2013

INDOOR COARSE PARTICULATE MATTER: EVALUATING EXPOSURES AND HEALTH EFFECTS IN ASTHMATIC CHILDREN

Marcy Lynn McNamara
The University of Montana

Follow this and additional works at: https://scholarworks.umt.edu/etd

Let us know how access to this document benefits you.

INDOOR COARSE PARTICULATE MATTER: EVALUATING EXPOSURES AND

HEALTH EFFECTS IN ASTHMATIC CHILDREN

By

MARCY LYNN MCNAMARA

B.S. Neuroscience, Allegheny College, Meadville, PA, 2008

Dissertation
presented in partial fulfillment of the requirements
for the degree of
Doctorate of Philosophy
in Toxicology
The University of Montana
Missoula, MT
July 2013

Approved by:

Dr. Sandy Ross
Dean of The Graduate School

Dr. Curtis W. Noonan, Chair
Center for Environmental Health Sciences

Dr. Tony J. Ward
Center for Environmental Health Sciences

Dr. Andrij Holian
Center for Environmental Health Sciences

Dr. Chris Migliaccio
Center for Environmental Health Sciences

Dr. Chris Palmer
Department of Chemistry and Biochemistry

INDOOR COARSE PARTICULATE MATTER: EVALUATING EXPOSURES AND
HEALTH EFFECTS IN ASTHMATIC CHILDREN

Chairperson: Dr. Curtis W. Noonan

ABSTRACT

The primary aim of the ARTIS study (Asthma Randomized Trial of Indoor Wood Smoke) is to improve the quality of life of asthmatic children living in wood stove homes by reducing in-home fine particulate matter ($PM_{2.5}$). In the original project, characterizing exposure to the coarse fraction of particulate matter (consisting of particles <10 μm and >2.5 μm; PMc) was not proposed. The coarse fraction has been shown to elicit increased pulmonary inflammation compared to $PM_{2.5}$. This may be due to biogenic constituents of PMc such as endotoxin, a component of the cell wall of Gram-negative bacteria. Scientific interest in the coarse fraction is on the rise due to its unique documented adverse health effects and potential for regulatory status. A novel filter-based sampler (coarse particle environmental monitor, CPEM) was used to characterize coarse fraction and airborne endotoxin concentrations in a subset of ARTIS homes. In 50 homes, only the presence of pets was associated with PMc concentrations. The frequency of wood stove use (loading/stoking) was not associated with either PMc or airborne endotoxin concentrations. Following 43 homes from pre- to post-intervention, homes that received an active filter intervention had a 27.4 μg/m^3 greater reduction in $PM_{2.5}$ relative to a placebo intervention with a treatment effect of ~90%. The placebo filters were unexpectedly efficient in significantly reducing PMc and airborne endotoxin concentrations. As a result, the active filter intervention showed no enhanced ability to reduce PMc and airborne endotoxin relative to the placebo intervention. These effects remained significant when the filter units were run at least 50% of the time. Finally, baseline health outcomes were investigated for association with PMc and airborne endotoxin concentrations in 38 asthmatic children living in the wood stove homes. As a whole we found a more general pro-inflammatory and oxidative stress response to increased endotoxin concentrations as opposed to the phenotypic allergic asthma response. We also found separate and unique health outcome associations with PMc and airborne endotoxin. Overall, these studies significantly advance our understanding of in-home exposures, health effects, and reduction efforts of asthma exacerbating agents in wood stove homes.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

This dissertation describes exposures, health effects, and reduction efforts of asthma exacerbating agents in wood stove homes. The introduction will 1) provide an in-depth review of the global importance of biomass smoke research, 2) describe the mechanisms of coarse fraction and endotoxin toxicity in relation to asthma, and 3) summarize previous research on indoor air quality interventions.

