduce inflammation in the body [71].

v. Malodor

AFO emissions can affect the health and well-being of nearby residents according to non-toxicological mechanisms as well. Ammonia, hydrogen sulfide, and hundreds of volatile organic compounds detected in AFO emissions in trace amounts have strong, unpleasant odors that contribute to overall malodor in vicinity of AFOs. The intensity of odor is often intense enough to register community complaints [72]. Malodor can cause feelings of unease, stress, and agitation in exposed individuals and may cause subsequent respiratory effects at concentrations below established toxicity thresholds [73].

d. AFO exposures and community health: Previous studies

A robust collection of epidemiologic research describes relationships between occupational AFO exposures and a variety of respiratory disorders, including asthma, chronic obstructive pulmonary disease (COPD), bronchitis, acute respiratory distress syndrome, hypersensitivity pneumonitis, and rhinosinusitis [74,75]. The earliest occupational surveys estimated prevalence of respiratory symptoms as high as 60% for workers in confined facilities [76].

It is difficult to generalize evidence from occupational studies to community-level health effects. Workers, especially those working in indoor AFO facilities, are exposed to concentrations of toxicants far higher than those detected in the outdoor air of neighboring communities. For example, average ammonia levels measured inside AFO buildings range from 2.3 ppm (1.6 mg/m³) to 34 ppm (23.6 mg/m³) [77-78] whereas ambient outdoor concentrations recorded in communities near AFOs generally average at least three orders of magnitude lower [79-80]. Concentrations of airborne toxicants released from AFOs decline rapidly with increasing distance as the contaminants disperse through the atmosphere [81]. Despite the striking difference in exposures likely experienced in a community as compared to the occupational context, there are many reasons to believe that AFO pollution may still cause health effects in nearby communities. AFO employees often represent a healthier subset of any given population, and they typically are exposed to AFO-related air pollution for a limited time every work week. In contrast, communities surrounding AFOs include highly susceptible individuals such as the very young or elderly as well as those with pre-existing respiratory diseases. Surveys in multiple regions of the US demonstrate that communities nearest AFO sites are more likely to be low income and of racial/ethnic minority groups [82]. In addition, community members may be subject to exposure periods longer than the typical work shift if a high proportion of time is

spent at home, especially given evidence that outdoor air pollution from AFOs penetrates to indoor spaces [79].

Evidence for community-level health effects attributable to airborne AFO exposures is limited, as summarized in Table I.1. Of all published studies (n=9), seven assessed outcomes related to respiratory system toxicity, including doctor-diagnosed asthma; medication for asthma or wheeze; self-report of wheezing, coughing or other respiratory symptoms; spirometry measures (peak expiratory flow, PEF, and forced expiratory flow in 1 sec, FEV1); bronchial hyperresponsiveness to metacholine; and missed school or activities due to asthma. Six of these seven studies of respiratory health were cross-sectional in design, and the correct temporal relationship between exposure and outcome therefore could not be confirmed. One exception was a panel study of healthy adults living near large AFOs in North Carolina, in which reports of respiratory symptoms and measures of lung function were analyzed with respect to time-varying exposures [83]. No existing studies have been conducted to investigate AFO pollution and short-term changes in respiratory health in a vulnerable subpopulation, such as children or individuals with pre-existing respiratory disease, like asthma.

Exposure assessment in these existing studies typically depended upon the location of subjects' homes or schools to regional AFOs, such as distance to nearest facility [89, 90], number of AFOs within a certain buffer distance [85], or a simple binary classification based on whether or not a subject lived on a farm [86]. A more sophisticated approach developed by Pavilonis et al. involved calculation of "relative" AFO exposure, as shown in Table I.1 [84]. In this case, each subject's exposure to airborne pollution from each AFO was assumed to scale with size of the facility, the inverse square of distance between home and AFO, and the fraction of the time over a recent 3 year period when daily average wind direction was directed from the

AFO to the home. Then, total relative AFO exposure for each subject was then calculated by summing contributions to this estimated exposure by all AFOs within 4.8 km from the home.

We have identified only two studies of respiratory outcomes that involved direct measurement of AFO-related pollutants at or near subjects' homes. In a cross-sectional study of healthy adults, Schulze et al. measured ammonia at 22 sites across a study area in rural Germany and interpolated the average values to all residences in the study [80]. The only existing panel study of respiratory outcomes included direct measurements of hydrogen sulfide and particulate matter of various sizes conducted at one central site with hourly temporal resolution [83].

Taken together, the results of the six cross-sectional studies indicate that adults and children who live or attend school closer to AFOs are more likely to have been diagnosed with asthma, self-report respiratory symptoms at a higher frequency, and, in some cases, have lower lung function. However, results of some investigations are inconsistent in dose-response patterns and several were limited in adjustment for potential confounders. A systematic review conducted in 2010 concluded that existing evidence for community-level health effects linked to AFO air releases was insufficient to infer a causal link [92]. The report of Schinasi et al. and related reports from the same research group [83,87,88,91] were published more recently than this systematic review, and provide the best evidence that elevated concentrations of directly-measured AFO pollutants are followed by community health effects, including respiratory symptoms as well as elevated blood pressure [87], increased stress and feelings of negative mood [91]. To our knowledge, there are no existing studies of AFO exposures focused upon children or individuals with pre-existing respiratory disease using a panel or similar longitudinal study design.

e. 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 (Figure I.1). The Yakima Valley is characterized by a high density of agricultural operations, including farm lands for the production of apples, corn, grapes and other crops, and farm animal confinement facilities, predominantly large dairy operations. Residents in Yakima County have an estimated per capita income of \$19,610 compared to \$30,661 for Washington State overall, and 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). A high proportion of the county population identifies as Hispanic/Latino (46.3%), four times higher than Washington State [93]. The Yakima Valley agricultural industry depends upon 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

AFARE was a community-based participatory research project conducted within the framework of El Proyecto Bienestar, a collaboration of academic researchers and community groups that aims to protect the health of agricultural workers and their families in the Yakima Valley [94]. Project partners included the University of Washington Pacific Northwest Center for Agricultural Safety and Health (PNASH); Yakima Valley Farm Workers Clinic (YVFWC), a network of federally-qualified health clinics serving migrant and seasonal farmworker families as well as other underserved populations in the region; and the Northwest Communities Education Center which includes Radio KDNA, a Spanish language public radio station that provides support and education for the Latino community in the Yakima Valley. The overall research goal of AFARE was to identify environmental factors that aggravate asthma in the rural

setting. Surveys of agricultural workers in the Yakima Valley have identified pediatric asthma as a priority public health concern [95].

Children were recruited for participation in AFARE from the YVFWC Asthma Outreach Program (AOP), a program involving home visits by community health workers to teach children and families how to identify and manage asthma symptoms, utilize medication, and mitigate potential triggers of asthma, with a primary focus upon indoor exposures [96]. Enrollment began in July of 2010 and continued through the first year of AFARE towards a goal of at least 50 participants. In total, 59 subjects were enrolled in the study, though 10 (17%) dropped out prior to the end of AFARE data collection in October 2012. Reasons for leaving the study included moving out of the study area, switching clinics (outside the YVFWC network), and becoming tired of study activities.

AFARE participants ranged in age from 6 to 16 years at enrollment, and the majority identified as Latino/Hispanic and were born in the United States (Table I.2). Participants and families completed questionnaires at baseline to assess potential environmental and occupational (including “take home” pathways of exposure) risk factors for asthma morbidity (Table I.3). Baseline surveys contained additional questions about recent and lifetime asthma health, including health care utilization for asthma events and current medication usage (Table I.4). Children underwent clinical examinations for general health as well as skin prick testing to assess allergic sensitization to 22 locally-relevant aeroallergens, measurement of exhaled nitric oxide, an indicator of airway inflammation, and clinical spirometry (Table I.4).

Longitudinal data collection consisted of health assessments as well as environmental monitoring (Figure I.2). Two tools were used to follow subjects’ time-varying asthma health:

the Asthma Control Questionnaire (ACQ), administered at approximately biweekly intervals, and daily home spirometry:

Biweekly Asthma Control Questionnaire (ACQ): Approximately every two weeks, phone interviews with either the child or an adult family member were conducted using the validated ACQ [97]. At the time of the interview, interviewees were asked to recall the one week period prior in their responses. The interview included five questions about recent asthma symptoms (shortness of breath, limitation of activities, wheezing, nighttime waking, and morning asthma symptoms), and ordinal categorical responses were used to represent increasing severity and frequency. A sixth question ascertained short-acting bronchodilator use as average number of “puffs” per day. Responses to each of the six ACQ questions were coded as integers ranging from 0 (representing no symptom/medication use) to 6 (representing highest symptom severity/medication use). Finally, these six integer values were averaged to produce one continuous number, the ACQ index, which indicated the degree of asthma control experienced over the previous week. Higher ACQ indices represent poorer asthma symptom control.

Daily home spirometry: At the time of enrollment, each child received a PikoNET handheld peak flow meter (PFM) with digital memory (nSpire Health, Inc; Longmont, CO) and was instructed by a trained staff member in proper use of the device according to guidelines of the American Thoracic Society. Children were asked to use the PFM daily every day of the study and to withhold use of short-acting bronchodilator medication in the period immediately prior to PFM use. At approximately six-week

intervals, a staff member from the YVFWC AP visited participants as part of regular AP activities, and uploaded PFM measurements from the participant's device at that time. During a 12-month AFARE follow-up clinic visit (Figure I.2), each subject's technique and ability to produce an error-free measurement was observed by AFARE staff members, and subjects were retrained in PFM use if necessary. FEV1 measurements acquired from the PFM were converted into percent of predicted values (FEV1%) based on standard reference equations [98].

Residential monitoring of outdoor air was conducted at 18 sites across the AFARE study region during the final 14 months of the study (Figure I.1). Fourteen monitoring devices were designed to actively sample air through six separate channels, allowing for analysis of six different airborne constituents during the same sampling period. Monitors were placed outside the homes of fourteen participants at the start of the sampling campaign (Figure I.2), though four were moved to new locations before the end of sampling because the subject moved (n=3) or because the subject dropped from the study and the monitor was relocated to the home of a different subject (n=1). Samples from air monitors were analyzed for ammonia, fine dust (PM_{2.5}), total dust, and pesticide compounds. Analyses described in this dissertation involve only the ammonia data collected by AFARE monitors.

f. Dissertation specific aims

Three specific aims were proposed to address the broad hypothesis that pediatric asthma morbidity is associated with airborne emissions of AFOs in the Yakima Valley. In each case, the study population consisted of the AFARE cohort of children with pre-existing asthma, and the health outcomes analyzed were repeated assessments of asthma symptom severity (biweekly) and measurements of lung function (daily). The first two aims below were each focused upon a specific component of air pollution attributable to AFO emissions in the Yakima Valley. In contrast, exposure in the final aim was calculated with a perspective on AFO emissions as complex mixtures of multiple toxicants.

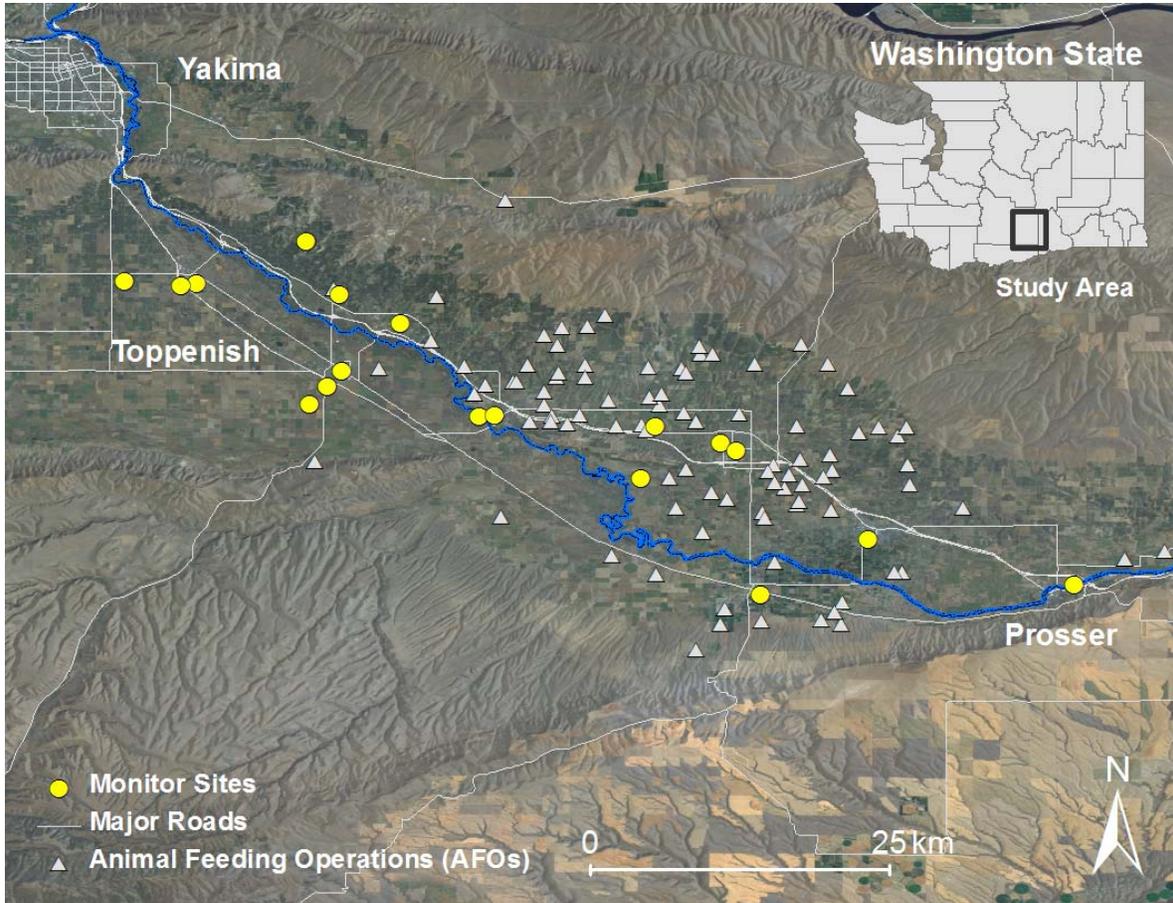
Specific aim 1: To determine if worsening of asthma health follows days of increased levels of fine particulate matter (PM_{2.5}) measured in the ambient outdoor air of the region.

Specific aim 2: To determine if worsening of asthma health follows days of increased concentrations of ammonia measured in the ambient outdoor air at children's homes.

Specific aim 3: To determine if worsening of asthma health follows days of increased exposure to airborne AFO "plumes" at subjects' homes and schools. Time-varying estimates of plume exposure will be calculated using a novel metric that incorporates characteristics of regional AFO sites (i.e. size and location) as well as hourly wind conditions.

The following three chapters (Chapters 2-4) of this dissertation summarize methodology and findings of each investigation in turn, and the final chapter consists of a discussion of overall findings, strengths, limitations, and suggestions for future research (Chapter 5).

Figure I.1: Map of the AFARE study region



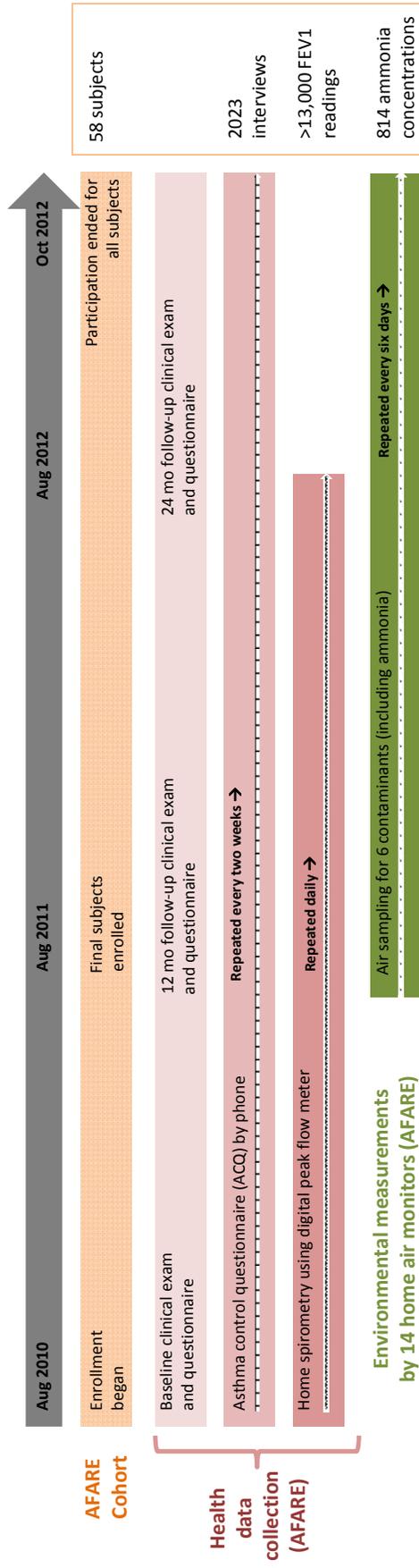


Figure 1.2: AFARE data collection timeline

Table I.1: Summary of previous studies of AFO exposure and community health

Citation	Study design	Study subjects	Outcome(s) of interest	Exposure assessment	Results	Limitations
A. AFO exposure and respiratory health outcomes						
Pavilonis 2013 [84]	Cross-sectional	565 children in rural Iowa age 0 to 17 years	Two self-reported outcomes: - physician dx asthma - physician dx asthma or medication for wheeze	Relative exposure to AFOs $E = \log \sum \frac{A}{d^2} f(w)$ Where: A=AFO area d=distance f(w)=fraction of time when wind blowing towards home - Exposure modeled as a continuous linear variable and in quartiles, separately	-Adjusted OR for both outcomes > 1 when E _{relative} modeled as continuous variable (p<0.05 in both cases) - Adjusted OR for physician-diagnosed asthma or wheeze medication = 2.40 (95%CI: 1.11, 5.21) for highest quartile vs lowest	- Weather station was 80 km from study area - Unclear interpretation of OR in linear models with respect to E _{relative} - Inconsistent results between continuous and quartile models - Small number of cases relative to number of covariates in multivariate model
Schulze 2011 [80]	Cross-sectional	457 adults in rural Germany with no farm exposure in childhood	All subjects: - self-report of wheezing and allergic rhinitis Subset of 149 subjects: - FEV1% - sensitization to relevant allergens	Interpolated mean ammonia concentration at residences based on monitored ammonia at 22 sites	- For subset of subjects with clinical exam (n=149), FEV1% was found to be -8.2% lower (95% CI: -14, -2.7) for ammonia in upper 3 quartiles compared to the lowest quartile	- Cut-point for exposure dichotomization was not justified - No investigation of dose-response across broader exposure levels - Subjects with higher ammonia exposure were also more likely to be sensitized to allergens, but associations were not adjusted for this factor
Schinasi 2011 [83]	Panel	101 healthy adults residing near swine CAFOs in North Carolina (16 communities)	Daily reports of upper respiratory symptoms, irritation, GI concerns and neurological complaints, as well as PEF and FEV1	Repeated measures averaged over 1 or 12 hour periods: - Direct measurement of air pollutants made at one community monitor: H ₂ S, PM10, semi-volatile PM10, PM2.5 - Reports of odor	- Several symptoms were associated with one or more exposures; - strongest associations observed with respect to odor and H ₂ S	- Each community observed for two weeks only - Reports of symptoms may be influenced by odor perception
Radon 2007 [85]	Cross-sectional	6937 healthy adults in rural Germany	For a random sample of 1000 subjects: - reported symptoms (wheezing without cold, physician dx asthma, allergic rhinitis) - Specific IgE to common allergens - Bronchial	Two exposure, both modeled as categorical: - Reported odor annoyance - Number of animal houses within 0.5 km of subject home	- Three self-reported symptoms were related to reported odor annoyance - Highest category of animal house proximity associated with decreased FEV1% and increased BHR	- Limited adjustment for potential confounding factors - Relationships with FEV1% were only observed for highest category of exposure (# animal houses) which contained a small number of subjects (22 vs 580 in reference) - Report of odor annoyance is subjective and was not strongly

Mirabelli 2006 [90]	Cross-sectional	58,169 students aged 12-14 years at 265 schools in N. Carolina	Hyperresponsiveness (BHR) to methacholine - FEV1% predicted All self-reported on surveys Primary: - current wheezing Also: - physician dx asthma - medical care utilization for asthma - activity limitations or missed school due to asthma	Several CAFO-related exposures based on school location: - Distance to nearest CAFO - Hog pounds w/in 3 miles of school - Hog pounds weighted by distance and wind direction - Odor reports by school staff	- Slightly increased prevalence of current wheeze reported at schools nearer to CAFOs - Association varied by whether child reported asthma and by method for categorizing exposure	related to number of animal houses nearby - Potential bias from low participation rate (21% surveys excluded due to missing responses) - SES is associated with prevalence of wheeze, yet method of controlling for SES was limited - Dose-response relationships were inconsistent - Health assessment based on children's reports on survey and might be inaccurate - Multiple comparisons of many exposures and outcomes, stratified by allergy status as well
Sigurdarson 2006 [89]	Cross-sectional	308 students between grades K and 5 at two schools in Iowa	Report of physician dx of asthma, ascertained by survey of parents	Children's exposure status was designated as exposed or unexposed based on which school attended	- Adjusted odds of asthma at school near CAFOs was 5.7 times higher (95%CI: 1.8, 18.4) than at school far from CAFOs - Asthma severity did not differ between schools.	- Only 2 schools in study and analysis did not take into account school-level effects - Low response rate and potential selection bias - Limited control for confounding
Merchant 2005 [86]	Cross-sectional	610 children living in Iowa	Four outcomes, reported by survey: - physician dx asthma - medication for wheeze or asthma - current wheeze - cough with exercise	Residence on farms that raise swine; additionally, farms raising swine that use antibiotics	- Relative to subjects who live on farms but do not raise swine, those who live on farms with swine were more likely to have one or more asthma outcome - Associations stronger for residence on farms using antibiotics	- Response bias is possible - Farms with antibiotics more likely to be larger; may confound association with asthma
B. AFO exposures and their health outcomes						
Wing 2013 [87]	Panel	101 healthy adults residing near swine CAFOs in North Carolina (Schinas 2011)	Systolic and diastolic blood pressure measured 2x day for 2 weeks	Community measurements of ambient hydrogen sulfide and PM10 and reports of hog odor	- Diastolic blood pressure increased with odor and H2S - systolic pressure increased with H2S	- Reports of odor are subjective - Some inconsistencies in associations between different outcomes and exposures

Horton 2009 [91]	Panel	101 healthy adults residing near swine CAFOs in North Carolina (Schinas 2011)	2x daily reports of stress and mood (anxious, angry, unhappy, and confused)	Community measurements of ambient hydrogen sulfide and PM10 and reports of hog odor	Several significant associations between feelings of stress/negative mood with increased H2S, PM10 and odor. Strongest associations observed with odor.	- Assessments of odor and stress/mood were both subjective and reported at the same time; vulnerable to bias - Associations with objective measures of exposure (PM10 and H2S) mostly nonsignificant
Avery 2004 [88]	Panel	15 healthy adults living within 1.5 miles of at least one hog operation	2x daily measure of secretory IgA	Self-reported odor measured 2x daily over two week period	For each unit increase in odor category, log sIgA concentration was 0.058 (std err = 0.032) units higher	- Small sample size - Estimate of salivary excretion rate is highly uncertain

Table I.2: Demographics of AFARE cohort at enrollment¹

Characteristics	N=59²	(%) or mean±SD
Age		10.4 ± 2.6
Total household members		5.3 ± 1.6
Sex		
Male	29	(49)
Female	30	(51)
Race/ethnicity		
Hispanic	55	(93)
Non-Hispanic White	4	(7)
Country of birth		
US	46	(78)
Mexico	10	(17)
Missing	3	(5)
Income		
<\$14,999	25	(42)
\$15,000-29,999	18	(31)
\$30,000-59,999	11	(19)
>\$60,000	2	(3)
Don't Know	3	(5)
Residence type		
Rural or farm	9	(15)
Rural, not farm	14	(24)
In town	35	(59)
Don't know	1	(2)

¹Characteristics assessed by surveys of subjects and family members at the time of enrollment.

² All 59 “ever enrollees” were included in this summary, though one subject dropped out immediately after enrollment and was not included in subsequent analysis of longitudinal data.

Table I.3: Asthma risk factors of AFARE cohort at enrollment

Risk factors ¹	N=59	(%)
A. Environmental risk factors		
Subject's home w/in 0.25 mile of:		
Farms raising animals	13	(22)
Crop farms	28	(47)
Busy road	28	(47)
Dusty road	32	(54)
Environmental tobacco smoke		
Subject smokes	0	(0)
One or more smokers in household	12	(20)
Carpet in child's bedroom	40	(68)
B. Occupational risk factors		
Child is employed or does informal farm work	11	(19)
Child accompanies an adult to workplace	21	(36)
Child is exposed to dust, gases, fumes, chemicals or odors while at a worksite	10	(17)
Adult in household believed to "take home" contamination from worksite	28	(47)
C. Asthma risk factors related to general health		
Report of flu shot in previous year	41	(69)
BMI-for-age > 85 th percentile	30	(51)
Born premature (before 37 weeks)	10	(17)
Atopy status²		
Allergic asthma	42	(71)
Nonallergic asthma	17	(29)
Family history		
Allergy	33	(56)
Asthma	31	(52)

¹All ascertained by surveys conducted with subjects and family members, with the exception of BMI for age and atopy status, determined based on clinical measurements.

²Atopic (or, allergic) asthma was diagnosed based on a positive reaction to one or more of 22 locally-relevant aeroallergens, as observed during skin prick testing.

Table 4: General asthma-related health of AFARE cohort at enrollment

Asthma health characteristic ¹	n	Mean ± s.d or (%)
Age at first asthma symptoms (yrs)		4.1±3.6
Age at doctor-diagnosis of asthma (yrs)		5.2±3.2
Reported asthma medication use		
Quick relief (e.g. albuterol)	53	(90)
<i>Controller medications</i>		
Inhaled corticosteroids (IC) only	26	(44)
Leukotriene antagonist (LTRA) only	3	(5)
Both IC and LTRA	14	(24)
No controller medications	16	(27)
Fraction exhaled nitric oxide (NO)		
Low (0-25 ppb)	35	(59)
Medium (26-50 ppb)	16	(27)
High (>50 ppb)	7	(12)
Missing	1	(2)
Clinical spirometry results		
Normal lung function	41	(69)
Reduced lung function	9	(15)
Severely reduced function	8	(14)
Missing	1	(2)
Lifetime history of healthcare utilization for asthma		
At least one hospital visit	38	(64)
At least one overnight hospital visit	16	(27)
Emergency department visits:		
None	22	(37)
1 or 2 visits	20	(34)
3 to 9 visits	12	(20)
10 or more visits	5	(8)
At least one ICU admission	9	(15)

¹All ascertained by surveys conducted with subjects and family members, with the exception of exhaled NO and lung function tests, determined based on clinical measurements.

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**CHAPTER II: REGIONAL PM_{2.5} AND SHORT-TERM EFFECTS ON PEDIATRIC
ASTHMA MORBIDITY**

a. ABSTRACT

Background: Elevated pediatric asthma morbidity has been observed in rural US communities, but the role of the ambient environment in exacerbating rural asthma is poorly understood.

Objectives: To investigate associations between particulate matter less than 2.5 μm in diameter (PM_{2.5}) and pediatric asthma exacerbations in an agricultural community of Washington State.

Methods: School-aged children with asthma (n=58) were followed for up to 25 months with repeated measures of respiratory health. Asthma symptom control was assessed biweekly using the Asthma Control Questionnaire (ACQ) (n=2023 interviews). In addition, subjects used home spirometers on a daily basis to measure forced expiratory volume in one second (FEV₁) (n=7830 measurements). Regional PM_{2.5} was measured at a single air monitor located centrally in the study region. To assess relationships between PM_{2.5} and these outcomes we used linear regression with generalized estimating equations, adjusting for meteorological and temporal confounders. Effect modification by atopy was explored as well.

Results: An interquartile increase (IQR) in weekly average PM_{2.5} of 6.7 $\mu\text{g}/\text{m}^3$ was associated with an increase in ACQ index of 0.04 (95%CI: 0.01, 0.07), indicating a decrease in asthma symptom control. When ACQ results were decomposed by symptom, wheezing, limitation of activities, and nighttime waking displayed the strongest associations. FEV₁ as a percent of predicted decreased by 0.9% (95%CI: -1.8, 0.0) for an IQR increase in PM_{2.5} one day prior (7.9 $\mu\text{g}/\text{m}^3$), and by 1.4% (95%CI: -2.7, -0.2) when restricted to children with atopic asthma.

Conclusions: This study provides evidence that PM2.5 in an agricultural setting contributes to elevated asthma morbidity. Further work on identifying and mitigating sources of PM2.5 in the area is warranted.

b. INTRODUCTION

Several longitudinal cohort studies have demonstrated that children with asthma experience short-term increases in symptoms as well as decrements in lung function following exposure to outdoor particulate matter with aerodynamic diameter of 2.5 μm or less (PM_{2.5}) (Ostro et al. 2001; Slaughter et al. 2003; Delfino et al. 2004; Moshhammer et al. 2006; Trenga et al. 2006; Dales et al. 2009; Gent et al. 2009). While this association has been well-described in urban settings, it remains largely unexplored in rural, agricultural communities.

Relationships between particulate matter (PM) exposure and respiratory health observed for urban children may not be generalizable to rural regions because of important differences in PM composition. Rural PM tends to contain a higher proportion of organic dust (Schenker et al. 1998), which is derived from plants, animal cells, insects, mold and fungi. In general, organic dusts are pro-inflammatory and cause airway inflammation and obstruction following inhalation (Schwartz 1999). These urban-rural differences in composition reflect varying PM_{2.5} sources. In cities, motor vehicle exhaust is a major source of PM_{2.5}, and wood burning and industrial point emissions contribute to lesser degrees (Maykut et al. 2003). In contrast, rural PM_{2.5} often is generated by agricultural activities, residential wood burning, and natural processes. Tilling, harvesting and field burning disperse an estimated 936 thousand tons of PM_{2.5} into the atmosphere every year, accounting for about 16% of all outdoor PM_{2.5} in the US (Aneja et al. 2009). In addition, large-scale agricultural operations emit gases that indirectly increase regional PM_{2.5} concentrations (NRC 2003). For example, large facilities for animal confinement release substantial amounts of ammonia gas, which subsequently reacts with nitric oxides and sulfuric

acid in the atmosphere to form ammonium salt aerosols, a component of PM_{2.5} (Aneja et al. 2009).

While past research indicated that asthma prevalence may be lower for children living on or near farms (Reidler et al. 2001; Gergen et al. 1988), recent investigations suggest that asthma morbidity in the rural US is as high or higher than in urban communities (Chrischilles et al. 2004; Pesek et al. 2010; Malik et al. 2012). Rural communities often face unique barriers to asthma diagnosis and management, such as limited access to health care, poor insurance coverage, poverty, and geographic isolation (Valet et al. 2009; Ownby 2005).

Aggravating Factors of Asthma in a Rural Environment (AFARE) is a community-based participatory research project aimed at identifying airborne asthma exacerbators in the ambient environment of an agricultural community. Here we describe relationships between community-wide temporal changes in PM_{2.5} and asthma morbidity for AFARE children using a longitudinal, repeated measures study design. We also explored whether atopy is associated with increased susceptibility to PM_{2.5} in this rural community.

b. METHODS

Study setting

The study took place in the Yakima Valley of Washington State, an area covering approximately 300 square miles and characterized by a high density of large-scale agricultural operations, including tree fruit orchards and dairy farms. The AFARE Study was conducted within El Proyecto Bienestar, a community-based participatory research partnership between the University of Washington Pacific Northwest Center for Agricultural Safety and Health; Yakima Valley Farm Worker Clinics (YVFWC), a network of federally-qualified health clinics serving migrant and seasonal farmworker families as well as other underserved populations in the region; and the Northwest Community Education Center which includes Radio KDNA, a Spanish language public radio station that provides support and education for the Latino community in the Yakima Valley.

Study subjects

AFARE subject recruitment began in August 2010 and continued throughout the first year of the study toward a goal of at least 50 participants. Subjects were invited to participate in the study if they were involved in the YVFWC Asthma Program, were of school age, had no other serious illnesses and intended to stay in the region during the two-year duration of the study. The Asthma Program is a longstanding clinical service delivered by community health workers at the patient's home, providing education about asthma management, including proper medication use and home indoor trigger identification and control (Postma et al. 2011). In total, 59 subjects were enrolled in the study, and 10 (17%) dropped out prior to the end of AFARE data collection. One subject who dropped out immediately following enrollment was excluded from analysis, leaving a sample size of 58 participants. Data from the other 9 subjects who left the study were

retained because they participated for a substantial amount of time and the decisions to end participation were unrelated to health or exposure status.

Research activities involving human subjects were approved by the University of Washington Institutional Review Board, and informed consent was obtained from all children prior to participation.

Baseline health assessment

At enrollment, subjects and caretakers completed a health history survey to determine clinical features of asthma status including current medication use. All subjects also underwent skin prick testing to identify children with atopic asthma. Subjects were told to withhold from antihistamine use for 72 hours prior to testing. Three disposable multiple test skin prick applicators were applied to the volar aspect of the subject's lower arm. (Multi-Test II, Lincoln Diagnostics, Decatur IL, USA). Antigens included 22 aeroallergens that comprised typical indoor inhalant allergens (mouse, cat, dog, dust mite mix, cockroach mix, mold mix) and area specific aeroallergens (cow, horse, western juniper, cottonwood, wheat, alfalfa, kochia, smut mix, sagebrush, alder, pigweed, western ragweed, johnson grass, russian thistle), as well as histamine (positive control), and saline (negative control) (ALK Technologies Inc., Princeton NJ, USA). The skin reaction was assessed 20 minutes after application, and a positive response was defined as a wheal size equal to or greater than the positive control.

Longitudinal health assessment

Longitudinal asthma morbidity was assessed with two tools: the Asthma Control Questionnaire and daily home spirometry.

Biweekly Asthma Control Questionnaire (ACQ): At approximately two-week intervals, phone interviews with either the child or an adult family member were conducted using the validated ACQ (Juniper et al. 2010). Interviewees were asked to recall the one week period prior to the interview date in their responses. The interview included five questions about asthma symptoms (nighttime waking, shortness of breath, limitation of activities, wheezing, and morning asthma symptoms) with ordinal categorical responses to indicate increasing severity and frequency. A sixth question ascertained frequency of short-acting bronchodilator use as average number of “puffs” per day. Responses to each of the six ACQ questions were coded as integers ranging from 0 (no symptom/medication use) to 6 (highest symptom severity/medication use) and averaged to produce one value, the ACQ index, to represent the degree of asthma control experienced over the previous week. The ACQ index can range from 0.0, representing the highest level of control, up to 6.0, which would indicate very poor control.

Daily home spirometry: At enrollment, each child received a PikoNET handheld peak flow meter (PFM) with digital memory (nSpire Health Inc., Longmont CO, USA) and was instructed in proper use of the device according to American Thoracic Society (ATS) guidelines. Children were asked to use the PFM twice daily on every day of the study, refraining from the use of short-acting bronchodilator medication immediately prior to PFM use. At approximately six-week intervals, a staff member from YVFWC visited participants and uploaded PFM measurements from the participant’s device. During a 12-month follow-up visit with the

research team at clinics, each subject's technique and ability to produce an error-free measurement was observed, and subjects were retrained in PFM use if necessary. Use of the PFM produced a value of FEV1, which was converted into the percent of predicted value (FEV1%) based on standard reference equations (Hankinson et al. 1999). The highest value of those recorded for a subject in a day was used in analysis.

PM2.5 measurements and meteorology

We obtained 24-hour average PM2.5 concentrations based on nephelometer measurements made at a central site air monitor in Toppenish, WA, managed by the Yakama tribe and included in the WA State Department of Ecology air monitoring network (<https://fortress.wa.gov/ecy/enviwa/>). Two local weather stations most central to the homes of participants (AgWeatherNet database for Toppenish and Snipes stations), provided data on 24-hour average temperature, relative humidity and precipitation.

Statistical analysis

Subject characteristics pertaining to demographics and baseline health status were summarized for the cohort overall as well for subgroups defined by atopy status. Comparisons of characteristics between atopic and nonatopic children were made using chi-squared tests of homogeneity for categorical quantities and t-tests with unequal variances for continuous values. Spearman correlation coefficients were calculated to assess pairwise correlations among meteorology variables and PM2.5.

We modeled associations between PM_{2.5} and each outcome using linear regression models based on generalized estimating equations (GEE) (Diggle et al. 2002) with autoregressive-1 (AR-1) or exchangeable working correlation structures to account for the correlation among the repeated measures for each subject. (Exchangeable correlation structures were used for FEV₁% models on account intermittently missing data, while AR-1 correlation was used in ACQ results. Results were qualitatively similar when correlation structures were varied in sensitivity analysis.) In all models, the exposure of interest was included in models as a continuous variable, and we present results as the mean change in outcome for an IQR increase in exposure, assuming a linear relationship. Covariates included in models as potential confounders were selected *a priori* based on existing evidence of relationships with both respiratory health and exposure. The effects of continuous adjustment variables such as temperature, relative humidity, precipitation, elapsed week of study, and seasonality (calendar month) were represented by cubic splines with five knots each. Other covariates used for adjustment were subject-specific characteristics associated with asthma health, including sex, age, atopy, use of inhaled corticosteroids at baseline, and BMI at baseline.

For models in which outcome was derived from biweekly ACQ surveys, exposure was calculated as the average PM_{2.5} over the seven days prior to the interview date, referred to as the weekly average PM_{2.5}. In exploratory analyses, associations between PM_{2.5} and individual components of the ACQ were estimated by dichotomizing the severity score for each ACQ component into *no* symptom or medication use versus *any* symptom or medication use. Logistic

regression with GEE was used to estimate the odds ratio (OR) for report of each symptom with an IQR increase in weekly PM2.5.

For models in which daily FEV1% was the outcome of interest, the 24-hour average PM2.5 measured one day prior to FEV1% measurement was used as the primary exposure of interest, and other lags were evaluated in sensitivity analyses (0, 2, 3, and 4 day lags). Values of FEV1% that were implausibly high (above 150%) or low (below 30%) were excluded from analysis. In addition, PFM measurements that were flagged by the device as potential errors were omitted from analysis even though inclusion of these measurements did not impact final results in a meaningful way. Subjects with 10 valid PFM readings or more were included in analyses of FEV1 in order to exclude participants with very poor compliance and/or PFM technique.

Model diagnostics were performed to determine whether the central assumptions of GEE were violated. Specifically, plots of residuals versus the linear predictor as well as exposure of interest were inspected to determine whether there existed a meaningful trend in the deviations of residuals from zero. The possibility of influential subjects was explored using the “leave one out” method, by which point estimates and corresponding standard errors were estimated after exclusion of each subject in turn and compared to results generated from analysis of the complete study sample. None of the results of these diagnostic tests indicated cause for concern about model assumptions. Finally, analyses were repeated using linear mixed models (LMM), which returned similar results to those obtained with GEE in all cases.

FEV1 readings were not available for all subjects on every day of the study due to data loss (e.g. technical problems with the laptop and software used to upload PFM data during home visits), broken or lost devices, imperfect compliance, and exclusion of flagged or implausible measurements. We explored patterns of missingness by comparing subjects' data completeness rates to characteristics associated with asthma morbidity, such as average ACQ index, average FEV1%, inhaled corticosteroid use at baseline, and atopy status using linear regression with robust standard errors. In addition, relationships between the odds of missing PFM data on specific day for each subject and both daily PM2.5 and the average of nonmissing FEV1 in the same week were separately assessed using linear regression with GEE.

FEV1 analyses were performed both on available data [i.e. complete case (CC) analysis] as well as on imputed data in order to address the problem of missing data. To generate imputed datasets, multiple imputation was performed using a regression model that included all covariates used in adjusted epidemiologic models, as well as dummy variables to indicate subject, and ordinal categorical responses to each question on the ACQ survey in the relevant time period. Fifty datasets with imputed values for each missing daily FEV1% measurement were generated (i.e. $m=50$). Analyses between FEV1% and one day prior PM2.5 were conducted on each dataset separately, and the estimated coefficients and standard errors derived from each were combined using Rubin's rules (Rubin 1987).

In sensitivity analysis, an interaction term was added to each model to assess the presence of effect modification by atopy. Finally, results of the two main analyses were repeated following stratification by distance between subject's residence and the stationary monitor. Distances of

homes to the monitor site were measured using the “sp” statistical package in R (version 2.14.2, The R Foundation for Statistical Computing), and each home was identified as being either closer or further to the monitor than the mean distance. The main epidemiologic analyses were repeated with restriction to children in either distance group.

All analyses were performed using Stata 12.0 (StataCorp LP, Texas Station TX, USA), with the exception of distance calculations.

d. RESULTS

Characteristics of AFARE cohort

The AFARE cohort consisted of an equal number of girls and boys ranging in age from 6 to 16 years at the time of enrollment (Table II.1). 41% (n=24) of participants were from families with annual household incomes below \$15,000, and half (n=29) had one or more parent employed as a farmworker. In addition to having a diagnosis of asthma, 50% (n=29) had a BMI-for-age greater than the 85th percentile at baseline. All but 4 children self-identified as Hispanic and only 17% (n=10) were born outside the United States. At baseline, subjects and their families were asked about residential proximity to possible environmental exposures associated with asthma, and nearly half (n=27) reported living within 0.25 mile of a “busy” road or a dusty, unpaved road; 38% (n=22) reported living near farms growing crops; and 19% (n=11) of families said they lived near farms raising animals.

Most AFARE participants had experienced a significant asthma exacerbation in the past: two-thirds (n=38) reported being hospitalized at some point for asthma, and 79% (n=46) reported having at least one unscheduled urgent care or emergency department (ED) visit for asthma in the 12 months prior to study enrollment. The majority of subjects were taking at least one controller medication at baseline, with the 71% taking inhaled corticosteroids, 29% taking leukotriene antagonists, and 24% taking both.