Global Biomass Smoke Exposure

Wood is the most common solid fuel burned worldwide for the 3 billion people that cook and heat their homes with biomass or coal (~43% of the world's current population with some estimates >50%). Biomass fuel refers to any living or recently living plant and/or animal-based material that is deliberately burned by people for fuel such as wood, twigs, dried animal dung, charcoal (a product of incomplete burning of wood), grass, or agricultural crop residues. Fuels lower in the energy ladder such as biomass fuels are less efficient and produce more pollution, but are less expensive (Figure 1.1) (Sood, 2012). Socioeconomic status is a key component in the global use of biomass fuels, with poor and rural areas dependent on biomass for fuel. People living at the bottom of the energy ladder often live on <$1-2 per day and have no access to cleaner burning gas or electricity (Mortimer, 2012).

Figure 1.1. The energy ladder.
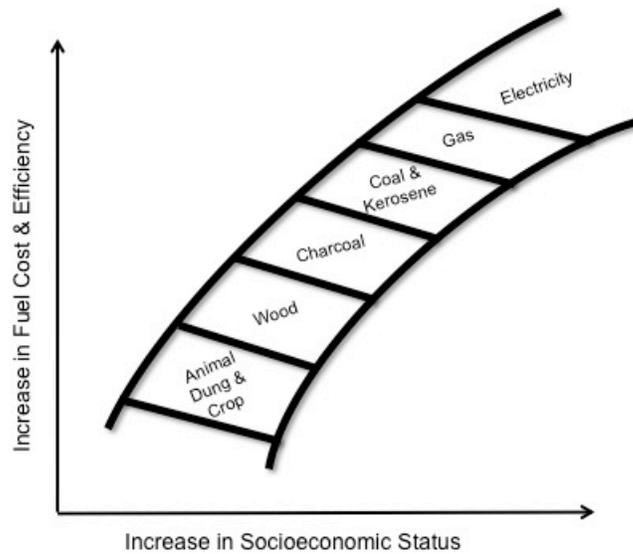
Figure 1.1. The energy ladder. Fuels lower in the energy ladder are less efficient and produce more pollution, but are less expensive. Conversely, fuels higher in the energy ladder are more efficient and produce less pollution, but are more expensive (Sood, 2012).

The impact of all this in-home biomass burning is that approximately 76% of all global particulate matter (PM) air pollution occurs indoors in the developing world (Fullerton, 2008). Biomass fuel smoke contains a complex mixture of a large number of pollutants including PM, carbon monoxide, oxides of nitrogen and sulfur, benzene, formaldehyde, polycyclic aromatic hydrocarbons such as benzo(α)pyrene, free radicals, aldehydes, volatile organic compounds, chlorinated dioxins, oxygenated and chlorinated particulate matter, and endotoxin (Kurmi, 2012). Among these pollutants, a comprehensive review by Naeher *et al.* concluded that fine particulate matter (having an aerodynamic diameter <2.5 µm; ($PM_{2.5}$)) serves as the best exposure metric for wood smoke and tends to be among the most elevated pollutants during exposure events for comparison with existing air quality standards (Naeher, 2007). The selection of an appropriate exposure metric for wood smoke is important as smoke from various solid fuels is not alike. For example, wood-burning stoves in low-income areas in Mozambique were associated with a significantly higher release of respirable particles during cook time (1260 µg/m^3) compared with either charcoal (540 µg/m^3) or liquefied petroleum gas stoves (200-380 µg/m^3) (Ellegard, 1996).

There are established associations between indoor air pollution from biomass smoke and a wide variety of adverse health effects. The strongest associations exist with chronic obstructive pulmonary disease (COPD; both emphysema and chronic bronchitis), acute respiratory tract infections, and lung cancer. Weaker associations exist with asthma, tuberculosis, and interstitial lung disease (Sood, 2012). Evidence also implicates exposure to indoor air pollution from biomass smoke with adverse effects on several birth outcomes including low birth weight, intrauterine growth retardation, and perinatal mortality (Pope, 2010). These associations are not limited to a particular geographical area. For example, reports of maternal exposure to biomass smoke and

reduced birth weight have been published in studies from Zimbabwe (Mishra, 2004), Guatemala (Boy, 2002), Pakistan (Siddiqui, 2008), and India (Tielsch, 2009).