Children with atopic asthma were more likely to be taking inhaled corticosteroids or leukotriene antagonist at baseline, to have been hospitalized with asthma, to have reported an unscheduled clinic or emergency department visit for asthma in the year before enrollment, and less likely to live with at least one adult farmworker, but none of these differences reached statistical significance.

Longitudinal asthma morbidity

Subjects participated in AFARE for an average of 92 weeks. After exclusion of one subject that completed fewer than 8 interviews, there were 1948 interviews with complete data from 57 children available for analysis. For each subject, a subject-specific ACQ index was calculated by averaging the ACQ indices resulting from all interviews the subject completed. The average subject-specific ACQ index was found to be 0.52 (Table II.2). In general, subjects' asthma control improved over the course of the study, with an average decrease in 0.22 units in ACQ index per year of participation. Atopic subjects had poorer asthma control than children with

nonatopic asthma (average ACQ index of 0.58 versus 0.39, $p = 0.004$).

Severity scores for each separate component of the ACQ were dichotomized as any symptom/medication use and summarized for all interviews collected (Table II.2). Overall, the presence of each symptom was reported in fewer than half of the interviews, though the frequency of each symptom was higher for atopic children compared to nonatopic children. Likewise, at least some bronchodilator usage was reported during about half of all interviews for the overall cohort, while atopic children were more likely to report use of this rescue medication.

During the study, 7830 lung function measurements were collected from children's PFM devices, and the subject-average FEV1 as percent of predicted (FEV1%) was 75% (s.d. = 15%). Participants' FEV1 increased across the study period, with average lung function growth estimated to be 0.26 L per year of participation; similarly, FEV1% increased by an average of 2.3% per year.

Subjects' PFM data completeness rates (i.e. percent of days with at least one PFM measurement) ranged from 12 to 80% (mean = 35%). True compliance rates, however, are likely substantially higher because these estimates were affected by loss of data in the field. We detected no statistically significant relationships between subject PFM completeness rates and average ACQ index, average FEV1%, atopy status, or use of inhaled corticosteroids at baseline. We also found no evidence that the odds of FEV1 missingness on a specific day of the study was related to average lung function during the same time period (week average FEV1%) or PM2.5 measured on the day before (results not shown).

Community PM2.5

The distance between subjects' homes to the central monitor ranged from 0.7 to 29 miles, with a median distance of 7.5 miles. Daily PM2.5 concentrations had a median (IQR) of 5.7 (7.9) $\mu\text{g}/\text{m}^3$ over the entire two-year study period. In general, PM2.5 was elevated during winter months due to occurrences of stagnation events in the valley, although high PM2.5 concentrations were observed in autumn of 2011 and 2012 as a result of forest fires in the region (Figure II.1). Log-transformed PM2.5 concentrations were found to be moderately and inversely correlated with windspeed ($r=-0.63$) and weakly correlated with relative humidity ($r=0.34$) and wind direction ($r=-0.36$).

Associations between asthma symptom control and PM2.5

The degree of overall asthma control, represented by the ACQ index (higher index corresponding to poorer control), was found to be lower in weeks following higher PM2.5 concentrations (Table II.3). On average for this study population, an IQR increase in weekly PM2.5 was associated with an increase in ACQ index of 0.04 (95%CI: 0.01, 0.07) after controlling for potential confounders. Restriction of analysis to subjects living within 11 miles of the monitors increased the magnitude of effect. For this half of the cohort, an increase in ACQ index of 0.06 (95%CI: 0.02, 0.09) was estimated for each IQR increase in PM2.5.

Among individual asthma symptoms assessed by the ACQ, the strongest association was observed with wheezing (Table II.4). Specifically, with each IQR increase in PM2.5, a 31% increase in odds of wheezing was observed (95% CI: 18%, 45%). Statistically significant associations were also evident for limitations of activities and nighttime waking (OR=1.21 and 1.13, respectively, for an IQR increase in PM2.5).

Associations between lung function (FEV1%) and PM2.5

In s the analysis of available FEV1% data [complete case (CC) analysis] and the analyses of 50 datasets in which missing FEV1% was imputed [multiple imputation (MI) analysis], we observed decrements in lung function associated with higher PM2.5 concentrations one day prior to FEV1% measurement (Table II.5). The point estimates from CC and MI analyses were similar though results derived from the MI dataset were more precise. For CC analysis, an IQR increase in 24-hour average PM2.5 on the day prior was associated with a change in FEV1% of -0.9% (95%CI: -1.8%, 0.0%), while MI results indicated a corresponding change in FEV1% of -1.2% (95%CI: -2.0%, -0.3%). In contrast to sensitivity analysis performed with ACQ results, restriction of the analysis to subjects living near the monitors did not affect results. Exploratory analysis of varying lag days yielded results that were lower in magnitude and with wider 95% confidence intervals in each case (results not shown).

Effect modification by atopy

Associations between ACQ index and weekly average PM2.5 were not modified by atopy status (Table II.3; p-value for interaction term=0.78). In contrast, the decrement in FEV1% estimated for increasing PM2.5 was significantly stronger for atopic subjects in the complete case analysis (p-value for interaction = 0.014) but not when the multiply imputed dataset was analyzed (p-value for interaction = 0.55) (Table II.5).

e. DISCUSSION

Our results indicate that children with asthma in the agricultural Yakima Valley region experience short-term increases in asthma morbidity associated with increases in regional PM2.5. Adverse effects upon subjective reports of asthma symptoms (limitation of activities, more wheezing, more nighttime waking) as well as objective measures of lung function (FEV1) were observed. Our observations bear some similarities to the adverse effects of ambient PM2.5 on pediatric asthma observed in urban settings, despite likely differences in sources and composition.

Compared to monitoring sites in an urban center of WA State (i.e. Seattle), PM2.5 concentrations in the Yakima Valley were similar. A monitoring site in downtown Seattle recorded levels nearly identical to the Toppenish site (median (IQR) = 5.8 (3.2) $\mu\text{g}/\text{m}^3$) during the same sampling period, while a monitor in the industrial region south of Seattle measured slightly higher PM2.5 concentrations (median (IQR) = 7.8 (4.0) $\mu\text{g}/\text{m}^3$). Higher PM2.5 days (i.e. above 20 $\mu\text{g}/\text{m}^3$) occurred more frequently in Toppenish than in Seattle. Forest fires in late 2012 were

severe enough to cause several days of PM_{2.5} levels that far exceeded the EPA 24-hour National Ambient Air Quality Standards (NAAQS) for PM_{2.5}.

This study contributes new findings to asthma research by describing relationships between respiratory health and PM_{2.5} exposure in a non-urban setting. Previous pediatric panel studies conducted in urban settings have yielded similar findings. For example, Ostro et al. (2001) found that African American children with asthma in Los Angeles reported a 10% increase in odds of various asthma symptoms with an IQR increase in PM_{2.5}, even though exposures were considerably higher than those measured in our study region, with a mean PM_{2.5} concentration of 41 $\mu\text{g}/\text{m}^3$. Dales et al. (2009) followed a cohort of children in a Canadian city characterized by high volume truck traffic, where children were exposed to PM_{2.5} concentrations similar to those measured in Toppenish (median (IQR) = 6.5 (6.0) $\mu\text{g}/\text{m}^3$), and observed that bedtime FEV₁% declined about 0.5% with each IQR increase in PM_{2.5} in the previous 24 hours. A small cohort (n=9) of children with asthma in Spokane, WA, a moderate sized city in Eastern Washington where some PM_{2.5} sources would be similar to those in the AFARE study region, were more likely to report asthma symptoms on days of higher PM_{2.5} with a one day lag (Mar et al, 2004). The effects measured were smaller than those observed in our study, however, with OR for symptoms generally around 1.1 for a 10 $\mu\text{g}/\text{m}^3$ increase in PM_{2.5}.

Our analyses returned mixed findings related to effect modification by atopy. Asthmatic individuals who are sensitized to one or more common aeroallergen may be more susceptible to air pollution, especially pollutants with oxidant potential (Tunnicliffe et al. 1994; Strand et al. 1998; Jenkins et al. 1999). Some investigators have hypothesized that airway inflammation

following PM exposure leads to heightened permeability of airway epithelia, which in turn may enhance the allergenic potential of aeroallergens for sensitized individuals (D'Amato et al. 2005). A handful of previous panel studies provide direct evidence that atopic children with asthma do experience higher susceptibility to PM_{2.5}. Delfino et al. (2004) measured personal PM_{2.5} as well as PM_{2.5} measured at a central site monitor and found that the observed decrements in FEV₁% were stronger for atopic boys. A study of children with asthma in Fresno, CA, where PM pollution is influenced by motor vehicle traffic as well as regional agricultural activities, revealed that atopic children constituted a vulnerable subgroup in links between asthma symptoms and exposure to coarse PM (Mann et al. 2010). In contrast, other researchers hypothesize that inhalation of organic dust will stimulate airway inflammation according to nonallergic mechanisms (Schwartz 1999), implying that children with asthma will be susceptible regardless of atopy status.

There are a number of important limitations to our study. Our exposure assessment was dependent upon measurements made at a single central monitoring site, and the same daily PM_{2.5} concentration was assigned to each child on every day of the study. This approach ignores the possibility of spatial variability in PM_{2.5} across the study area or the existence of “personal dust clouds” found in the local environment of a child, which may significantly contribute to the true PM_{2.5} exposure experienced by each child (Delfino et al. 2004). In using central site measurements of PM_{2.5} to represent exposure, we assumed that day-to-day changes in regional PM_{2.5} would correlate well with corresponding changes in individual-level exposures for AFARE children. If the exposure error caused by spatial variability in outdoor PM_{2.5} was nondifferential in the AFARE study region, this error would have the effect of

biasing observed associations towards null. In sensitivity analysis, we found that associations with asthma symptom control for the half of the cohort living in closer proximity to the monitor were larger in magnitude and more precise compared to the children living further away.

Our approach to exposure assessment is further limited by the method of measuring PM_{2.5} concentrations. Nephelometer measurements provide mass concentration alone, affording no insight into concentrations of the specific chemical and biological PM constituents shown to be associated with respiratory effects, such as elemental carbon, wood smoke, fungal species, pollen proteins, or endotoxins. Furthermore, we cannot rule out the possibility that our observed associations between asthma morbidity and PM_{2.5} concentration arose due to confounding with other outdoor pollutants that frequently co-exist with PM_{2.5}. Use of a multi-pollutant model would mitigate the influence of co-pollutant confounding, and our future analyses of pollutants measured at AFARE subject homes may permit such an analysis.

Data completeness rates for one of our outcomes, home spirometry, were variable and relatively low. This is a common limitation in collecting subject-initiated measures such as home peak flow (Redline et al. 1996). It is important to note that factors other than subjects' lack of participation compromised completeness of FEV₁ measurements, including loss of data during the process of uploading measurements from the PFM device or long wait times before replacement of broken devices, and these mechanisms are likely completely random with respect to health and exposure. We explored patterns of missingness and subjects' data completeness rates and found no evidence that missingness and noncompliance are related to health or exposure in this study.

Despite these limitations, our study has a number of strengths. AFARE data collection included thousands of repeated measures of two distinct asthma health metrics collected over longer periods of time than many other pediatric panel studies. Panel studies are demanding of resources but are especially well suited for the study of time-varying exposures that result in short-term, reversible health effects. Because each subject is observed repeatedly during periods of relatively high and low exposure, within-subject associations between exposure and health can be analyzed, and the influence of between-subject confounding is thereby mitigated. We chose to control for temporal and meteorological variables aggressively in analysis in order to minimize the possibility that associations between PM_{2.5} and asthma morbidity are confounded by other covariates, a common concern in air pollution epidemiology (Lumley and Sheppard 2003). We selected regression with GEE as our primary statistical method to account for correlation of measurements within subjects, and we compared the results to those obtained using linear mixed models. Finally, we recognized the fact that missing spirometry data could introduce bias to GEE results if the missingness mechanism was not completely at random (Diggle et al. 2002), and we used multiple imputation to impute informed guesses for missing values. Our multiple imputation model was strengthened by inclusion of ACQ responses within the relevant time periods.

f. CONCLUSIONS

Our results contribute evidence that PM_{2.5} pollution in this agricultural setting impacts the health of children with asthma, which is a significant finding in light of increasing PM_{2.5} levels in this region, which have been approaching nonattainment of EPA NAAQS in recent years. Further work on identifying and mitigating the dominant sources of PM_{2.5} in this area as well as similar agricultural settings is warranted, especially given the vulnerability of rural communities to adverse effects of pediatric asthma.

Table II.1: Demographics and baseline health description of AFARE cohort by atopy^a.

	All subjects (n=58)	Atopy status (Skin Prick Test Result) ^b	
		Positive (n=42)	Negative (n=16)
<i>Demographics</i>			
Female	29 (50%)	17 (41%)	12 (75%)
Age at baseline (years)	10.4 +/- 2.7	10.4 +/- 2.7	10.3 +/- 2.8
Household income <\$15k/year	24 (41%)	18 (43%)	6 (38%)
Born outside US	10 (17%)	8 (19%)	2 (13%)
Hispanic/Latino ethnicity	54 (93%)	38 (91%)	16 (100%)
Parent(s) employed as farmworker	29 (50%)	19 (45%)	10 (63%)
Residence within ¼ mile of:			
<i>Farms growing crops</i>	22 (38%)	14 (33%)	8 (50%)
<i>Farms raising animals</i>	11 (19%)	9 (21%)	2 (13%)
<i>Unpaved dusty roads</i>	27 (47%)	18 (43%)	9 (56%)
<i>High traffic roadway</i>	24 (41%)	19 (45%)	5 (31%)
<i>Asthma and general health</i>			
Daily controller medication use at baseline:			
<i>Inhaled corticosteroids (IC)</i>	41 (71%)	32 (76%)	9 (56%)
<i>Leukotriene antagonist (LTRA)</i>	17 (29%)	15 (36%)	2 (13%)
<i>Both IC and LTRA</i>	14 (24%)	13 (31%)	1 (6%)

Ever hospitalized with asthma	38 (66%)	29 (69%)	9 (56%)
Unscheduled visit for asthma to urgent care or ED in 12 months prior to enrollment	46 (79%)	34 (81%)	12 (75%)
Atopic asthma ^b	42 (71%)	42 (100%)	0 (0%)
At least one adult smoker in household	8 (14%)	5 (12%)	3 (19%)
BMI for age >85th percentile at baseline	29 (50%)	21 (50%)	8 (50%)

Abbreviations: US, United States; ED, Emergency department.

^a Categorical variables summarized as N (%) and continuous measures as mean +/- standard deviation

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

Table II.2: Summary of longitudinal health data collection^a

	All subjects (n=58)	Atopy status ^b	
		Allergic (n=42)	Nonallergic (n=16)
Subject-average ACQ index	0.52 +/- 0.31	0.58 +/- 0.33	0.39 +/- 0.22
Percent of interviews in which each symptom reported:			
<i>Woken by asthma</i>	45.4%	51.5%	27.8%
<i>Limited in daily activities</i>	19.6%	21.5%	14.1%
<i>Shortness of breath</i>	34.8%	38.2%	24.9%
<i>Symptoms in morning</i>	38.7%	42.7%	27.0%
<i>Wheezing</i>	24.5%	28.9%	21.7%
Percent of interviews in which puffs of bronchodilator reported	50.7%	55.3%	37.4%
Subject average FEV1, % predicted	75 +/- 15%	75 +/- 16%	76 +/- 11%

Abbreviations: ACQ, asthma control questionnaire; FEV1%, forced expiratory volume in 1 second as a percent of predicted value.

^a Continuous values presented as mean +/- standard deviation and categorical results as percent of responses.

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

Table II.3: Association between asthma symptom control index and IQR increase in weekly PM2.5

Subjects	Coefficient (95%CI) ^a	p-value
All subjects (n=57) ^b	0.04 (0.01, 0.07)	0.003
Atopy subgroups ^c		
Atopic subjects (n= 42)	0.04 (0.01, 0.08)	0.01
Nonatopic subjects (n= 15)	0.03 (-0.01, 0.08)	0.25

Abbreviations: IQR, interquartile increase; PM2.5, particulate matter of 2.5 µm or greater in aerodynamic diameter.

^a Coefficient is the estimated change in ACQ index associated with an IQR increase in weekly PM2.5 (6.9 µg/m³) after controlling for temperature, relative humidity, precipitation, seasonality and elapsed time in study (all as splines) as well as age, BMI, inhaled corticosteroid use at baseline, and sex. Results were derived from GEE model with AR1 correlation matrix.

^b One subject with fewer than 8 interviews was excluded from this analysis.

^c Atopy was defined as at least one positive result in skin prick testing performed at baseline. p-value for interaction = 0.78.

Table II.4: Odds of specific asthma symptoms associated with an IQR increase in weekly PM2.5

Symptom or medication use	OR (95%CI) ^a	p-value
Limitation of activities	1.21 (1.00, 1.46)	0.05
Wheezing	1.31 (1.18, 1.45)	<0.001
Nighttime waking	1.13 (1.01, 1.26)	0.03
Shortness of breath	1.10 (0.96, 1.26)	0.17
Symptoms worse in morning	1.00 (0.91, 1.11)	0.97
Use of short-acting “relief” medication	1.09 (0.99, 1.20)	0.09

Abbreviations: IQR, interquartile increase; PM2.5, particulate matter of 2.5 μm or greater in aerodynamic diameter.

^a OR is the odds ratio for report of any symptom/medication use regardless of severity associated with an IQR increase in weekly PM2.5 ($6.9 \mu\text{g}/\text{m}^3$) after controlling for temperature, relative humidity, precipitation, seasonality and elapsed time in study (all as splines) as well as age, BMI, inhaled corticosteroid use at baseline, and sex. Results were derived from GEE model with AR1 correlation matrix.

Table II.5: Association between FEV1% and IQR increase in 24-hour-average PM2.5 measured one day prior

Subjects	Complete case analysis		Multiple imputation	
	Coefficient (95%CI) ^a	p-value	Coefficient (95%CI) ^a	p-value
All subjects (n=50) ^b	-0.9 (-1.8, 0.0)	0.06	-1.2 (-2.0, -0.3)	0.01
Atopy subgroups ^c				
Atopic subjects (n= 36)	-1.4 (-2.7, -0.2)	0.03	-1.2 (-2.1, -0.3)	0.01
Nonatopic subjects (n= 14)	0.5 (-0.7, 1.7)	0.43	-1.0 (-2.1, 0.1)	0.10

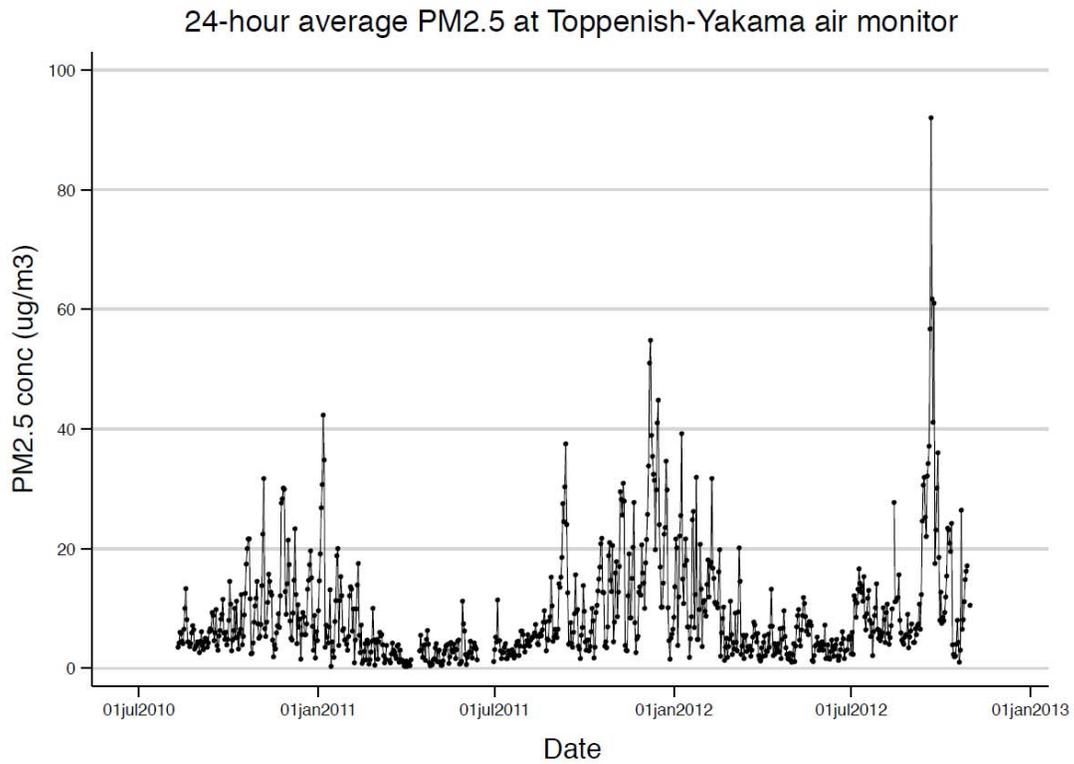
Abbreviations: IQR, interquartile increase; PM2.5, particulate matter of 2.5 µm or greater in aerodynamic diameter.

^a Coefficient is the estimated change in FEV1% associated with an IQR increase in daily PM2.5 (7.9 µg/m³) after controlling for temperature, relative humidity, precipitation, seasonality and elapsed time in study (all as splines) as well as age, BMI, inhaled corticosteroid use at baseline, and sex. Results were derived from GEE model with exchangeable correlation matrix.

^b Eight subjects with fewer than 10 valid FEV1% readings on file were excluded from analysis.

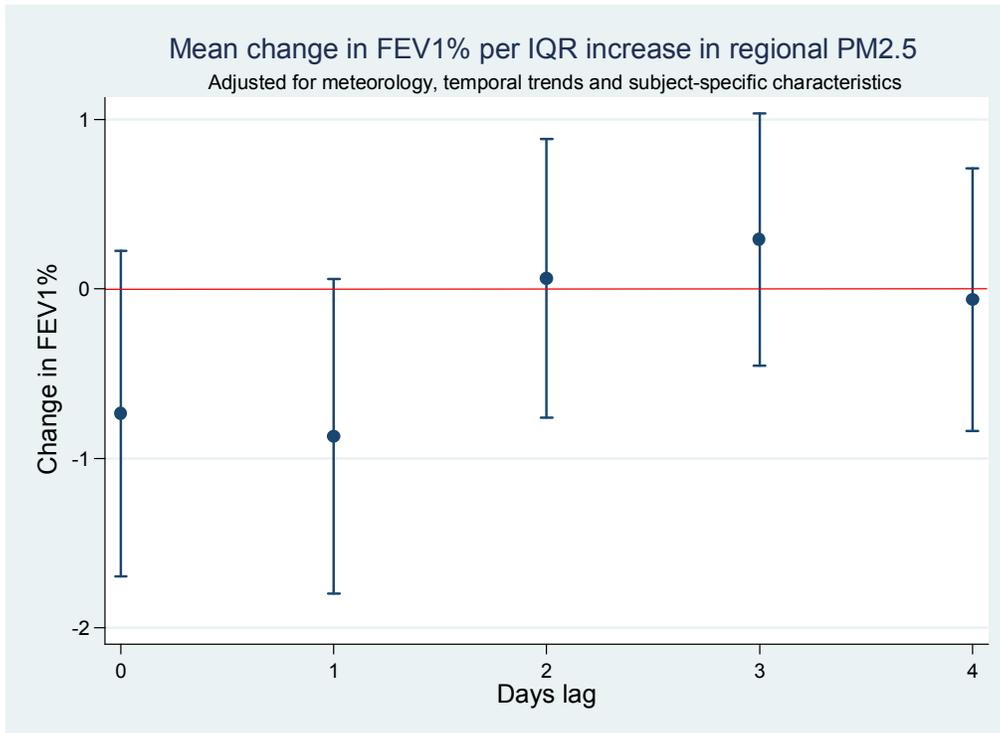
^c Atopy was defined as at least one positive result in skin prick testing performed at baseline. p-value for interaction = 0.014 by complete case analysis and 0.55 using multiple imputation dataset.

Figure II.1: 24-hour-average PM2.5 concentrations measured at the Toppenish-Yakama air monitor in the WA State Department of Ecology air monitoring network.



Abbreviations: PM2.5, particulate matter of 2.5 μm or greater in aerodynamic diameter; WA, Washington.

Figure II.2: Associations between FEV1% and PM2.5 measured on multiple lag days for complete case (CC) data^a.



Abbreviations: PM2.5, particulate matter of 2.5 μm or greater in aerodynamic diameter; IQR, interquartile range.

^a Coefficient is the estimated change in FEV1% associated with an IQR increase in daily PM2.5 ($7.9 \mu\text{g}/\text{m}^3$) after controlling for temperature, relative humidity, precipitation, seasonality and elapsed time in study (all as splines) as well as age, BMI, inhaled corticosteroid use at baseline, and sex. Ranges represent 95% confidence intervals around each point estimate. Results were derived from GEE model with exchangeable correlation matrix.

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**CHAPTER III: AMBIENT AMMONIA AND SHORT-TERM EFFECTS ON
PEDIATRIC ASTHMA MORBIDITY**

a. ABSTRACT

Background: Large-scale animal feeding operations (AFOs) compromise regional air quality in the rural United States through emission of pollutants such as ammonia gas. Exposure to airborne AFO pollution may cause pediatric asthma exacerbations in surrounding communities.

Objectives: To describe spatial and temporal patterns in ambient ammonia concentrations in an agricultural region, and to investigate associations between short-term fluctuations in ammonia and subsequent changes in respiratory health in children with asthma.

Methods: For 13 months in the Yakima Valley of Washington State, 14 monitors sampled ammonia in outdoor air for 24-hour periods every six days. School-age children with asthma (n=51) were followed for two health outcomes: biweekly reports of asthma severity using the Asthma Control Questionnaire (ACQ), and daily measurements of forced expiratory volume in one second (FEV1). Associations between each outcome and ammonia were assessed using generalized estimating equations.

Results: 24-hour ammonia concentrations varied from 0.2 to 238.1 $\mu\text{g}/\text{m}^3$ during the study period and displayed a strong correlation with AFO proximity. FEV1% was 3.8% lower (95% CI: 0.2, 7.3) per interquartile increase in one-day lagged ammonia concentration and 3.0% lower (95% CI: 0.5, 5.8) for two-day lagged concentration. No associations between self-reported asthma severity assessed by the ACQ outcomes and estimated ammonia exposure were observed.

Conclusions: Ammonia concentrations were elevated in this community and strongly predicted by AFO proximity. Exposure to ammonia might cause lung function decrements in children with asthma in the surrounding community. Alternatively, ammonia may serve as a marker for other AFO pollutants responsible for respiratory effects.

b. INTRODUCTION

In recent decades the industrialization of commercial agriculture has affected environmental quality in the rural United States, in part due to the rise of large-scale facilities for confinement of livestock and poultry, termed animal feeding operations (AFOs).¹ The largest AFOs house over a hundred thousand animals in a relatively small area free of vegetation, and substantial amounts of animal manure are generated continuously on site. Liquid and solid animal wastes combine to form a slurry that is commonly stored at AFO sites in waste lagoons and may eventually be applied to adjacent growing fields as fertilizer. During manure storage as well as application to farmlands, a variety of chemical and biological pollutants are released to the atmosphere, and resultant impacts on regional air quality have been observed.¹⁻⁵

Several components of AFO emissions may cause or exacerbate respiratory disease for nearby residents, including ammonia gas. Ammonia is water soluble and dissolves in the mucosa of the upper respiratory system upon inhalation, causing eye, nose and throat irritation,⁶ a suggested trigger for lower respiratory tract dysfunction, including bronchospasm.⁷ In addition, ammonia may penetrate to the lower respiratory tract following absorption to particulate matter.⁸ Animal studies show that ammonia causes epithelial damage and impairment of the lung's mucociliary clearance mechanism, potentially leading to increased susceptibility to inhaled particles and infectious organisms.⁶ In addition, ammonia emissions degrade air quality following secondary transformations in the atmosphere as ammonia and acid aerosols react to produce particulate matter of 2.5 microns or less (PM_{2.5}), an air pollutant linked to significant morbidity and mortality worldwide.⁹ Elevated ambient ammonia can affect the health and well-being of nearby

residents according to non-toxicological mechanisms as well. Ammonia has a strong, unpleasant odor that contributes to the overall malodor in vicinity of AFOs, often intense enough to register community complaints.¹⁰ Malodor can cause feelings of unease, stress, and agitation in exposed individuals and may cause subsequent respiratory effects at concentrations below established toxicity thresholds.¹¹

Previous epidemiologic investigations of community-level AFO exposures and respiratory health are limited to cross-sectional studies,¹²⁻¹⁷ with the exception of one longitudinal, repeated measures study of healthy adults living near swine AFOs.¹⁸ Though some cross-sectional studies have included pediatric subjects, none involved repeated measures of respiratory health for assessment of time-varying asthma health and none included direct measurements of ambient ammonia in the community.^{12-14,17} Here we describe results of a longitudinal study of pediatric asthma exacerbations using health and environmental data collected from the Aggravating Factors of Asthma in a Rural Environment (AFARE) project set in the Yakima Valley, an agricultural region of Washington State characterized by a high density of large dairy operations.

The primary objectives of our investigation were to describe spatial and temporal patterns in community-level ammonia concentrations in the Yakima Valley, and to estimate associations between time-varying ammonia concentrations outside subjects' homes and short-term fluctuations in asthma health, measured as daily lung function and biweekly reports of symptom severity. Secondary analyses were conducted to assess the relationships between ammonia and asthma symptom severity at longer time lags, to explore the hypothesis that sub-chronic exposure predisposes children with asthma to respiratory illness.

c. METHODS

Study setting

AFARE was conducted within El Proyecto Bienestar (EPB), a community-based participatory research partnership seeking to protect the health of agricultural workers and their families in the Yakima Valley. Project partners include the University of Washington Pacific Northwest Center for Agricultural Safety and Health (PNASH); Yakima Valley Farm Workers Clinic (YVFWC), a network of federally-qualified health clinics serving migrant and seasonal farmworker families as well as other underserved populations in the region; and the Northwest Communities Education Center which includes Radio KDNA, a Spanish language public radio station that provides support and education for the Latino community in the Yakima Valley. The study region is characterized by a high density of large-scale agricultural operations, including dairy AFOs, largely concentrated in the southern half of the valley.

Study subjects

Beginning in August 2010, AFARE subjects were recruited from the YVFWC Asthma Program (AP).¹⁹ Inclusion criteria included being of school age and having no serious illnesses other than asthma. A total of 59 children were enrolled in the AFARE study; however, for the analyses described here we consider only the 51 children who participated for at least three months during the period of air monitoring (September 2011 through October 2012; see Figure I.2). At enrollment all subjects underwent skin prick testing to 22 common and locally relevant inhalant allergens in order to assess atopy, and families completed a health history survey to describe

clinical features of past and present asthma health. Research activities were approved by the University of Washington Institutional Review Board, and informed consent was obtained from all children prior to participation.

Identification of AFOs

We used the WA State Department of Agriculture (WSDA) database of dairy operations registered in 2012 through the Dairy Nutrient Management Program (DNMP) to identify AFOs in the region. We systematically inspected aerial photography images of the entire study region available online via Google Maps and Google Earth for characteristics of AFOs such as large dirt areas containing cattle, feeding and milking shelters, and animal waste storage ponds in order to confirm -- and when necessary, correct -- the location of the 67 dairy operations registered with the DNMP. We also identified additional non-dairy AFOs, including heifer, beef and poultry operations not included in the WSDA database. The area of all land that appeared to be part of each AFO was estimated in units of m^2 and the geographic location was approximated as the center of the facility.

Outcome variables

Biweekly Asthma Control Questionnaire (ACQ): At approximately two-week intervals, phone interviews with either the child or an adult family member were conducted using the validated ACQ.²⁰ Interviewees were asked to recall the one week period prior to the interview date in their responses. The interview included five questions about asthma symptoms (nighttime waking,

shortness of breath, limitation of activities, wheezing, and morning asthma symptoms) with ordinal categorical responses to represent increasing severity and frequency. A sixth question ascertained short-acting bronchodilator frequency of use as average number of “puffs” per day. Responses to each of the six ACQ questions were coded as integers ranging from 0 (no symptom/medication use) to 6 (highest symptom severity/medication use) and averaged to produce one value, the ACQ index, to represent the degree of asthma control experienced over the previous week.

Daily home spirometry: At the time of enrollment, each child received a PikoNET handheld peak flow meter (PFM) with digital memory (nSpire Health, Inc; Longmont, CO) and was instructed in proper use of the device according to guidelines of the American Thoracic Society. Children were asked to use the PFM twice daily, in the morning and evening, every day of the study and to withhold use of short-acting bronchodilator medication in the period prior. At approximately six-week intervals, a staff member from the YVFWC AP visited participants and uploaded PFM measurements from the participant’s device. During a 12-month AFARE follow-up visit with the research team at clinics and immediately prior to the start of ammonia monitoring, each subject’s technique and ability to produce an error-free measurement was observed, and subjects were retrained in PFM use if necessary. FEV1 measurements from the PFM were converted into percent of predicted values (FEV1%) based on standard reference equations.²¹ Values of FEV1% that were implausibly high (above 150%) or low (below 30%) as well as measurements that were flagged by the device as potential errors were omitted from analysis.

Ammonia measurements and meteorology

We have described the design, deployment and performance of the air sampling device previously.²² Fourteen devices were placed outside the homes of a subset of the AFARE participants that were selected based on property accessibility and security as well as consideration of overall spatial variability across the study region. Four monitors were moved during the air monitoring period because study subjects changed residences (n=3) or the family dropped out of the study (n=1), and one monitor was destroyed in a home fire three months prior to the end of the study and was not replaced. The monitoring device was constructed to conduct simultaneous measurement of multiple contaminants for 24-hour periods at six-day intervals. Devices actively sampled outdoor air for ammonia using a silica bead sampling tube (SKC Inc; Eighty Four, PA) with flow rates ranging from 0.10-0.32 L/min according to the Occupational Safety and Health Administration (OSHA) method ID-188. Flow rates of sampling pumps were calibrated once a month and were adjusted to meet the intended flow rate range when necessary. Sampling tubes were transported to the University of Washington Environmental Health Laboratory at 0°C, where they were desorbed with deionized water and analyzed for ammonium ion using ion chromatography. Ammonia masses below the limit of detection (LOD) (either 1 or 0.5 µg, depending on the date of analysis) were approximated as LOD/2.

Two local weather stations central to the study region provided data on 24-hour average temperature, relative humidity and precipitation (Washington State University AgWeatherNet stations at Toppenish and Snipes).

Statistical analysis

Relationships between ammonia concentrations and AFO proximity were assessed using four metrics calculated relative to each monitor location: distance to nearest AFO, count of AFOs within a 5 km buffer, count of AFOs within a 10 km buffer, and a weighted sum of regional AFOs calculated according to Equation 1:

$$AFO\ proximity = \sum_{All\ AFOs} \frac{\log(Area)}{distance} \quad (Equation\ 1)$$

Pearson correlation coefficients (r) were calculated to assess pairwise correlations between each of the four estimates of AFO exposure and median ammonia measured at the 18 monitoring sites.

We modeled associations between time-varying ammonia exposure and each of the two outcome measures separately using generalized estimating equations (GEE) with an exchangeable correlation matrix.²³ For analyses of FEV1%, lagged relationships ranging from 0 (same day) to 5 days were investigated and exposure was approximated as the 24-hour average ammonia concentration measured at the air monitor nearest to the location of the child's home at the time of FEV1 measurement. For the biweekly ACQ outcomes, weekly average ammonia concentrations were approximated using measurements obtained at nearby monitors within a window of time between eight days and one day prior to the interview date. All measurements collected at the five nearest neighbor monitors during this period were identified and averaged using inverse-square distance weighting into one quantity referred to as the weekly average ammonia. (Inverse distance weighting was not possible for FEV1 analysis because of a one-day

shift in sampling schedules for monitors in the north and south regions.) In all epidemiologic models, the mean outcome was modeled to be linear in response to the primary exposure of interest, and we presented the final results as effect sizes per IQR increases. In sensitivity analysis, the main analyses were repeated following restriction to children living within the median distance to the nearest air monitor (1.0 km).

Covariates included in models as potential confounders were selected *a priori* based on existing evidence of relationships between the covariate and both respiratory health and exposure: temperature and relative humidity (averaged over the week prior for ACQ outcomes) as well as two variables related to temporal trends, days elapsed in study and seasonality, represented by cubic splines. Also included were subject-specific characteristics potentially associated with asthma: sex, age, atopy, use of inhaled corticosteroids at baseline, BMI at baseline and presence of adult smoker in household.

Model diagnostics were performed to determine whether the central assumptions of GEE were violated. Specifically, plots of residuals versus the linear predictor were inspected, and the possibility of influential subjects was explored using the “leave one out” method, by which point estimates and corresponding standard errors were estimated after exclusion of each subject in turn and compared to results generated from analysis of the complete study sample. These analyses did not indicate the presence of any issues related to model assumptions. Analyses were repeated using linear mixed models (LMM) to assess robustness of results to choice of statistical model, and in all cases produced similar results to those obtained with GEE.

All analyses were performed using Stata 12.0 IC (StataCorp LP; College Station, TX), with the exception of distance and nearest neighbor determinations, which were conducted using R (version 2.14.2, The R Foundation for Statistical Computing).

d. RESULTS

Cohort characteristics

Of the 51 children enrolled in AFARE in the second year, many were from low income families with one or more adults employed as a farm worker, and self-identified as Hispanic/Latino (Table III.1). The majority of children were taking controller medications at the time of enrollment (i.e., inhaled corticosteroids and/or leukotriene antagonists), and 38 subjects (74%) were identified to have atopic asthma on the basis of skin prick testing performed at baseline. Only 7 children (14%) lived with at least one adult smoker. Based on a clinical exam performed at baseline, nearly half of the subjects (49%) were classified as overweight, defined as BMI-for-age above the 85th percentile.

Longitudinal health data

In the period during which residential air monitors collected samples for ammonia analysis, subjects completed an average of 20 interviews each, from which a subject-average ACQ index of 0.42 ± 0.30 was determined. Each individual symptom assessed by the ACQ was reported to

have occurred in fewer than 50% of the interviews. Children with atopic asthma had average ACQ scores significantly higher than children without atopy (0.49 vs 0.23; $p=0.004$), indicating poorer control. An average of 87 FEV1 measurements were collected for each child. FEV1 increased for the cohort across the study period on average, with the population-average lung function growth estimated to be 0.26 L per year (s.d. = 0.37 L).

Community ammonia concentrations

There were 18 locations where monitoring was conducted throughout the Yakima Valley region (Figure III.1). For AFARE subjects without monitors placed at their homes, the distance from subject home to the nearest AFARE monitor ranged from 0.01 to 9 km. The median ammonia measured at each site ranged from 2.9 to 72.7 $\mu\text{g}/\text{m}^3$ with higher concentrations observed in the southern region of the study area (Figure III.2). Concentrations at each site exhibited a high degree of temporal variability on short time scales (i.e. days to weeks). Ammonia concentrations were weakly and negatively correlated with both daily wind speed ($r=-0.29$) and direction ($r=-0.22$), weakly correlated with relative humidity ($r=0.17$) but not correlated with temperature or precipitation ($r<0.05$). Some evidence for seasonal patterns in ammonia concentrations was observed, with slightly elevated concentrations measured during the winter months (Table 2).

Our scan of aerial images of the region identified 97 likely AFO sites, ranging in size from 6500 to 900,000 m^2 in estimated surface area (mean area = 190,000 m^2). Median ammonia measured at each monitoring site exhibited a statistically significant correlation with each measure of AFO proximity (Table III.3). Distance to the nearest facility explained the smallest amount of

variability in site median ammonia ($R^2 = 0.56$), while the sum of inverse distances accounted for 78% of the variability in ammonia. Each of the four metrics of AFO proximity displayed a high degree of pairwise correlation.

Ammonia exposure and asthma morbidity

We found no significant relationship between asthma symptom control and the weekly ammonia exposure estimated for the week prior to the interview date (Table III.4). An IQR increase in ammonia of $18 \mu\text{g}/\text{m}^3$ was associated with a 0.01 unit increase in the ACQ index (95%CI: -0.04, 0.05). Expanding the exposure window to include two and four weeks prior to the interview data had no meaningful effect on the results.

Ammonia exposure was associated with FEV1% at various lag intervals, with FEV1% being lower following days of elevated ammonia; this relationship was statistically significant only on lag days 1 and 2 (Figure III.3). FEV1% was decreased by 3.8% (96%CI: 0.2, 7.3) per IQR increase in ammonia one day following ammonia measurement and decreased by 3.0% (96%CI: 0.5, 5.8) two days later. When analyses were repeated following restriction to children living within 1.0 km of an air monitor, associations estimated for all lag intervals were found to be larger in magnitude (i.e. more negative) but generally less precise. FEV1% decreased 6.3% (95%CI: 2.3, 10.3) two days after exposure, for example, for the subset of children living near monitors.

e. DISCUSSION

Our results provide unique insight into population-level ammonia exposures occurring in an agricultural community and potential effects on the respiratory health of children with asthma. We found that ambient ammonia concentrations were elevated in the southern half of the Yakima Valley where most AFO's were located. At the monitoring site with the highest density of surrounding AFO's, the 75th percentile of 24-hour ammonia concentrations was 101 $\mu\text{g}/\text{m}^3$, exceeding the EPA reference concentration for chronic inhalation exposure of 100 $\mu\text{g}/\text{m}^3$. Ammonia concentrations measured in our study were higher than those reported by some investigators in similar study regions. For example, in a region of Iowa with a high density of swine AFO's, researchers measured average levels of 6.7 $\mu\text{g}/\text{m}^3$ outside homes next to animal facilities.²⁴ These numbers are similar to those reported by Williams et al. at homes located within 0.4 km from the nearest dairy facility in the same region of our study.⁴ In contrast, ammonia monitoring conducted in areas of intensive animal husbandry in Europe found higher concentrations: for example, in Lower Saxony, Germany, mean annual levels were between 16 and 24 $\mu\text{g}/\text{m}^3$,¹⁶ and between 10 and 25 $\mu\text{g}/\text{m}^3$ in the Netherlands.²⁵

Our findings indicate that geographic differences in ammonia concentrations were explained in large part by proximity to animal operations, with 77% of the between site variability in time-averaged ammonia concentrations explained by a distance- and size-weighted measure of proximity (Equation 1). This is not surprising given that the majority of anthropogenic ammonia emissions worldwide are attributable to animal production facilities, which release ammonia during the production and handling of animal manure, as well as during application of liquid and solid wastes to nearby fields.¹ We found that ambient concentrations were slightly higher in

winter months, the time of year when fertilization occurs in this region, though ammonia emission rates are observed to increase at higher temperatures.²⁶ We observed a high degree of temporal variability in ammonia concentrations with six day sampling intervals, a feature that is important to consider when estimating epidemiologic associations with short-term health effects.