The disease burden of indoor air pollution from household solid fuel use is extraordinary. An estimated 2 million people die prematurely from illnesses attributable to indoor air pollution from household solid fuel use annually (Sood, 2012), with almost one-half of these deaths resulting from pneumonia in children under the age of 5 years (World Health Organization, 2009). Worldwide, indoor air pollution from solid fuel use is the number one global cause of COPD (Sood, 2012), and along with lung cancer accounts for 1.1 million deaths every year in non-smoking women, occurring almost exclusively in lower- and middle-income countries (Mortimer, 2012).

Despite these staggering statistics, household air pollution historically has not received sufficient attention from the scientific, medical, public health, development, and policy-making communities. However, in recent years there have been increasing efforts to make a large impact on the current burden of disease. In 2010 an initiative led by the United Nations Foundation called the Global Alliance for Clean Cookstoves was launched to achieve the goal of 100 million homes adopting clean cooking technology by 2020 (Martin, 2011). Another large intervention study known as RESPIRE (Randomized Exposure Study of Pollution Indoors and Respiratory Effects) has also taken place over the last decade resulting in multiple publications characterizing exposures and health impacts of long-term reductions in wood smoke exposure in rural Guatemala (Smith, 2006, Smith, 2009, McCracken, 2007, McCracken, 2011, Thompson, 2011, Smith, 2011).

Biomass burning is not exclusive to developing countries. Rural areas of the United States (Ward, 2010), Canada (Levesque, 2001), the United Kingdom (Semple, 2011), Scandinavia (Molnar, 2005, Gustafson, 2008), Australia (Johnston, 2013) and New Zealand (McGowan, 2002) have regions where winter air pollution is dominated by

particulate matter from solid fuel domestic heating. Summertime exposures to biomass smoke can also occur during wildland fire events, resulting in exposures for wildland firefighters (Booze, 2004, Reinhardt, 2004), base camp personnel (McNamara, 2011), and the residents of nearby communities. Alterations in weather patterns, such as warmer temperature trends brought about by global climate change, can influence the frequency and duration of wildland fires (Dale, 2001). Warmer temperatures have also contributed to the size and severity of the mountain pine beetle outbreak, which in the western United States alone is estimated to have killed over 2 million acres of coniferous trees, thereby leaving standing fuel for future forest fires (Williams, 2002). Coupled together, these warmer temperatures and increased fuel loads in our forests are providing for increased risks of large wildland fires. As a result, exposure of wildland fire fighters, incident command and support personnel, and residents of nearby communities to biomass smoke will likely increase. As exposure to biomass smoke is not limited to a geographical area, more studies are needed to characterize exposures and health effects in a variety of settings.

The Coarse Fraction and Endotoxin

Fine particulate matter has been established as the best exposure metric for wood smoke (Naeher, 2007). Larger respirable particles, classified as PM_{10} (aerodynamic diameter <10 μm) are also found at elevated concentrations in homes burning solid fuels in developing countries (Ezzati, 2002) and have known negative health effects (Pope, 1995). However, the coarse size fraction, consisting of particles < 10 μm and >2.5 μm (PMc), has arisen as a new class of interest due to its distinct epidemiological and toxicological findings. A systematic review of studies investigating both $PM_{2.5}$ and PMc concluded that little evidence exists establishing a relationship between PMc and mortality. However, in studies of COPD, asthma, and respiratory hospital admissions PMc has a stronger or as strong short-term effect as $PM_{2.5}$ (Brunekreef, 2005).

Several characteristics make PMc a unique size fraction of PM. Unlike PM_{10}, PMc is not as strongly correlated with $PM_{2.5}$ in the ambient environment (Tager, 2010). This allows for distinct health effects of PMc to be determined independent of $PM_{2.5}$ concentrations. From a toxicological perspective, PMc has been shown to cause a more significant pulmonary inflammatory response than $PM_{2.5}$ in an animal model (Tong, 2010) as well as increased inflammatory potential in multiple respiratory cell lines (Gualtieri, 2010, Oeder, 2012). PMc captured during wildfire activity has shown more proinflammatory and oxidative stress potential on an equal-dose basis than $PM_{2.5}$ particles (Wegesser, 2010). This indicates that PMc from biomass smoke may elicit a stronger respiratory response than $PM_{2.5}$.