We found no evidence that increased ammonia exposure was linked to poorer asthma control as measured by symptoms and medication usage. In contrast, we found significant associations between a measure of lung function, FEV1%, and estimated ammonia exposure at subjects' homes. Effect sizes were largest in magnitude and most precise in relation to ammonia measured one and two days prior to spirometry, supporting our hypothesis that short term respiratory effects would occur. In sensitivity analysis, we restricted this analysis to children who lived within 1 km of the nearest air monitor in order to address the likelihood of increasing nondifferential error in exposure assessment with increased distance from the nearest monitoring site. As predicted, this restriction resulted in larger estimates of effect.

Our findings may reflect a true causal relationship between ammonia exposure and decreased lung function, although further studies are necessary to infer causality. One previous study of pediatric respiratory health and ambient ammonia took place in a region with a high density of fertilizer production plants, where mean ambient ammonia concentrations ranged from 54 to 113 $\mu\text{g}/\text{m}^3$, exceeding most exposure levels experienced by our cohort.²⁷ No differences were found in FEV1 of children living in high ammonia communities when compared to children in a "control" neighborhood with no known ammonia sources. However, lung function was assessed only twice for the cohort, and the concentrations of ammonia in the control area were elevated as

well, ranging from 30 to 63 $\mu\text{g}/\text{m}^3$. We have found no other published studies of population-level exposures to ambient ammonia and pediatric respiratory health. A cross-sectional study of healthy adults residing near biodegradable waste sites did report a significant relationship between modeled exposure to ambient ammonia and increased frequency of self-report respiratory and sensory irritation effects, including coughing.²⁸

Ammonia may serve as a marker for the complex airborne emissions from AFOs, and the observed decreases in lung function may have resulted from exposure to one or more co-pollutants with established respiratory system toxicity, such as endotoxin, particulate matter, or hydrogen sulfide.²⁹⁻³¹ Occupational studies of AFO workers have established a strong link between respiratory disease and exposure to AFO air pollutants.³²⁻³⁴ Regardless of whether the decreases in lung function reported here were caused by exposure to ammonia or to one or more of other the toxicants emitted from AFOs, our findings add to the existing body of evidence for a causal relationship between community-level AFO exposure and respiratory disease.³⁵⁻³⁷

Previous investigations have described relationships between prevalence of childhood asthma, either lifetime or current, and AFO exposures;^{12-14,17} however, all previous epidemiologic studies involving pediatric subjects have been cross-sectional in design. In addition, most existing studies relied upon measures of time-invariant AFO exposure, such as distance from subject's home to nearest facility¹² or number of AFOs located within a certain distance of subjects' schools.^{13,14} In contrast, we made direct measurements of an AFO-related pollutant in the ambient environment of children's homes repeatedly for over a year. Our work can be compared to a panel study of AFO exposures and respiratory health in a cohort of healthy adults for which

two AFO-related pollutants, hydrogen sulfide and PM of 10 μm or less in diameter, were repeatedly sampled in subjects' communities.¹⁸

To our knowledge, we have conducted the first longitudinal, repeated measures study of community-level ammonia exposures and respiratory health, as well as the first study of this design to address AFO exposures in relation to pediatric health. A longitudinal repeated measures study is the ideal design to investigate exposures and outcomes that vary in time, and the within-subject comparisons help mitigate the influence of between-subject confounding. This is important in the context of AFO proximity and asthma morbidity because residence on or near a farm raising animals may be associated with other subject characteristics linked to risks of respiratory disease such as socioeconomic status, atopy, and early-life exposures to a farming environment.³⁸⁻³⁹

Despite these strengths, we acknowledge several limitations. As mentioned previously, it is uncertain whether the relationships with lung function are due to ammonia or other components of AFO emissions. Secondly, our exposure assessment was based solely on children's residences; we did not collect information about time-activity patterns. It is possible that children were less likely to play outdoors on days of higher ammonia exposures, but we are unable to account for such avoidance behavior in our analyses. In addition, our weekly estimates of ammonia exposure assigned to each ACQ survey date were based on only one, and sometimes two, measurement(s) of ammonia made at some time in the week prior to the interview.

Because ammonia concentrations vary significantly on a time scale of days in this study region, this method is likely influenced by a large degree of nondifferential measurement error. This

nondifferential error may have masked any true relationships between asthma symptom control and ammonia exposure by biasing results towards the null. Finally, our analysis of FEV1% and ammonia may be biased on account of missing spirometry data. Results of GEE in analysis of correlated data is susceptible to bias when missingness mechanisms are not missing completely at random.²³ Linear mixed models are more robust to such a bias, and in sensitivity analysis we found that results of either modeling technique to be similar.

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Table III.1: Characteristics of AFARE children enrolled during air monitoring (n=51)

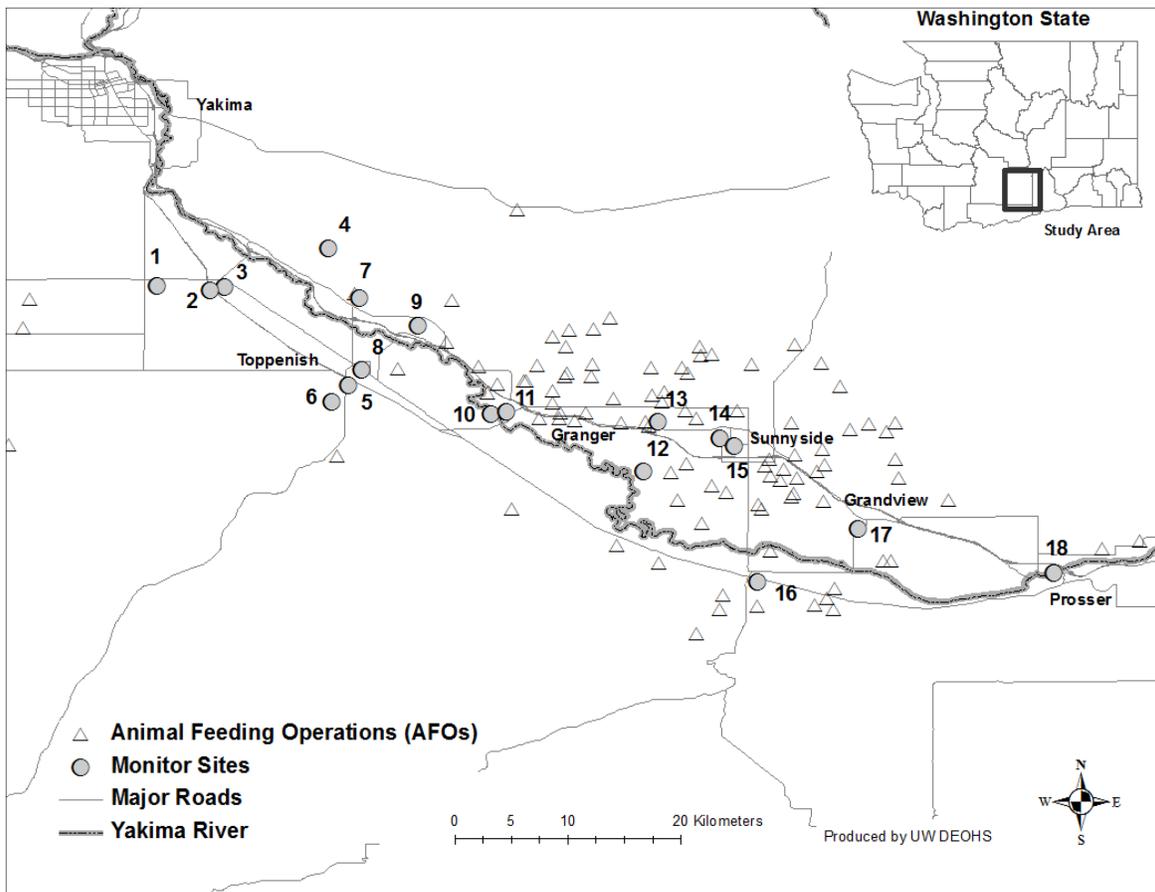
	N (%) or mean +/- sd
<i>Demographics</i>	
Female	25 (49%)
Age at enrollment (years)	10.3 +/- 2.7
Household income <=\$15k/year ^a	20 (39%)
Born outside US	10 (20%)
Hispanic/Latino ethnicity	47 (92%)
Parent(s) employed as farm worker	23 (45%)
<i>Asthma and general health at baseline</i>	
Medication use at baseline:	
<i>Inhaled corticosteroids (IC) only</i>	22 (43%)
<i>Leukotriene antagonist (LTRA) only</i>	2 (4%)
<i>Both IC and LTRA</i>	14 (27%)
Ever hospitalized with asthma	34 (67%)
Unscheduled visit for asthma to urgent care	
or ED in 12 months prior to enrollment	39 (76%)
Atopic asthma ^b	38 (74%)
At least one adult smoker in household	7 (14%)
BMI for age >85th percentile	25 (49%)
<i>Longitudinal asthma health</i>	
Subject-average ACQ index	0.42 +/- 0.30
Subject-average FEV1, % predicted	81 +/- 17%

^aTwo subjects did not respond.

^bIndicated by positive skin prick test to at least one of 22 common inhalant allergens.

Abbreviations: ACQ = Asthma control questionnaire; FEV1% = forced expiratory volume in 1 second as percent of predicted; BMI = body mass index; ED = emergency department

Figure III.1: Map of study region, including 18 monitoring locations and 97 regional animal feeding operations (AFOs)¹



¹ Locations of monitors are jittered in random directions and distances (up to 1 km) to protect subject identities.

Figure III.2: 24-hour average ammonia concentrations (n=814) measured at six-day intervals over a 14 month sampling period. Monitoring sites are numbered east to west across the study area. The width of each box is proportional to the sample size.

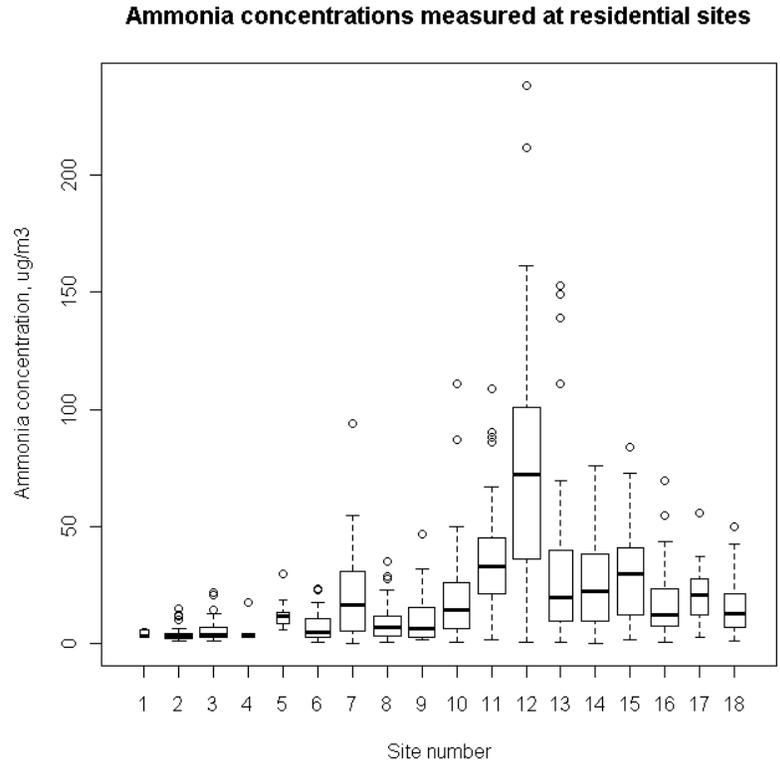


Table III.2: Ammonia (NH₃) concentrations by season

Season	Median NH₃ (µg/m³)	IQR
Dec, Jan, Feb	17.2	3.8, 40.8
Mar, Apr, May	11.6	3.5, 22.1
Jun, Jul, Aug	11.8	3.5, 27.8
Sep, Oct, Nov	12.9	4.3, 31.2

Abbreviations: NH₃ = ammonia; IQR = interquartile range

Table III.3: Median ammonia (NH₃) and metrics of AFO proximity: Correlation matrix

	Log (median NH₃ (μg/m³))	Distance to nearest AFO (km)	# AFOs < 5 km	# AFOs < 10 km	Σ log(area)/distance for all AFOs in region (Equation 1)
Log (median NH ₃ (μg/m ³))	1.00				
Distance to nearest AFO (km)	-0.75^b	1.00			
# AFOs < 5 km	0.84^c	-0.64 ^a	1.00		
# AFOs < 10 km	0.80^b	-0.64 ^a	0.97 ^c	1.00	
Σ log(area)/distance for all AFOs in region	0.88^c	-0.73 ^b	0.98 ^c	0.97 ^c	1.00

^a p<0.01, ^b p<0.001, ^c p<0.0001

Abbreviations: NH₃ = ammonia; AFO = animal feeding operation

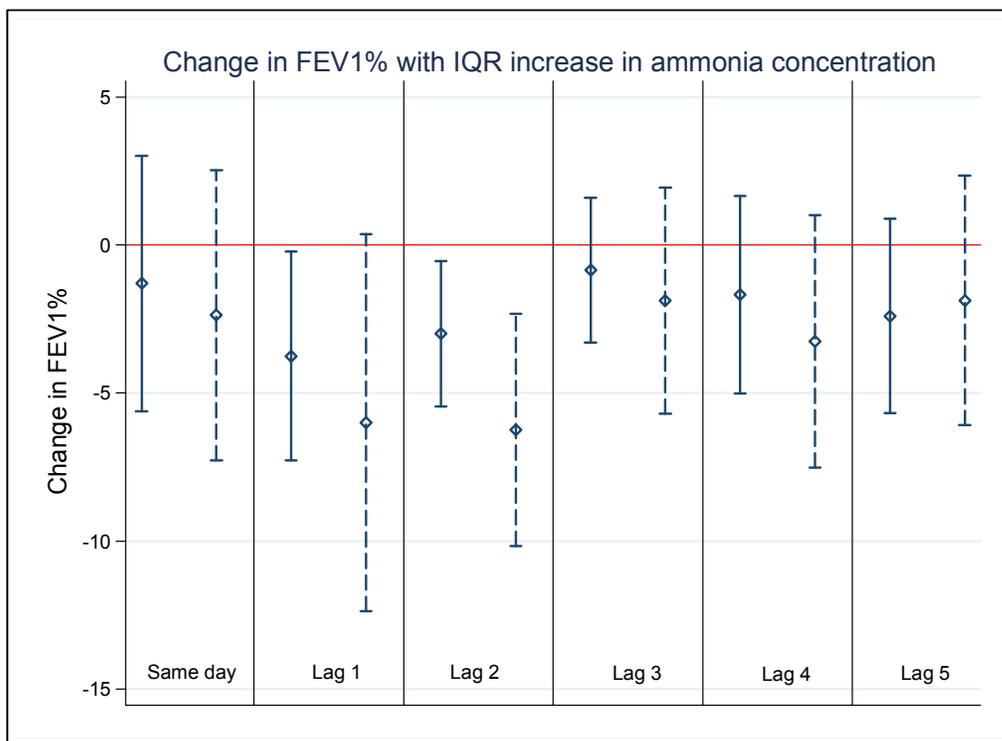
Table III.4: ACQ index and average ammonia concentrations

Averaging time for ammonia exposure prior to interview date	Coefficient (95%CI)^a	p-value
One week prior	0.01 (-0.04, 0.05)	0.55
Two weeks prior	-0.02 (-0.05, 0.02)	0.34
Four weeks prior	-0.02 (-0.06, 0.03)	0.46

^a Coefficient is the estimated change in ACQ index associated with an IQR increase in weekly ammonia ($18 \mu\text{g}/\text{m}^3$) after controlling for temperature and relative humidity, cubic splines for time elapsed and seasonality, as well as age, BMI, inhaled corticosteroid use at baseline, sex, and adult smokers in household. Results were derived from GEE model with an exchangeable correlation matrix.

Abbreviations: ACQ = Asthma control questionnaire; IQR = interquartile range; GEE = generalized estimating equations; BMI = body mass index; CI = confidence interval

Figure III.3: Associations between FEV1% and ammonia (NH₃) concentrations measured at the nearest neighbor monitors.



For each lag relationship, results for the entire cohort (solid line) as well as those obtained after restriction to subjects living within 1 km of the nearest monitor (dashed line) are displayed. Point estimates and 95% CI represent changes associated with an IQR increase in 24-hour average ammonia ($25 \mu\text{g}/\text{m}^3$), and adjusted for cubic splines of elapsed time and seasonality, temperature and humidity, as well as subject age, sex, BMI, atopy status, report of adult household smoker, and inhaled corticosteroid use at baseline. Results were derived from GEE model with an exchangeable correlation matrix.

Abbreviations: FEV1% = forced expiratory volume in 1 second as percent of predicted; IQR = interquartile range; GEE = generalized estimating equations; BMI = body mass index; CI = confidence interval

**CHAPTER 4: AFO PLUME EXPOSURES AND SHORT-TERM EFFECTS ON
PEDIATRICA ASTHMA HEALTH**

a. ABSTRACT

Background

Industrial-scale dairy operations and other animal feeding operations (AFOs) adversely impact regional air quality in the rural United States. Previous studies of AFO emissions and community health indicate that AFO exposure may cause or exacerbate respiratory disease, but existing methods for exposure assessment are limited. Longitudinal, repeated measures studies of this topic are scarce and have been conducted only in healthy adult populations to date.

Methods

We developed metrics to describe both spatial and temporal variations in AFO plume exposure in the Yakima Valley region of eastern Washington State. Estimates of exposure were compared to a chemical marker of AFO emissions, ammonia gas, measured repeatedly at 18 sites across the study region for 14 months. This method of AFO plume exposure assessment was then applied to epidemiologic analyses of health outcomes collected in the Aggravating Factors of Asthma in a Rural Environment (AFARE) study, a longitudinal repeated measures study of pediatric asthma morbidity. School age children with asthma (n=58) were followed for up to 26 months with biweekly surveys of asthma symptom control and daily measures of forced expiratory volume in one second (FEV1). Short-term relationships with AFO exposure were assessed using linear regression with generalized estimating equations based on separate models for home and school locations.

Results

Estimates of plume exposure captured a moderate degree of spatial variability in ammonia ($r=0.54$) and a small amount of temporal variability ($r=0.62$ after time-varying parameters added). We observed reductions in lung function following days of elevated AFO exposures with largest effects occurring one day following exposure: FEV1% changed -1.4% (95%CI: -2.7, -0.9) and -2.4% (95%CI: -4.4, -0.4) for each IQR increase in daily plume exposure assessed at homes and schools, respectively. No significant associations with asthma symptom control were detected.

Conclusions

Daily changes in AFO exposure across the Yakima Valley can be described fairly well with a simple exposure metric incorporating wind speed and direction. Children with asthma may experience decrements in lung function following exposure to airborne plumes of AFO pollutants.

b. INTRODUCTION

The rise of industrial-scale agriculture in the United States threatens environmental quality in a number of ways, including by pollution of regional airsheds [1]. Large-scale facilities where livestock and poultry are raised for food production, called animal feeding operations (AFOs), emit numerous biological and chemical pollutants to the surrounding atmosphere. Previous studies have documented elevated concentrations of hydrogen sulfide [2, 3], ammonia [4-6], endotoxins [7-8], bioaerosols [3], odors [3, 9], and total dust [4,10] in the vicinity of AFOs.

Many of the individual components of AFO emissions are toxic to the respiratory system, and some may produce synergistic effects during co-exposure [11, 12]. Increased risks of several respiratory diseases for AFO workers are well-documented [13, 14]; in contrast, relationships between AFO airborne emissions and community health are less certain. Previous epidemiologic studies have evaluated associations between community-level AFO exposures and respiratory effects [15-23], and some results indicate that community exposures to airborne AFO pollution may adversely impact respiratory health in nearby residential areas. However, nearly all prior studies were cross-sectional in design and were dependent upon time-averaged estimates of AFO exposure such as distance to nearest AFO [15, 16, 20], count of AFOs within a certain distance [17], a count of nearby AFOs with consideration of average wind speed and direction [18], or modeled ambient concentrations of an AFO-related pollutant [19]. Based in part on these limitations, a 2010 systematic review of research linking community health effects to AFO exposures concluded that the accumulated evidence was insufficient to infer causal relationships [24].