The increased inflammatory potential of PMc relative to $PM_{2.5}$ may be linked to the biological components of PMc. The biological components of PM, also known as biogenics, can be evaluated through quantification of mold spores, (1,3)-β-d-glucan (a fungal marker), total protein, and endotoxin. Endotoxin, lipopolysaccharide derived from the outer membrane of Gram-negative bacteria, has long been recognized to cause both

immediate and sustained airflow obstruction in asthmatics (Liu, 2004). A temporo-spatial analysis detected ambient endotoxin in PMc at concentrations 10-fold higher than found in $PM_{2.5}$ (Heinrich, 2003) and this distribution is consistent in indoor environments as well (Menetrez, 2009).

To evaluate the biological activity of endotoxin in air and dust samples, dry heat sterilization treatment of 250°C for 30 minutes will depyrogenate a sample, effectively inactivating the endotoxin (Hecker, 1994). This method is also accepted for removal of endotoxin from pharmaceutical agents by the Food and Drug Administration (FDA, 1984). Comparing standard samples to heat-treated samples reveals inactivation of endotoxin within the coarse fraction that significantly attenuates inflammatory mediators in healthy humans (Alexis, 2006). This supports *in vitro* data which reveals heat-treatment of house dust reduces the capacity of human alveolar epithelial cells to produce IL-8, a proinflammatory mediator (Mathiesen, 2004).

As previously mentioned, endotoxin is one of many pollutants found in biomass smoke. Endotoxin is found to be particularly elevated where maize crop residue or cow dung is burned (Kurmi, 2012). Elevated airborne endotoxin levels were also seen in a study conducted in Nepal and Malawi with airborne endotoxin concentrations at 24 endotoxin units (EU)/m^3 in charcoal-burning homes and 40 EU/m^3 in wood-burning homes (Semple, 2010). In Nepal, short-term measurements during cooking indicated average inhalable endotoxin levels of 365 EU/m^3 for dung-burning homes and 43 EU/m^3 for wood-burning homes (Semple, 2010). These concentrations are orders of magnitude higher than similar studies reporting airborne endotoxin in non-biomass burning homes (Thorne, 2007). Another study found wood-burning homes in Scotland and Ireland had higher airborne endotoxin concentrations than homes burning coal, peat, or using gas cooking (Semple, 2011).

In addition to biomass smoke, in-home endotoxin concentrations have been consistently attributed to several home characteristics. Rural homes have been associated with higher levels of indoor endotoxin than urban homes (Lawson, 2011, Barnig, 2012). Increased number of residents has been found to be significantly associated with increased endotoxin concentrations in dust samples in two large studies sampling 184 and 2,552 homes, respectively (Singh, 2011, Thorne, 2009). In addition, number of pets has also been identified as a predictor of in-home endotoxin levels (Thorne, 2009, Park, 2001, Sordillo, 2011). Park *et al.* investigated the presence of dogs in homes and found a 96% difference in airborne endotoxin in total suspended particulate between homes currently having a dog and homes that had never had a dog (Park, 2001). Thorne *et al*. and Sordillo *et al.* reported similar findings by evaluating number of pets via presence/absence of dogs and cats (Thorne, 2009, Sordillo, 2011). Dampness/moisture sources have been identified as predictors of in-home endotoxin qualitatively by survey data on humidifier use, report of water damage, visible mold, use of central air, and living in an apartment (Sordillo, 2011, Park, 2001). When possible these primary factors (rural setting, people, pets, and dampness) should be evaluated in studies where indoor endotoxin is an exposure of interest.

<u>Toxicity of the Coarse Fraction/Endotoxin</u>

The exact mechanism by which particulate matter exerts its adverse effects is still unclear. It is likely a combination of factors stemming from local inflammation in the lungs (responsible for adverse pulmonary effects) as well as a systemic inflammatory response that impacts coronary arteries (resulting in adverse cardiovascular events) (Hoffmann, 2007). Once inhaled, larger particles (such as PMc) are likely deposited at the tracheobronchial tree level while smaller particles ($PM_{2.5}$) reach the alveolar spaces (van Berlo, 2012). Mucosal host immune defense systems work to eliminate the foreign material through physical and chemical clearance processes. PMc is hypothesized to induce innate immune responses via the endotoxin-toll-like receptor (TLR) 4 pathway while $PM_{2.5}$ likely acts via generation of reactive oxygen species by transition metals and/or polyaromatic hydrocarbons (Miyata, 2011). The innate immune responses are characterized by activation of transcription factors such as nuclear factor (NF)-κB and the production of downstream proinflammatory cytokines (Miyata, 2011).

Endotoxin is interchangeably referred to as LPS, or lipopolysaccharide, which is a structural component of the outer membrane of gram-negative bacteria (Figure 1.2). The lipid region of LPS (lipid A) anchors the LPS into the bacterial membrane in living bacteria. When the bacteria dies or the cell membrane is damaged, the lipid A portion of LPS is the antigenic surface recognized by the innate immune system and is essential for its characteristic toxicity as variations in lipid A structure are associated with variations in its toxic potency over a wide range (Duquenne, 2012). The outer portion of the polysaccharide (O-specific chain) varies among serotypes of a single bacterial species while the core oligosaccharide chain is relatively conserved (Medicine, 2010, Song, 2012).

Figure 1.2. Chemical structure of LPS.
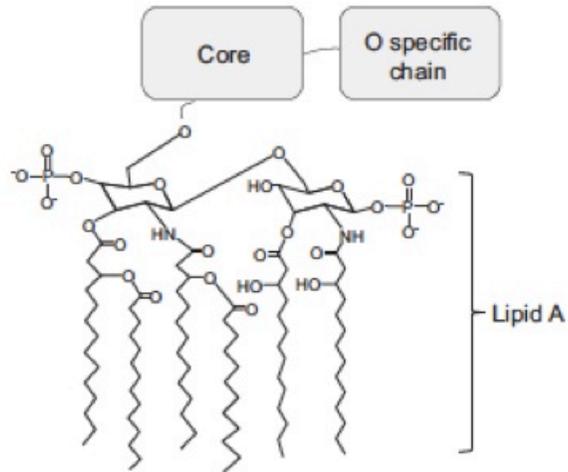
Chemical structure of LPS. O specific chain varies across bacterial species,
Lipid A region responsible for toxic effects. From Song 2012

Figure 1.2. Chemical structure of LPS. O specific chain varies across bacterial species,
while lipid A region is responsible for toxic effects (Song, 2012).

The pathway for endotoxin binding and cell activation is a classic example of the innate immune system encountering, recognizing, and setting forth processes to eliminate a foreign pathogen (Figure 1.3) (Cohen, 2002). The mechanism includes an opsonin, LPS binding protein (LBP), which binds free LPS and presents it to either membrane-bound or soluble CD14. After binding LPS-LBP complexes, membrane-bound CD14 activates myeloid cells such as dendritic cells and macrophages while soluble CD14 activates nonmyeloid (epithelial or endothelial) cells. The signal is transduced via toll-like receptors (TLR) TLR4 and TLR2 in myeloid and nonmyeloid cells, respectively. CD14 and TLRs are known as pattern receptors and are involved in the recognition of other bacterial and fungal infections via pathogen-associated molecular patterns (PAMPs) (Song, 2012). The activation of the TLR signaling pathway results in the synthesis of proinflammatory cytokines and therefore prepares the immune system for infection and foreign pathogen removal (Song, 2012).