More recently, a longitudinal repeated measures study of healthy adults residing near swine AFOs found that time-varying AFO exposures may be related to short-term respiratory effects (i.e. temporary decreases in lung function and self-reports of difficulty breathing) [21] as well as other health outcomes, including increased blood pressure [22] and negative mood [23]. This study design, also referred to as a “panel study,” is demanding of resources but is especially well suited for the study of time-varying exposures that result in short-term, reversible health effects. Because each subject is observed repeatedly during periods of relatively high and low exposure, within-subject associations between exposure and health can be analyzed, and the influence of between-subject confounding is thereby mitigated.

We aimed to conduct a panel study of AFO-related exposures using health and environmental data collected during the Aggravating Factors of Asthma in a Rural Environment (AFARE) project, a community-based participatory research study of pediatric asthma morbidity set in an agricultural region of Washington State. The AFARE research framework provides a unique opportunity to investigate time-varying AFO exposures and short-term effects upon the respiratory health of children with asthma, a susceptible population. We developed a metric to estimate children’s time-varying exposures to airborne contaminant “plumes” from AFOs similar to a time-invariant metric used in a recent epidemiology analysis of pediatric asthma [18], and we compared estimates of plume exposure to levels of a chemical marker of AFO pollution, ammonia, measured repeatedly across the study region. Finally, we applied this new method of AFO exposure assessment to the epidemiologic study of daily and weekly changes in asthma health for the AFARE cohort.

c. METHODS

The AFARE study setting and cohort

The AFARE study took place in the Yakima Valley of Washington State, a region spanning from the city of Yakima in the northwest to Prosser in the southeast and roughly 42 miles in length from corner to corner (Figure I.1). Approximately 70-80% of the region is used for agriculture, with a high density of animal feeding operations, predominantly large dairies, located in the southeastern half of the valley (i.e. the Lower Yakima Valley). In 2012 there were 65 dairies in the Lower Yakima Valley licensed through the WA State Department of Agriculture, housing over 150,000 cows in total [25].

AFARE was conducted within El Proyecto Bienestar, a community-based participatory research partnership between the University of Washington Pacific Northwest Center for Agricultural Safety and Health; Yakima Valley Farm Worker Clinics (YVFWC), a network of federally-qualified health clinics serving migrant and seasonal farmworker families as well as other underserved populations in the region; and the Northwest Community Education Center which includes Radio KDNA, a Spanish language public radio station that provides support and education for the Latino community in the Yakima Valley. All research was approved by the University of Washington Institutional Review Board and consent was obtained from all subjects prior to participation.

AFARE subject recruitment began in August 2010 and continued throughout the first year of the study toward a goal of at least 50 participants. Subjects actively involved in the YVFWC

Asthma program were invited to enroll if they were of school age, had no other serious illnesses and intended to stay in the region during the two-year duration of the study. The Asthma Program is a longstanding clinical service delivered by community health workers at the patient's home, providing education about asthma management, including proper medication use and home indoor trigger identification and control [26]. In total, 58 subjects were enrolled in AFARE and 9 (16%) dropped out prior to the end of AFARE data collection in October 2012. Data from subjects who left the study were retained in analysis because in each case the decision to end participation was unrelated to health or exposure status.

Longitudinal health assessment

Two distinct methods were used to assess subjects' respiratory health repeatedly across the study duration: the Asthma Control Questionnaire (ACQ) at biweekly intervals and daily home spirometry.

Biweekly Asthma Control Questionnaire (ACQ): At approximately two-week intervals, phone interviews with either the child or an adult family member were conducted following the format of the ACQ [27], a survey of asthma symptom control that has been validated in pediatric populations. Interviewees (either the child or parent) were asked to recall the one week period prior to the interview date in their responses and asked five questions about asthma symptoms (nighttime waking, shortness of breath, limitation of activities, wheezing, and morning asthma symptoms) with ordinal categorical responses to represent increasing severity and frequency. A

sixth question ascertained frequency of short-acting bronchodilator use as average number of “puffs” per day. Responses to each of the six ACQ questions were coded as integers ranging from 0 (no symptom/medication use) to 6 (highest symptom severity/medication use) and averaged to produce one value, the ACQ index, to represent the average level of asthma control experienced over the previous week.

Daily home spirometry: At the time of enrollment, each child received a PikoNET handheld peak flow meter (PFM) with digital memory (nSpire Health, Inc; Longmont, CO) and was instructed in proper use of the device according to American Thoracic Society (ATS) guidelines. Children were asked to use the PFM twice daily in the morning and evening on every day prior to the use of short-acting bronchodilator medication, and the stored measurements were downloaded by YVFWC staff at approximately six-week intervals. During a 12-month AFARE follow-up visit with the research team at clinics and immediately prior to the start of ammonia monitoring, each subject’s technique and ability to produce an error-free measurement was observed, and subjects were retrained in PFM use if necessary. Use of the PFM produced a value of FEV1, which was converted into the percent of predicted value (FEV1%) based on standard reference equations [28]. Values of FEV1% that were implausibly high (above 150%) or low (below 30%) as well as measurements that were flagged by the device as potential errors were omitted from analysis. When multiple FEV1 values were available on a given day, the highest FEV1% on each day was chosen to represent a child’s daily FEV1%.

Ammonia monitoring

Air monitoring was conducted during the second year of the AFARE study only; we have described the deployment and performance of air sampling devices specifically designed for this purpose previously [29]. Fourteen devices were placed outside the homes of a subset of the AFARE participants selected based on accessibility and security as well as in consideration of overall spatial variability across the study region (Figure 1). Four monitors were moved to a new location during the air monitoring period because families changed residences (n=3) or the subject dropped out of the study (n=1), and one monitor was destroyed in a home fire three months prior to the end of the study and was not replaced. As a result, there were 18 monitoring sites during the course of the study. Every six days, devices actively sampled outdoor air for 24 hours using a silica bead sampling tube (SKC Inc; Eighty Four, PA) with flow rates ranging from 0.10-0.32 L/min according to the Occupational Safety and Health Administration (OSHA) method ID-188. The flow rates of sampling pumps were calibrated once a month and were adjusted to meet the intended flow rate range when necessary. Sampling tubes were transported to the University of Washington Environmental Health Laboratory at 0°C, where they were desorbed with deionized water and analyzed for ammonium ion using ion chromatography. Ammonia masses below the limit of detection (LOD) (either 1 or 0.5 µg, depending on the date of analysis) were replaced by LOD/2 for the calculation of concentration. Because the volume of air sampled varied, the concentrations calculated from ammonia masses below the LOD were highly variable. A total of 814 ammonia concentrations were measured during the study period.

Calculation of daily plume exposure (E_{plume})

To identify the location of all AFOs in the study region, we started with the WA State Department of Agriculture (WSDA) database of dairy operations registered in 2012 through the Dairy Nutrient Management Program (DNMP) because the majority of animal operations in the Yakima Valley are dairies. Then, we systematically inspected aerial photography images of the entire study region available online via Google Maps and Google Earth for characteristics of AFOs such as large dirt areas containing cattle, feeding and milking shelters, and animal waste storage ponds in order to confirm -- and when necessary, correct -- the location of the 65 dairy operations registered with the DNMP. We identified 32 additional non-dairy AFOs, including heifer, beef and poultry operations not included in the WSDA database. The area of all land that appeared to be part of each AFO was estimated in units of m^2 and the geographic location was approximated as the center of the facility. The distance and bearing (360°) from each AFO to every subject location was determined using the “geosphere” package in R (version 2.14.2, The R Foundation for Statistical Computing).

Four equations were used to calculate exposure to AFO plumes at a subject’s home or school location (Table IV.1). The first two versions, $E_{\text{plume},1}$ and $E_{\text{plume},2}$, were time-invariant and were calculated based upon characteristics of regional AFOs. $E_{\text{plume},1}$ was calculated using distance to regional AFOs alone, and $E_{\text{plume},2}$ incorporated facility size, a proxy measure for number of animals, in addition to distance. $E_{\text{plume},3}$ and $E_{\text{plume},4}$ were time-dependent quantities calculated with daily resolution based on wind speed and direction measured at each AFO location during a 24-hour period. $E_{\text{plume},3}$ included a term to account for changing hourly wind directions and the

bearing from AFO to home, and $E_{\text{plume},4}$ additionally included a term to account for wind speed during the hours when wind was blowing in the direction from AFO to subject location.

Hourly meteorological conditions (wind speed and direction) at each AFO on every day of the study were obtained from the Washington State University AgWeatherNet database [30], which includes historical meteorology data for ten weather stations within the region. For each AFO, the nearest weather station was identified and used as the source of hourly wind measurements. The term in equations for $E_{\text{plume},3}$ and $E_{\text{plume},4}$ pertaining to wind direction, $f(t, s, a)$, was calculated as the proportion of hours when the wind was blowing from the AFO to the subject location (home or school) based on an eight-point wind rose. Finally, the term describing daily wind speed in $E_{\text{plume},4}$, $u(t, s, a)$, was calculated as the average wind speed only during the hours of the day in which the wind was blowing in the direction from the AFO to subject location. Hourly wind speeds lower than 0.5 m/s were considered to be “calm conditions” and replaced by 0.5 m/s [31].

Statistical analysis

All statistical analyses were performed using Stata 12.0 IC (StataCorp LP; College Station, TX). Relationships between log-transformed ammonia concentrations and E_{plume} estimates were summarized using Pearson correlation constants (r). Correlations were determined using all ammonia concentrations as well as following exclusion of those derived from an ammonia mass less than the LOD.

We modeled associations between time-varying estimates of plume exposure ($E_{\text{plume},4}$) and each of the two outcome measures separately and using linear regression with generalized estimating equations (GEE) [32] and an exchangeable correlation matrix. $E_{\text{plume},4}$ was calculated for each subject's home and school locations on every day of the study, and associations with health outcomes were assessed in separate models for home- and school-based exposure. For exposures estimated using school locations, we considered only months during which school was assumed to be in session (i.e. September through June), and we took into account any year-to-year changes in school location reported during annual clinic visits.

For analyses of FEV1% and daily plume exposure, we investigated lagged exposures ranging from 0 (same day) to 4 days prior to spirometry measurement. In biweekly ACQ interviews, subjects were asked to recall the average level of symptoms experienced over the week prior; therefore, we calculated an average AFO exposure over the seven days prior to the interview date for the primary exposure in ACQ models. In all epidemiologic models, the mean outcome was modeled to be linear in response to the primary exposure of interest, and we present the final results as effect sizes per IQR increases. Covariates included as potential confounders were selected *a priori* based on existing evidence of relationships between the covariate and both respiratory health and exposure. The effects of continuous adjustment variables such as temperature, relative humidity, elapsed week of study, and seasonality (calendar month) were represented by cubic splines. Also included were subject-specific characteristics potentially associated with asthma morbidity: sex, age, atopy, use of inhaled corticosteroids at baseline, BMI at baseline and presence of adult smoker in household.

Model diagnostics were performed to determine whether the central assumptions of GEE were violated. Specifically, plots of residuals versus the linear predictor were inspected, and the possibility of influential subjects was explored using the “leave one out” method, by which point estimates and corresponding standard errors were estimated after exclusion of each subject in turn and compared to results generated from analysis of the complete study sample. These analyses did not reveal any obvious violations of the model assumptions. Analyses were repeated using linear mixed models (LMM) to assess robustness of results to choice of statistical model, and in all cases returned qualitatively similar results to those obtained with GEE.

d. RESULTS

Characteristics of AFARE cohort and AFOs in the region

AFARE children ranged in age from 6 to 16 years old at the time of enrollment with a mean age of 10.4 years (s.d. = 2.7) and were split evenly by gender (Table I.2). The majority of subjects self-identified as Latino/Hispanic in ethnicity, and half reported that one or more parents were employed in farmwork. 24 (41%) of children were from families with an annual household income of \$15,000 or less.

At baseline, most children reported a significant lifetime burden of asthma as well as serious exacerbations in the recent past. Two thirds (n=38) had been hospitalized for asthma at one point in their lives, and 46 (n=79%) had visited an urgent care clinic or the emergency department for asthma in the 12 months prior to enrollment. The majority of children were taking daily maintenance medications (i.e. “controller” medications) for asthma at baseline, of which the most common was inhaled corticosteroids. 42 (71%) tested positive to at least one aeroallergen in skin prick testing and were classified as having atopic asthma. Half of the children had a BMI-for-age at the 85th percentile or higher, and 8 (14%) lived with at least one adult smoker.

In 2012, there were 65 dairy operations licensed by the WSDA, 5 of which (7.7%) were classified as small, with up to 199 mature animals each; 16 (25%) as medium, with 200-699 animals; and 44 (68%) as large, where over 700 animals were confined [25]. Our scan of aerial

images of the region identified 97 potential animal facilities, ranging in size from 6500 to 900,000 m² in estimated surface area (mean=190,000 m²).

Longitudinal asthma health

The average ACQ index for all subjects was 0.52 (s.d. = 0.31) based on 2,023 interviews conducted over 25 months (Table IV.2). Subjects' asthma control improved over the course of the study, with an average decrease in 0.22 units in ACQ index per year of participation. In all interviews conducted, subjects reported the presence of each symptom at any level of severity less than half the time. For example, limitation of daily activities to any extent was reported least frequently (19.6% of interviews), and the most frequent symptom was nighttime waking due to asthma (45.4%). In 50.7% of all interviews, children reported using a short-acting bronchodilator medication during the week prior.

The subject-average FEV1 as percent of predicted (FEV1%) was 75% (s.d. = 15%) based on 3852 error-free measurements collected. FEV1 increased for the cohort across the study period, with average lung function growth estimated to be 0.26 L per year of participation, and FEV1% increased by an average of 2.3% per year during the study.

Correlation between measured ambient ammonia and E_{plume}

Figure 2 displays relationships between each metric of plume exposure and measured ammonia concentrations at 18 sites across the region. Both time-invariant estimates of exposure ($E_{\text{plume},1}$ and $E_{\text{plume},2}$) displayed a moderate degree of positive correlation with measured ammonia concentrations ($r=0.54$ in both cases).

Adding the term to account for daily changes in wind direction (in $E_{\text{plume},3}$) increases the correlation to 0.61. In other words, incorporation of the term for changes in wind direction increased the model R^2 from 0.29 to 0.37, which means that the wind direction term alone accounts for 8% of the variability in daily ammonia. In contrast, addition of the wind speed term with $E_{\text{plume},4}$ increased correlation from 0.61 to 0.62 (an increase in R^2 from 0.37 to 0.38), and therefore only accounted for an additional 1% of the variability in ammonia. Ammonia measurements and $E_{\text{plume},4}$ were plotted for each monitoring site separately to assess whether the success of $E_{\text{plume},4}$ in predicting temporal trends in ammonia varied by location (Figure IV.3) At every site location, linear regression revealed a positive trend between ammonia concentrations and calculated values of $E_{\text{plume},4}$, though goodness-of-fit and slope varied by site.

All four metrics of E_{plume} appear to substantially over-predict ammonia in a number of cases, as evident in the data points in the lower right quadrant of all four graphs. In exploratory analysis, these apparent outliers were labeled by daily precipitation, temperature, relative humidity, season, and monitoring site (figures not shown). Qualitatively, the outliers did not appear to differ from the other points according to any of these characteristics.

Associations between asthma health and $E_{\text{plume},4}$

Associations between asthma health outcomes and AFO exposure were estimated based on children's home and school locations in separate models. Biweekly measurements of asthma symptom control, represented as the ACQ index, displayed no relationships with averages of $E_{\text{plume},4}$ for the eight days prior to the day of interviews (Table IV.3). Similar results were obtained whether the calculation of $E_{\text{plume},4}$ was performed based on home or school locations. In exploratory analysis, we assessed relationships between each of the six components of the ACQ survey and AFO exposure separately and found no meaningful relationships (results not shown.)

Analysis of daily FEV1% with respect to $E_{\text{plume},4}$ calculated on the same day as lung function measurement as well as one to four days prior indicated that decrements in lung function occurred following elevated exposures to AFO plumes (Figure IV.2). Statistically significant decrements were observed lagged one day after exposure: -1.4% (95%CI: -2.7, -0.9) and -2.4% (95%CI: -4.4, -0.4) when $E_{\text{plume},4}$ was determined at home and school locations, respectively.

e. DISCUSSION

We estimated individual-level exposures to airborne emissions from regional AFOs using four methods of increasing complexity ($E_{\text{plume},1}$ through $E_{\text{plume},4}$) and compared these estimates to measured concentrations of outdoor ammonia. We found that the simplest metric of plume exposure ($E_{\text{plume},1}$), calculated solely upon distances to regional AFOs, displayed a moderate degree of correlation with daily ammonia concentrations. The addition of facility size in the equation for $E_{\text{plume},2}$ did not improve correlation with ammonia by a meaningful amount, which indicates that the surface area of AFOs in this region might not be an accurate proxy for number of animals, an important determinant of ammonia emission rates from dairy operations [33].

With the objective of capturing temporal variations in AFO exposure, we developed two additional metrics of plume exposure ($E_{\text{plume},3}$ and $E_{\text{plume},4}$) using measurements of daily wind direction and wind speed. This increased the correlation modestly, indicating that our exposure metrics were more successful at describing spatial variations in ammonia concentrations than day-to-day variations at individual monitoring sites. With $E_{\text{plume},3}$ we attempted to account for changing directions in wind at AFO locations, and we found that this term accounted for an additional 8% in the daily variation in ammonia beyond the predictive value of the two simpler, time-invariant metrics of plume exposure. For $E_{\text{plume},4}$ an additional term in the denominator was included in order to attenuate the estimated exposure with increased wind speeds, a concept derived from theories of atmospheric dispersion stating that pollutants in plumes become less concentrated with increased wind speed [31]. Counter to our expectations, $E_{\text{plume},4}$ did not exhibit an improvement in correlation with ammonia above that of $E_{\text{plume},3}$, a possible reflection of the fact that the volatilization rates of ammonia from AFO confinement areas and waste

lagoons increase with wind speed [34]. Therefore, the relationship between ambient ammonia concentrations and wind speed may not be simple, as faster winds increase the amount of ammonia emitted from an AFO but also decrease the resultant downwind concentrations. One investigation of swine AFO odor in residential communities found that odor intensity increased at low speeds and high speeds relative to moderate wind speeds [9], implying a nonlinear relationship between wind speed and AFO plume exposures in this context. Future work could include parameterization of the separate components of $E_{\text{plume},4}$ in order to explore the relationships between individual components, such as wind speed, and ammonia. This information could be used to develop a more accurate version of E_{plume} .

We chose to compare our metrics of AFO plume exposure to ammonia measurements based on the assumption that ammonia is a specific marker of AFO emissions. However, ambient ammonia levels are not necessarily an accurate representation of “true” AFO plume exposure. Airborne emissions from AFOs contain a wide variety of potential toxicants with disparate chemical and physical properties. The fate of each component following release to the atmosphere depends upon pollutant-specific characteristics such as deposition rates and reactivity. Concentrations of ammonia, for example, decrease as ammonia reacts with acid aerosols in the atmosphere, absorbs to airborne particulate matter, and deposits upon soil and vegetation [35]. The levels of other AFO-associated components with respiratory system toxicity, including endotoxin, PM_{2.5}, malodor, and hydrogen sulfide may not correlate well with ammonia concentrations, and therefore $E_{\text{plume},4}$ may predict overall AFO plume exposure better or worse than it predicts ammonia.

We chose *a priori* to use $E_{\text{plume},4}$ as our time-varying exposure measure in analysis of health outcomes. In epidemiologic analysis, we observed no significant relationships between asthma symptom control and average plume exposure in the week prior, and these results did not differ by location of exposure assessment. In contrast, we found that lung function was lower following days of elevated plume exposure and that effect sizes were stronger when $E_{\text{plume},4}$ was calculated based on school locations. Using either school- or home-based exposure assessments, a one-day lag relationship exhibited associations of greatest magnitude and largest statistical significance. If this result represents a true relationship between AFO exposures and respiratory health for children with asthma, the absence of an association between ACQ results and plume exposure might be explained by the differences in temporal resolution of the two health outcomes. During ACQ surveys, children were asked about symptoms experienced over a seven-day time period while FEV1 measurements represent lung function at one moment in time.

Our results are similar to those of our previous analysis of asthma health in the AFARE cohort relative to ammonia concentrations at subjects' residences (Chapter III of this dissertation). However, this current work represents a significant contribution beyond the previous study on account of increased time resolution of exposure assessments and a much larger sample size of exposure and health measurements available for epidemiologic models. In addition, we report here associations with exposure to AFO plumes as an aggregate mixture of toxicants, in contrast to the previous analysis focused upon ammonia alone.

Our current findings contribute support to the hypothesis that AFO pollution exacerbates respiratory disease in surrounding communities. Previous cross-sectional studies have

established links between prevalence of childhood asthma or wheezing disorders and residential proximity to AFOs [15], school proximity to AFOs [16, 20], and metrics of AFO exposure that takes into account number of animals within a buffer distance [17] and time-averaged wind conditions [18]. A panel study of healthy adults found that decrements in FEV1 and increases in subjective reports of upper respiratory symptoms followed periods of elevated AFO exposures, indicated by monitored hydrogen sulfide and reports of AFO odors from the same subjects [21].