Figure 1.3. Principal LPS mechanism.
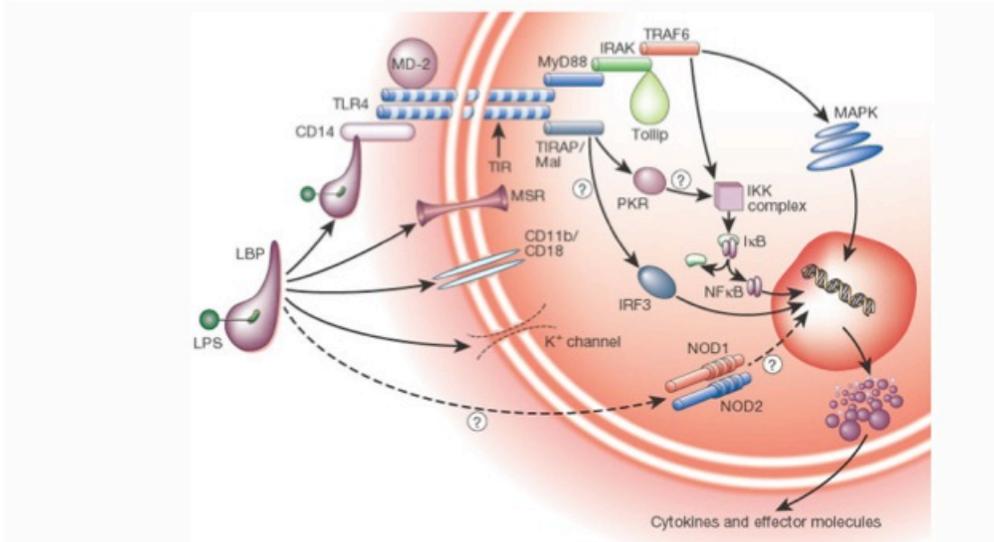
Figure 1.3. Principal LPS mechanism. The principal mechanism by which LPS is sensed is via an LPS-binding protein (LBP)–LPS complex and then signaling through the Toll-like receptor 4 (TLR4)–MD-2 complex at the surface of an alveolar macrophage (Cohen, 2002).

Macrophages and dendritic cells have abundant membrane-bound CD14 and exhibit sensitive and prolific responses to endotoxin. Alveolar macrophages have been shown to produce the proinflammatory cytokines IL-8, IL-6, GM-CSF, and IL-1β as well as IFN-γ, which is critical in innate immune responses to bacterial infections in response to LPS exposure (Liu, 2004, Zhu, 2010). LPS is also a B cell mitogen and promotes isotype switching from IgM to IgE in the presence of IL-4 (Medicine, 2010). Thus, endotoxin not only serves as a potent stimulus to innate immune responses but also serves as a stimulus and bridge to adaptive immunity.

This concept introduces a unique paradox faced by endotoxin researchers. On one hand, the mechanisms described above could explain why residential endotoxin exposure has been significantly associated with an increase in prevalence of wheeze (Horick, 2006, Ryan, 2009) and has been identified as a significant risk factor for increased asthma prevalence (Thorne, 2005). This is in agreement with worker health effects observed in such diverse industries as the cotton industry, animal husbandry, wastewater treatment, coffee curing, and wood processing (Duquenne, 2012, Moen, 2012, Pipinic, 2010). A health-based guidance limit of 50 EU/m^3 has been recommended for occupational settings in the Netherlands for an 8-hr time-weighted average exposure (Heederik, 1997). There is no doubt that *in vitro, in vivo*, and *ex vivo* studies consistently show proinflammatory effects of endotoxin exposure.

On the other hand, early life exposure to endotoxin (i.e., rural upbringing) is thought to explain the lower risk of atopy and asthma found in farmers' children and children with childhood pets (Douwes, 2002, Sordillo, 2010). This potential protection from atopy and asthma is in agreement with the hygiene hypothesis, which attributes the increase in global asthma prevalence to increased cleanliness, reduced family size, and subsequent decrease in microbial exposures (Brooks, 2013). However, evaluation of this potentially protective effect is challenging to generalize to larger populations. Although

evidence suggests that endotoxin exposure may protect against the development of atopy, at most only 50% of asthma cases appear to be attributable to mechanisms involving atopy (Pearce, 1999). Atopy is characterized by the production of specific IgE in response to common environmental allergens for which skin prick testing is convenient in epidemiological studies (Peden, 2000). A recent review of this topic proposed the term "early immune challenge hypothesis" (Kramer, 2013). This hypothesis encourages parents to allow their children to play with one another in the natural environment without protecting them from a perceived 'dirty' environment while at the same time honoring hygiene standards and strategies, such as washing hands after using the bathroom (Kramer, 2013). The role of endotoxin in the etiology of asthma has yet to be fully elucidated. However, children and adults with existing asthma have a unique immune profile that may respond differently to endotoxin exposure than non-asthmatics.