To our knowledge there exist no investigations of AFO emissions and exacerbations of existing respiratory disease among community members in the published literature, and no published reports of AFO emissions and pediatric respiratory health using a panel study design. Pediatric asthma contributes a significant public health burden for rural communities in the United States because families in rural areas tend to be poorer and less well-educated, have less insurance coverage, and have more limited access to quality health care in comparison to urban and suburban counterparts – all characteristics conveying increased risk of elevated asthma morbidity [36-38]. For children with asthma, disease management is focused on control of symptoms and avoidance of acute exacerbations, which can be life-threatening in severity. Previous research has shown that increased frequency of exacerbations is directly related to lower quality of life for children with asthma [39], and exposure to air pollution is one of many possible factors that may trigger an exacerbation [40].

Beyond the focused topic of AFO exposures and community health effects, our work demonstrates a unique approach to time-varying exposure assessment based on daily variations in wind conditions in the context of agricultural pollutant dispersion. Particular strengths of our

study include the use of a panel study design, which helps mitigate the influence of between-subject confounding on results. We collected thousands of repeated measures of two related, but distinct, health outcomes. Self-reports of asthma symptom severity and lung function can be inaccurate, especially for pediatric populations [41, 42]. However, our overall findings were influenced heavily by within-subject relationships between exposure and outcome, and therefore we expect that any subject-specific biases in outcome assessment would be reduced in comparison to a study in which outcome was assessed once for each subject. An additional strength of our study was the opportunity to compare our modeled estimates of AFO exposure to ambient concentrations of ammonia, a component of AFO plumes, measured repeatedly at 18 sites across the region.

There are many limitations to our approach that should be considered when interpreting our results. Our plume exposure metrics were informed by principles of Gaussian dispersion of point-source atmospheric pollution, but AFOs are area sources rather than true point sources. Violations of this assumption would affect exposure estimates calculated for subject locations very close to AFOs [31]. Another potential limitation is that all equations used to calculate E_{plume} were dependent on the assumption of a constant pollutant emission rates from each AFO. This is a reasonable assumption considering that most toxic components of AFO plumes are emitted by animal manure, and animals produce waste daily. However, our exposure estimates did not account for the fact that emission rates from individual AFOs can differ according to several facility-specific characteristics, such as the type and age of animals confined, manure treatment practices, duration of manure storage in waste lagoons, and protein content of animal feed [33]. Also, our time-varying exposure estimates did not account for episodes of manure spraying on

nearby fields, an event likely to significantly increase airborne emissions. Further limitations in exposure assessment include the fact that we did not collect information on time-activity patterns, and we cannot rule out the possibility that avoidance behaviors affected children's exposures to AFO plumes because the strong, unpleasant odors may drive residents to spend more time indoors or away from home on days of elevated exposures. Children spend a large proportion of their day indoors at school and home, yet we did not account for differences in air conditioning or ventilation systems between subject locations, and we analyzed no samples of indoor air. Finally, it should be noted that despite the large number of repeated measurements collected the number of children in our cohort was rather small, which may limit generalizability of results across broader pediatric populations.

f. CONCLUSIONS

We developed a method for estimating exposure to AFO plumes that captured spatial as well as temporal variability in exposure. Application of this exposure method in epidemiologic analyses of short-term changes in respiratory health of children with asthma indicate that children may experience lung function decrements following elevated exposure to AFO airborne emissions at their homes and schools.

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Table IV.1: Variations on the calculation of daily AFO plume exposure

E_{plume}	Formula	Description
1	$E_{\text{plume},1}(s, a) = \log \sum_{a=1}^{97} \frac{1}{d_{a,s}^2}$	Exposure depends on proximity to AFOs alone (time invariant)
2	$E_{\text{plume},2}(s, a) = \log \sum_{a=1}^{97} \frac{A_a}{d_{a,s}^2}$	Exposure depends on proximity to AFOs and estimated size of facility (time invariant)
3	$E_{\text{plume},3}(t, s, a) = \log \sum_{a=1}^{97} \frac{A_a f(t, s, a)}{d_{a,s}^2}$	Exposure depends on distance, size and the percent of time on that day when wind was blowing towards subject location (time varying)
4	$E_{\text{plume},4}(t, s, a) = \log \sum_{a=1}^{97} \frac{A_a f(t, s, a)}{d_{a,s}^2 * u(t, s, a)}$	Same as (3) but with an additional term in denominator for average wind speed (time varying)

Where:

s = subject location (home or school)

a = animal operation (AFO)

t = day of study

A_a = estimated size of AFO facility (in m^2)

$d_{a,s}$ = AFO to subject distance

f(t, s, a) = fraction of hours in day when wind is blowing from AFO to home on 8-point wind rose

u(t, s, a) = average wind speed during the hours when wind is blowing from AFO to home

Table IV.2: Summary of longitudinal health data collection

	% or mean +/- std. deviation
Subject-average ACQ index	0.52 +/- 0.31
Percent of interviews in which each symptom reported:	
<i>Woken by asthma</i>	45.4%
<i>Limited in daily activities</i>	19.6%
<i>Shortness of breath</i>	34.8%
<i>Symptoms in morning</i>	38.7%
<i>Wheezing</i>	24.5%
Percent of interviews in which any short-acting bronchodilator use reported	50.7%
Subject-average FEV1, % predicted	75 +/- 15%

Abbreviations: ACQ, asthma control questionnaire; FEV1%, forced expiratory volume in 1 second as a percent of predicted value.

Figure IV.1: Correlation between monitored ammonia concentrations and four versions of E_{plume}

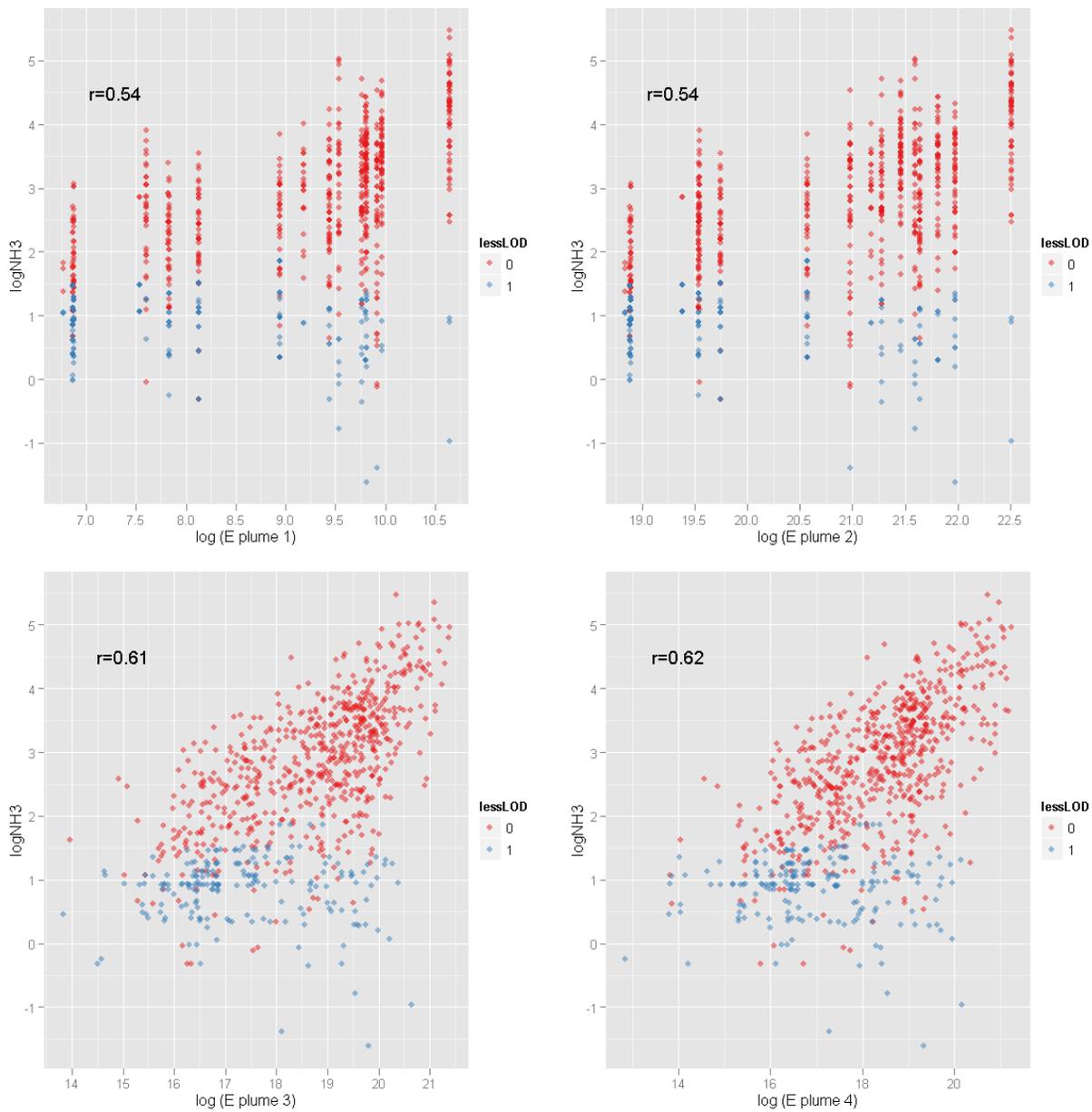


Table IV.3: Associations between asthma symptom control (ACQ index) and estimated AFO plume exposure

Location of exposure	Coefficient (95%CI)^a	p-value
Homes	-0.01 (-0.06, 0.03)	0.63
Schools	-0.01 (-0.09, 0.06)	0.71

Abbreviations: IQR, interquartile increase; $E_{\text{plume}, 4}$, estimated plume exposure calculated using equation 4 (Table IV.1)

^a Coefficient is the estimated change in ACQ index associated with an IQR increase in weekly $E_{\text{plume}, 4}$ after controlling for weekly temperature, weekly relative humidity, weekly precipitation, seasonality and elapsed time in study (all as splines) as well as sex, age, BMI, inhaled corticosteroid use at baseline, and presence of adult smoker in household. Results were derived from linear regression with GEE using an exchangeable correlation matrix.

Figure IV.2: Associations between FEV1% and estimated plume exposure

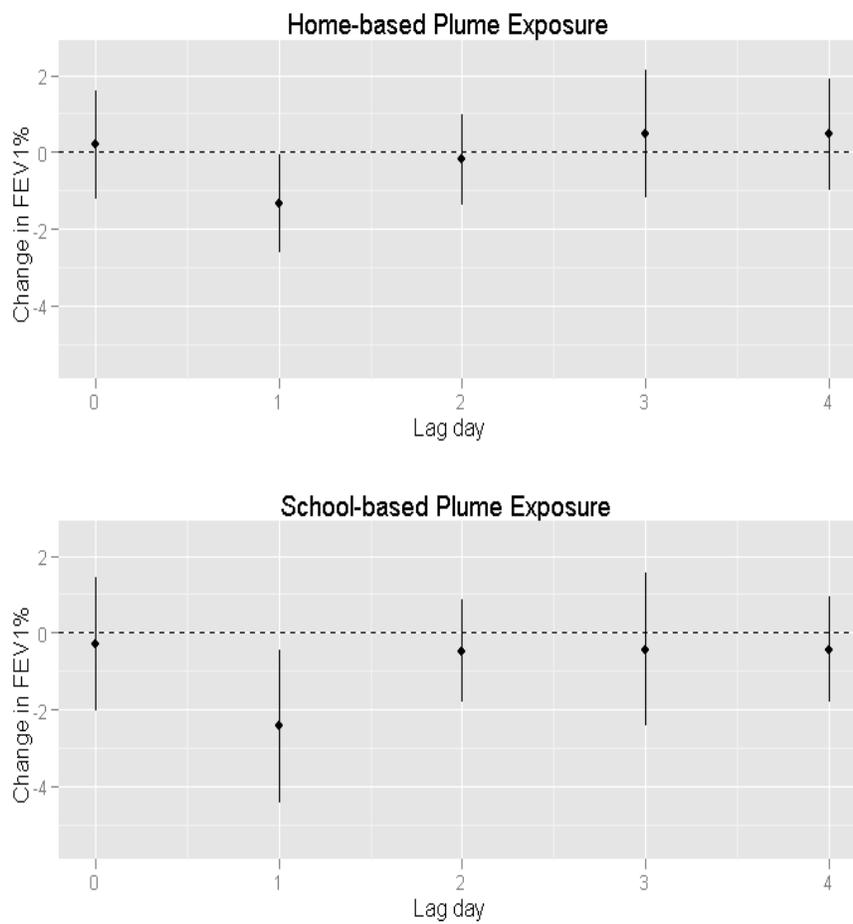
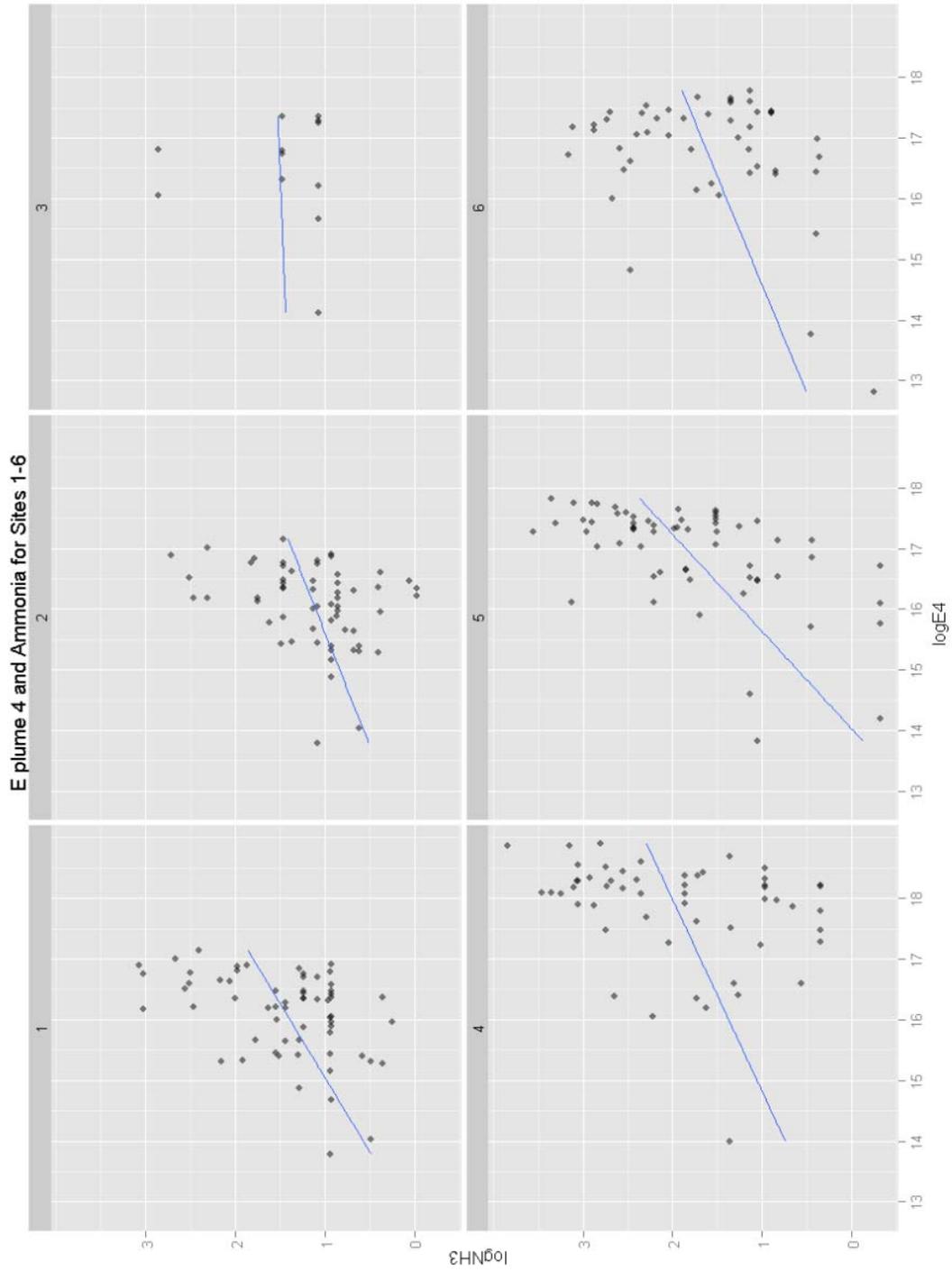
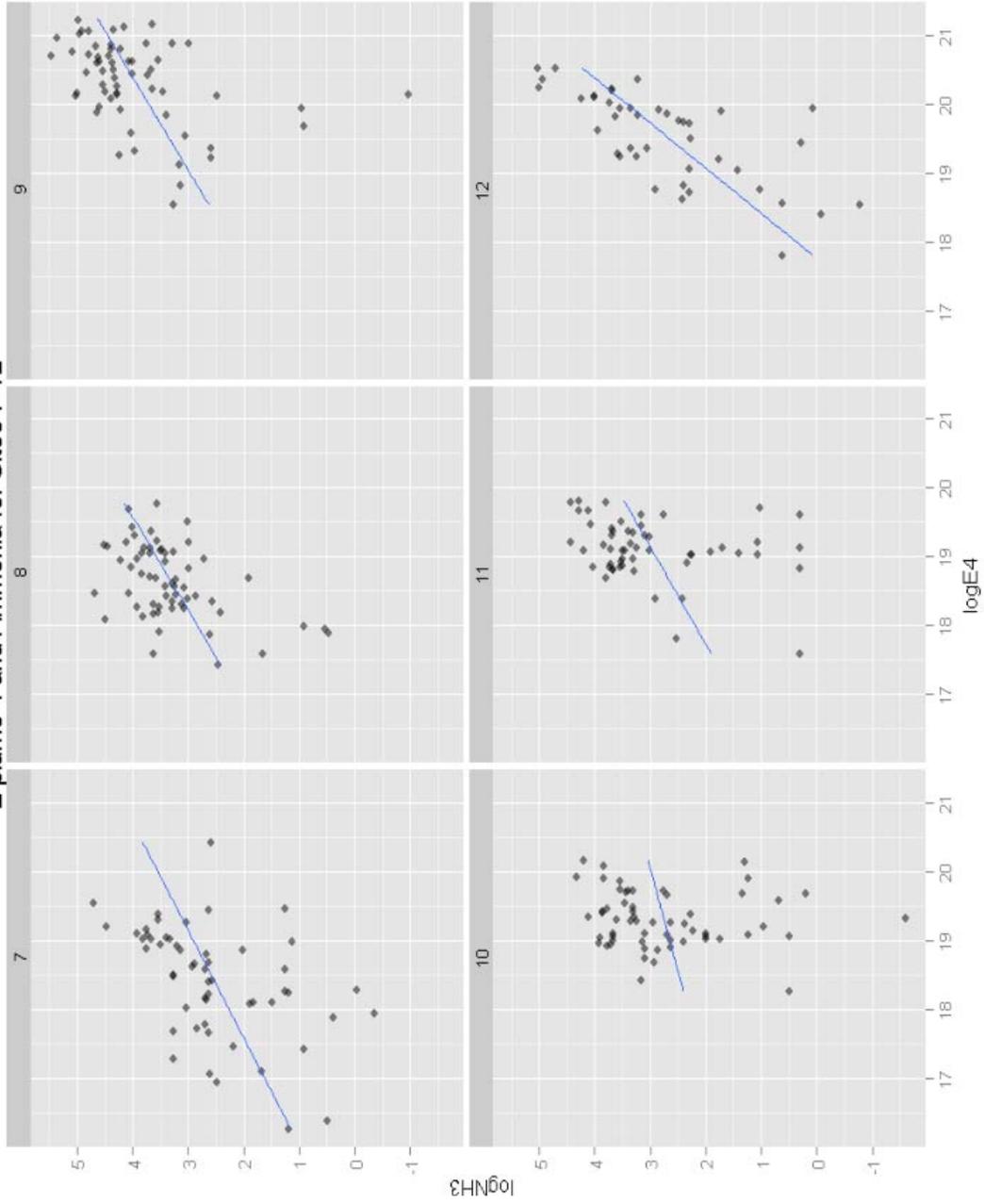