Asthma is a complex multifactorial disease that is characterized by reversible airway obstruction, airway hyperresponsiveness, and eosinophilic airway inflammation (Peden, 2000). Allergies and atopic asthma are considered type 1 hypersensitivies, which are reactions provoked by re-exposure to a specific type of antigen referred to as an allergen (Crinnion, 2012). Once inhaled, antigens are recognized and engulfed by alveolar macrophages, the first line of immune defense in the lung. The alveolar macrophage then sends out chemical messengers to alert the immune system to the presence of this antigen. The chemical messengers responsible for communication in the immune system are known as cytokines or lymphokines. T-helper cells (Th cells) are also immune system messengers that activate or direct other immune cells (Crinnion, 2012).

Th cells can develop into effector Th cells, memory Th cells, or regulatory Th cells. Effector Th cells subsequently differentiate into two major subtypes known as Type 1 and Type 2 helper T cells (Th1 and Th2 cells, respectively) (Crinnion, 2012). Th1 cells are involved in maximizing the killing efficacy of macrophages and play a vital role in fighting viral and bacterial pathogens. Th2 cells stimulate the adaptive immune system and influence B-cell proliferation and the production of neutralizing antibodies (Crinnion, 2012). Th2 cytokines activate eosinophils, a hallmark of the asthmatic phenotype (Peden, 2000).

The Th1/Th2 balance can be investigated via cytokine profiles. IL-10 and IL-4 are cytokines derived from Th2 cells while IFN-γ is a product of Th1 cells. IL-10 suppresses the activity of Th1 cells and IFN-γ suppresses the differentiation of Th2 cells (Chung, 2001). This feedback typically keeps infection fighting (Th1) and eosinophilic inflammation (Th2) in check, however studies investigating bronchalveolar lavage fluid and lung biopsies have confirmed that a Th2 dominated lung environment is seen in atopic asthmatics (Robinson, 1992, Walker, 1992). As a result, there is elevation of IL-4

and depression of IFN- γ (Tang, 1995), which is commonly described with the IL-4/IFN-γ ratio.

As mentioned above, at most only 50% of asthma cases appear to be attributable to mechanisms involving atopy, deemed extrinsic asthma (Pearce, 1999). Those with intrinsic, or nonatopic asthma, tend to have onset of symptoms later in life ("adult-onset asthma"), are more commonly female, have disease that is more difficult to control, and more frequently have rhinosinusitis (Corrigan, 2004). Intrinsic asthma is clinically handled as exercise induced or chemical (i.e., smoke) induced because intrinsic asthmatics mount no response to skin prick tests for common allergens (Corrigan, 2004). Overall, eosinophilic infiltration and local expression of cytokines thought to be important in asthma pathogenesis (i.e., IL-4 and IL-5) are similar between atopic and nonatopic asthmatics (Corrigan, 2004). This supports the hypothesis that IgE-mediated mechanisms play no critical role in the pathogenesis of asthma but do exacerbate symptoms in sensitized, atopic patients (Corrigan, 2004). In all, the atopic status of asthmatics is a crucial descriptor to account for in health-based studies.

Indoor Air Quality Interventions for Asthma

Prevalence of childhood asthma has drastically increased in the past several decades, especially in industrialized nations (Kay, 2001). The estimated number of U.S. children diagnosed with asthma was 10 million in 2011 with 1.8 million emergency room visits with asthma as the primary diagnosis (Bloom, 2012). Health care for children with asthma consumes >$2.0 billion annually in the U.S. alone (Landrigan, 2002). Due to this disease and health care burden there have been a diverse array of strategies to reduce exposures that exacerbate asthma symptoms. Indoor asthma triggers have been the targets of many intervention studies as people spend a majority of their time indoors, averaging 86-87% of time for the general population and 89-90% for children (Klepeis, 2001). The home environment is ideal for health-based interventions because it can be altered with fewer resources and fewer barriers than the outdoor environment. A recent review determined the economic value of home-based multicomponent interventions for reducing asthma morbidity to have a benefit-cost ratio ranging from 5.3-14.0, implying that for every dollar spent on the intervention the monetary value of the resulting benefits, such as averted medical costs, is $5.30 - $14.00 (Nurmagambetov, 2011).