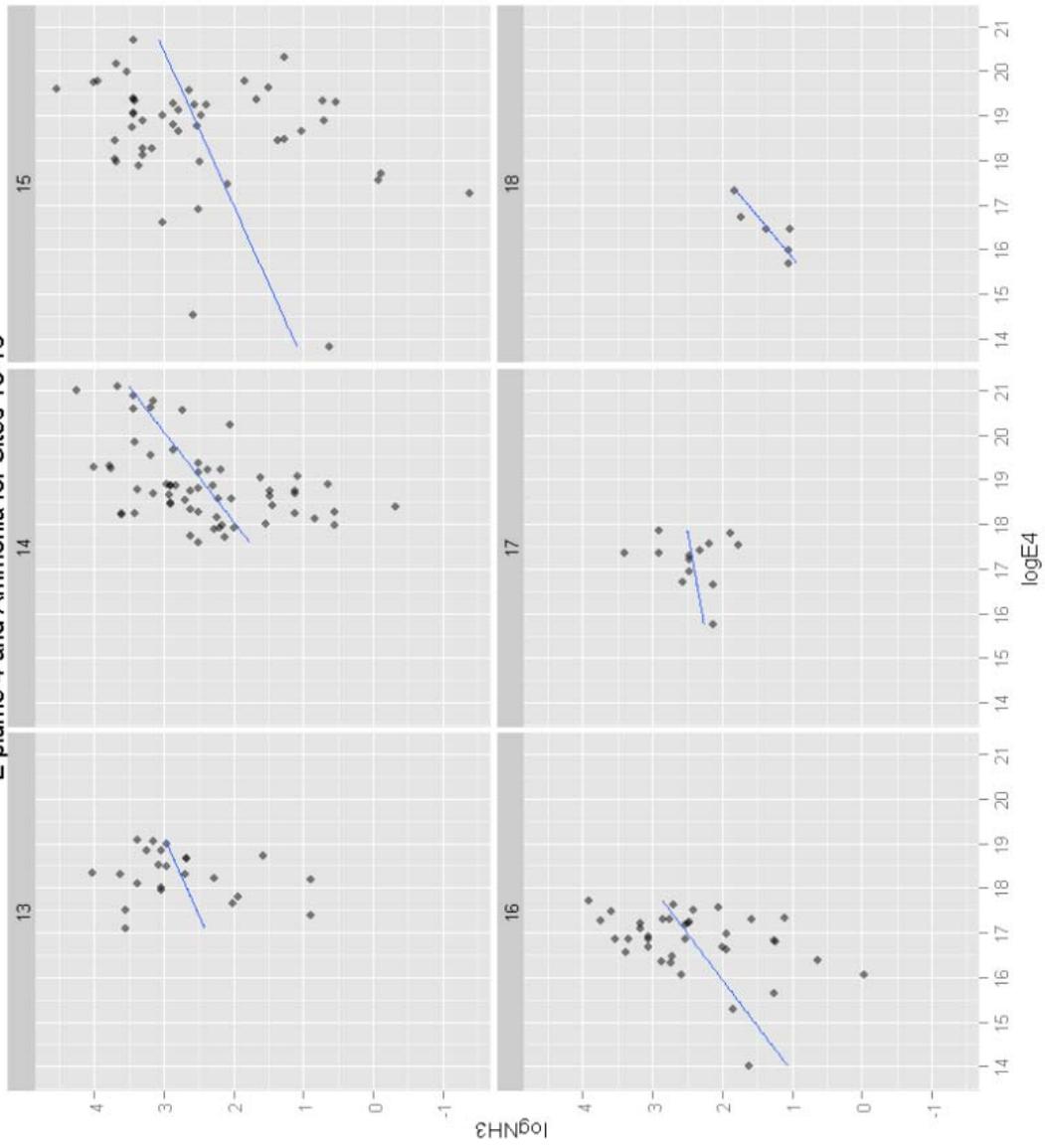
Figure IV.3: Measured ammonia and $E_{\text{plume},4}$ by monitoring site



E plume 4 and Ammonia for Sites 7-12



E plume 4 and Ammonia for Sites 13-18



CHAPTER V: DISCUSSION

We have conducted three investigations of outdoor air pollution and pediatric asthma morbidity in the Yakima Valley. Air quality in this rural community is compromised by emissions from industrial-scale agricultural operations, including large animal feeding operations (AFOs), and Yakima Valley residents have expressed concerns about the potential impacts on community health and well-being. During the AFARE project, a cohort of school-age children with asthma (n=58) was followed for 26 months with repeated measurements of airborne air contaminants as well as repeated assessments of two health outcomes. This unique depth of individual-level data pertaining to exposure and outcome allowed us to assess whether short-term, reversible changes in asthma symptom severity or lung function occur following days and weeks of elevated AFO exposures. Previous studies of AFO-related air pollution and community-level respiratory health have been conducted in a variety of settings (Table 1); however, our work is the first of these to analyze pediatric asthma as a variable disease and to assess relationships between time-varying AFO exposures and temporary occurrences of asthma worsening.

a) Overall summary of results

For the children with asthma who participated in the AFARE study, relationships between AFO exposures and asthma worsening at short time scales were observed. There were similarities as well as differences among the observed short-term health effects of the three exposures (Table V.1). All three exposures were associated with lung function decrements within one to two days of exposure measurement. Associations with regional PM_{2.5} were lowest in magnitude, while associations with residential ammonia exposures displayed the

largest effect sizes. The discrepancy in effect sizes may reflect true differences in the biological effects of PM_{2.5}, ammonia, and AFO plumes. At the same time, magnitudes of the measured FEV₁ associations may have been influenced to varying degrees by differing amounts and types of exposure measurement error present. While PM_{2.5} was determined only at one site in the study region, ammonia was monitored at 18 homes distributed across the valley. Therefore, estimated associations with PM_{2.5} may have been affected by more nondifferential misclassification of exposure. Likewise, our calculations of daily plume exposure depended upon several simplifying assumptions that likely contributed substantial amounts of imprecision to exposure measurements. Two types of exposure measurement error relevant to air pollution time series studies have been described: classical error, which is defined as random “noise” in exposure measurements, and Berkson error, the error that arises when estimated exposure represents only some of the true exposure experienced by research subject. Classical error is believed to inflate standard errors and bias estimated health effects towards the null in most circumstances, while Berkson error should increase variance but likely will not contribute bias in health effect estimates [1]. Typically, both types of errors will be present at the same time in a study of air pollution and health effects, and it can be difficult to quantify the amounts and the exact effect of each error upon the results. For our three investigations, we propose that the PM_{2.5} and AFO plume exposures may have been influenced by more exposure measurement error overall than the ammonia estimates, and some components of the measurement error in those cases may have attenuated the observed effects with FEV₁ more strongly in the analysis with ammonia.

In addition to lung function, regional PM_{2.5} was associated with asthma symptom control as assessed by the ACQ. Increases in weekly average PM_{2.5} were associated with a slight increase

in ACQ index [a 0.04 unit increase per IQR increase in weekly PM_{2.5} (95%CI: 0.01, 0.07)]. When ACQ results were broken down into six individual components of the survey (i.e. five questions about asthma symptom severity and one assessing frequency of “relief” medication use), we observed a 31% increase in odds of wheezing (95%CI: 18, 45%); a 21% increase in odds of activity limitations (95%CI: 0, 46%); and a 13% increase in nighttime waking due to asthma (95%CI: 1, 26%), each for an IQR increase in mean weekly PM_{2.5}.

We offer several explanations for the lack of associations observed between ACQ index and both ammonia and estimated plume exposure in the case that true associations do indeed exist. First, ammonia was measured during the second year of the AFARE study only (Figure I.2) and therefore the epidemiologic analysis of ACQ results and ammonia incorporated only half of the available ACQ data. We found that for the AFARE cohort, the magnitude and variability of reported asthma symptom severity and medication usage on ACQ surveys declined significantly between the first and second years of the study. This trend could be attributable to the effectiveness of that YVFWC Asthma Program in helping children attain better asthma control, or it could be the result of “study fatigue.” Whichever the cause, there was much less within-subject variability in ACQ indices observed across the second year of the study, which may have limited the probability that analyses would reveal statistically significant associations between asthma control and ammonia exposure. In contrast, estimated plume exposure (E_{plume}) was analyzed for the entire period of AFARE health data collection. In this case, an alternate explanation for the lack of observed effects with ACQ index is that by averaging estimated E_{plume} exposure over week-long periods we substantially decreased the within-subject variability in exposure. Mean weekly PM_{2.5} averages, in contrast, still varied significantly week-to-week on account of seasonal variations in PM_{2.5}.

Finally, it should be noted that the direct comparisons among health effects invited by Table 5 must take into account an inherent difference in the way exposure was measured in the PM2.5 analysis compared to ammonia and plume exposures. Because PM2.5 exposure was based on measurements made at a single location in the Yakima Valley, subjects' exposures varied in time (i.e. day-to-day) but did not reflect geospatial variations in exposure. Therefore, the effect sizes summarized in Table 5 for PM2.5 reflect temporal variability in exposure alone. In contrast, results for the two other exposures (ammonia and AFO plumes) were based upon both temporal and spatial variations in exposure. This difference in exposure variability affects the interpretation of results derived from the three distinct investigations.

The effect sizes of AFO exposures upon respiratory health reported in Table 5 are relatively small from a clinical perspective but agree well with similar panel studies of outdoor air pollution and pediatric health. The minimal clinically significant change in ACQ index has been judged to be 0.5 [2], significantly larger in magnitude than the increase in ACQ index we detected for an IQR increase in regional PM2.5 [0.04 (95%CI: 0.01, 0.07)]. We have identified only one panel study in the published literature that analyzed ACQ indices in relation to outdoor air pollutants [3], and the reported increase in ACQ index per IQR in PM2.5 was nearly identical to ours [0.035 (95%CI: -0.027, 0.092)]. In analyses of lung function, we observed deficits up to about 4%, which matches well with prior panel studies of outdoor air pollution and pediatric asthma [4-6]. Even though the absolute magnitude of effects observed in our study were small, minor worsenings in lung function and increases in asthma symptom severity will potentially develop into a more severe exacerbation, a serious and potentially life-threatening event for children with asthma [7].

Other notable findings of this dissertation research pertain to the field of environmental exposure science. We have described spatial and temporal patterns in community-level ammonia exposures in a region where air quality is heavily impacted by emissions of AFOs. We found that residents in the Yakima Valley are exposed to higher ambient ammonia concentrations than those measured in similar studies. In addition, we developed an easy-to-calculate indicator of time-varying AFO plume exposure determined based on location of a subject's home or school. Even though we found that simpler, time-invariant measures of AFO proximity explained a large proportion of variability in ammonia concentrations, the addition of information related to daily wind conditions successfully captured an additional proportion of daily variations in ammonia. The AFARE research framework offered a unique opportunity to compare estimates of plume exposure to a chemical marker of AFO emissions as well as to apply the metric in epidemiologic analysis of thousands of repeated measures of health outcomes.

b) Strengths and limitations

Investigations described in this dissertation all involved a longitudinal, repeated measures study design. This study design is resource intensive and burdensome for participants, but it is the ideal method for investigating short-term, reversible changes in health that occur in association with time-varying exposures. Asthma is a variable disease, and most children in the AFARE cohort displayed a good degree of asthma control for most of the two-year study period, yet also experienced fluctuations in asthma control and lung function that represent mild-to-moderate worsenings or more serious asthma exacerbations. Likewise, PM_{2.5}, ammonia and AFO plume exposure are environmental exposures that change significantly on short time scales

in this region. On account of this temporal variability, the collection of a single measure of asthma health and AFO-related exposure per subject, as often is the approach in cross-sectional studies, likely would not be adequate to determine whether AFO exposures are causing asthma exacerbations.

Our study design also allows for an analysis of *within*-subject changes in outcome and health over time, which mitigates the influence of between-subject confounding on measured associations, another important limitation of cross-sectional studies. For example, individuals who reside very close to large animal facilities are more likely to be low income, an independent risk factor for increased risk of acute exacerbations in children of asthma [8]. The cross-sectional studies of AFO proximity and asthma in Table I.1 included adjustment for socioeconomic status to account for the potential of confounding, but residual confounding may have persisted in each case.

To our knowledge, the investigations summarized here are the first to describe short-term changes in respiratory health in relation to elevated AFO exposures for a pediatric study population. In addition, we believe we are the first to conduct an analysis of AFO exposures and pediatric health using direct measurements of a specific component of AFO emissions (i.e. ammonia) at residential locations. Comparable research includes a set of panel studies conducted in North Carolina involving a cohort of healthy adults [9-11]. In these studies, ambient air pollution and malodor attributable to AFO emissions was directly and repeatedly measured in subjects' communities, and investigators observed relationships between AFO exposures and a variety of health outcomes, including respiratory symptoms.

The availability of repeated measures of AFO-related contaminants (ammonia and PM_{2.5}) is a major strength of this dissertation work, but there are limitations associated with our exposure assessment methodologies. PM_{2.5} measurements were taken from a single location near the center of the study region, and these measurements were used to represent each child's daily exposure to PM_{2.5}. Even though this approach has been used in several other air pollution epidemiology studies, the use of central site measurements in the presence of spatial variability in exposure can lead to biased results [1]. For our investigation of ammonia, in contrast, we made direct measurements of the pollutant in the outdoor environment of subjects' home, and therefore our exposure assessment accounted for variation in ammonia by location. However, we used inverse-distance weighting to extend ammonia measurements made at a limited number of sites across the study area to estimates at all subjects' homes. Inverse-distance weighting is a relatively crude method of spatial interpolation and is dependent upon the assumption that concentrations vary between monitoring locations in a manner predicted by distance (in our case, inverse-squared distance.) A more sophisticated approach to geospatial analysis would include modeling the relationships between concentration and distance using observed data and then applying model results in prediction of concentrations across the region.

Similarly, our methods of exposure assessment employed in the investigation of AFO plume exposure were limited in a number of important ways. We used a highly-simplified approach to dispersion modeling that took into account wind speed and direction between source (AFO) and receptor (subject location) in a dichotomous manner. In other words, each AFO was considered to contribute to overall plume exposure during a given hour only if the wind was blowing within a 45 degree "cone of acceptance" from the AFO to subject location. In addition, we did not account for several important meteorological conditions that would influence the dispersion of

pollutants, such as stagnation events, precipitation and relative humidity. True atmospheric concentrations of the various components of AFO plumes would depend strongly on the chemical and physical properties of each component – for example, rates at which particles settle out from the air and the reactivity of gases. We have not attempted to account for this complexity in our models of E_{plume} . Furthermore, we identified AFO sites and sizes based on aerial scans of satellite imagery, and we may have missed existing facilities and/or falsely assigned AFO sites to locations without any animals – for example, AFO sites that have been decommissioned. This type of misclassification is likely to contribute nondifferential misclassification in calculation of E_{plume} .

Additional limitations pertain to our methods of outcome assessment. Self-reports of asthma symptom severity and lung function can be inaccurate, especially for pediatric populations [12]. However, our statistical methods depended highly upon within-subject relationships between exposure and outcome in all models, and therefore we expect that any subject-specific biases in outcome assessment would be reduced in comparison to a study in which outcome was assessed once for each subject. For example, even though some children may under- or over-report asthma symptoms on a consistent basis, our statistical analysis was focused upon short-term changes in symptom severity over time for individual subjects. Similarly, the accuracy of home spirometry is known to be effort dependent, but it is feasible that each subject exerts a similar amount of effort across the study period and that subject-specific *changes* in FEV1 more accurately reflect true responses in lung function than individual measurements. Our FEV1 analyses are additionally impacted by missing spirometry data, a potential source of bias for associations measured by GEE. In order to address the possibility that this bias may have affected our results, we completed our FEV1 dataset using multiple imputation and repeated our

analysis of lung function and PM_{2.5}, revealing similar results. We also repeated all analyses using linear mixed models as an alternative to GEE and observed qualitatively similar findings in all cases. Finally, exploration of subject-specific compliance relative to a variety of health and exposure characteristics indicated no significant relationships between compliance and either health or outcome.

c) Suggestions for future research

The results reported here suggest that children with asthma in the Yakima Valley may experience temporary decrements in respiratory health following elevated exposures to airborne AFO pollutants. Further research is necessary to confirm these relationships as well as to determine possible directions for effective translation to public health practice.

Our findings could be confirmed and extended through additional investigations of pediatric asthma and AFO exposure. Our method of AFO plume estimates could be applied to broader time periods in the Yakima Valley and/or other agricultural communities in Washington State in a time series investigation of pediatric hospitalization events. Hospitalizations for pediatric asthma are not common, but the calculations of E_{plume} could easily be extended to larger study populations over longer durations of time to increase statistical power. In addition, given the findings from other studies that link AFO exposures to increased blood pressure and feelings of stress (Table I.1), it might be interesting to use E_{plume} in a study of hospitalizations due to nonrespiratory health outcomes, such as cardiovascular events.

Our analysis of regional PM_{2.5} did not include speciation of the PM_{2.5} to which the AFARE children were exposed. Previous epidemiologic research indicates that PM_{2.5} is a heterogeneous

mixture of chemical and biological constituents and that the specific composition of PM_{2.5} is an important determinant in impacts on respiratory health [13]. Speciation of PM_{2.5} collected upon filters by AFARE residential monitors could help determine which component of PM_{2.5} in this region is most strongly associated with asthma health. Such work could help describe differences between the composition of PM found in this rural community and implicated in health effects and the PM generally found in urban areas. Likewise, PM of larger sizes was collected at AFARE homes as well, and stored samples could be analyzed for endotoxin content. Endotoxin is a component of AFO emissions with potent inflammatory effects upon the airway when inhaled. Analyses described here in this dissertation did not account for endotoxin exposure in any manner beyond its presumed inclusion in AFO airborne plumes.

Other extensions of exposure assessment could include a more sophisticated approach to modeling AFO-related air contaminants. We propose to use AERMOD, a dispersion modeling software package developed by the EPA and American Meteorological Society, to predict the fate and transport of ammonia after it is released by AFO sources and disperses across the study region [14]. Based on the estimated emission rates of ammonia from each source, local meteorology conditions, and an assumption that ammonia transport is described by a Gaussian atmospheric dispersion model, AERMOD can calculate the estimated daily ammonia concentration at any specific location in the study region. The accuracy of AERMOD predictions is heavily influenced by the accuracy of model input, especially the source emission rates. Ambient ammonia concentrations repeatedly measured in the study area allow us the opportunity to estimate these emission rates rather than depending on a surrogate measure, such as the number of animals or surface area of facility. AERMOD predictions could then be

incorporated into additional epidemiologic analysis of AFARE health data or in analysis of other health outcome datasets, such as those suggested above.

Future research on this topic as well as translation to applied public health practice should include a perspective on indoor air quality. A recent study conducted in the same study region by a separate group of researchers demonstrated that elevated ammonia, dust, and cow antigens are found not only outside but also inside residences near AFO locations. Given that people tend to spend large proportions of the day indoors, these indoor exposures may have stronger relationships with asthma health than outdoor exposures. Future study should include repeated measures of indoor concentrations of ammonia, endotoxin, and other AFO-related pollutants as well as methods for reducing these indoor air contaminants.

Finally, our methods and results can serve as an example of epidemiology research conducted within a community-based participatory research framework. Several limitations that we encountered in analysis could be avoided in future investigations. For example, missing data on account of imperfect subject compliance was been found to be problem in our study as well as other pediatric studies with similar designs [15], and future research could include mechanisms to encourage subject compliance in daily spirometry. Ideas include small incentive programs that continue throughout the duration of a long follow-up period with frequent points of contact and sizes of incentives scaled to subject compliance. For health outcomes that are assessed very frequently, like daily home spirometry, subjects could be assigned randomly to specific and limited periods of time during which they are asked to take measurements. For example, rather than expecting subjects to conduct spirometric measurements at home over a long study period (26 months, in our case), each subject could be assigned to several two week periods during the AFARE study in which he/she is asked to pay very close attention to spirometry compliance.

Finally, questions could be added to surveys of subjective asthma symptoms (i.e. ACQ surveys) in order to assess the possibility that subject-fatigue reduces the variability in outcomes measured after several months of participation.

Overall, the studies described in this dissertation support the hypothesis that increased risk of short-term asthma worsenings and exacerbations occur following exposure to AFO-related outdoor air pollution. Associations are most consistent for short-term deficits in lung function. Our work represents the first such set of investigations in a pediatric study population and therefore warrants additional and confirmatory research in alternative settings. Future work could include more advanced methods for spatio-temporal exposure modeling and improved outcome assessment. As industrialized agriculture continues to compromise outdoor air quality in this region as well as similar communities worldwide, the health of children with asthma in surrounding communities should be monitored and protected.

Table V.1: Results of three AFO-related exposures and AFARE health outcomes¹

Mean change in outcome	Regional PM2.5	Ammonia at home	Estimated plume exposure (E_{plume})	
	IQR = 7.9 ug/m ³	IQR = 25 ug/m ³	Home	School
FEV1%				
Lag 0 (same day)	-0.7 (-1.7, 0.2)	-1.3 (-5.6, 3.0)	0.2 (-1.2, 1.6)	-0.3 (-2.0, 1.4)
Lag 1	-0.9 (-1.8, 0.0)	-3.8 (-7.3, -0.2)	-1.4 (-2.6, -0.1)	-2.4 (-4.4, -0.4)
Lag 2	0.1 (-0.8, 0.9)	-3.0 (-5.4, -0.6)	-0.2 (-1.4, 1.0)	-0.5 (-1.8, 0.9)
Lag 3	0.3 (-0.5, 1.0)	-0.9 (-3.3, 1.6)	0.5 (-1.2, 2.1)	-0.4 (-2.4, 1.6)
Lag 4	-0.1 (-0.8, 0.6)	-1.7 (-5.0, 1.7)	0.5 (-1.0, 1.9)	-0.4 (-1.8, 0.9)
ACQ index (week prior)	0.04 (0.01, 0.07)	0.01 (-0.04, 0.05)	-0.01 (-0.06, 0.03)	-0.01 (-0.09, 0.06)

¹All results adjusted for meteorological covariates (temperature, relative humidity and precipitation), temporal trends (seasonality and time elapsed) and subject-specific characteristics (age, BMI, sex, atopy, inhaled corticosteroid use, presence of adult household smoker.) Associations that were statistically significant at a 95% confidence level are highlighted in bold.

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