Multiple reviews have established the effectiveness of indoor air filtration systems on reducing asthma symptoms (McDonald, 2002), including a review of recent intervention studies which determined air filtration to be responsible for ~7-25% health improvement in adverse allergy and asthma outcomes (Fisk, 2013). These air filters can reduce exposure to asthma triggers such as airborne pet allergen (van der Heide, 1999) and particulate matter. Air filters have been shown to significantly reduce breathing problems, allergy attacks (Johnson, 2009), and asthma symptoms scores (Eick, 2011) in asthmatic children. Air filtration has also been shown to be effective in adults, reducing biomarkers of inflammation in healthy adults (Allen, 2009) and medication requirement in asthmatic adults (McDonald, 2002). Therefore, low-cost air filtration interventions have

the potential to greatly improve indoor air quality (IAQ) while reducing health costs for the family of an asthmatic child.

Our research group has previously attempted to reduce indoor $PM_{2.5}$ concentrations in wood stove homes by replacing older model wood stoves with cleaner burning EPA-certified wood stoves (Ward, 2008, Noonan, 2012a). Crude overall indoor $PM_{2.5}$ concentrations were reduced by 56% following the wood stove changeouts but there was substantial variability in efficacy. For example, five of the 21 homes investigated in that study demonstrated no average reduction in indoor $PM_{2.5}$ and seven homes had at least one post-changeout sampling with higher indoor $PM_{2.5}$ than the concentrations observed in that home during pre-changeout sampling (Noonan, 2012a). While wood stove changeouts can be inconsistent, the use of a stand alone filter unit has been shown to reduce indoor $PM_{2.5}$ in a wood stove home by 76% (Hart, 2011). The lack of consistent indoor air quality improvements following wood stove changeouts and the efficacy of stand alone filter use in a wood stove home supports the use of an air filtration intervention to reduce in-home particulate levels in wood stove homes.

Title: Indoor coarse particulate matter: Evaluating exposures and health effects in asthmatic children

My overall hypothesis is that indoor coarse fraction levels are associated with adverse health outcomes in asthmatic children living in wood stove homes and that an air filter intervention is effective in reducing indoor coarse fraction concentrations.

Specific Aim 1 (Chapter 2): Characterize airborne coarse fraction (PMc) concentration and biological content in homes with wood stoves. Pre-intervention sampling will be used to characterize these exposures (n=50). I hypothesize that home characteristics previously identified as predictors of indoor endotoxin content (people, pets, and dampness/humidity) as well as frequency of wood stove use will be predictors of airborne endotoxin in these homes. Additional home characteristics such as square footage, ambient temperature, and $PM_{2.5}$ concentrations will be evaluated for their influence on PMc and airborne endotoxin concentrations.

Specific Aim 2 (Chapter 3): Evaluate efficacy of filtration unit intervention on reduction of coarse fraction and airborne endotoxin concentrations. Homes with pre- and post-intervention CPEM data (n=43) will be evaluated for PMc and endotoxin content reduction based on assigned intervention. I hypothesize that, compared to their baseline winter concentrations, indoor PMc concentrations will be more reduced in active filtration homes (n=21) than in placebo filtration homes (n=22). Particle reduction will also be evaluated through data collected with a particle counter which will provide simultaneous counts of particles in multiple size ranges (0.3-0.49, 0.5-0.99, 1.0-2.49, 2.5-4.9 and 5.0-10.0 μm).

Specific Aim 3 (Chapter 4): Establish relationship between coarse fraction and airborne endotoxin concentrations to physiological and overall health outcomes. All primary subjects from homes with CPEM and health data will be included in the analyses (n=38). I hypothesize that elevated coarse fraction and associated airborne endotoxin concentrations will correlate with adverse health outcomes in asthmatic children. Overall health will be evaluated through the use of a Pediatric Asthma Quality of Life Questionnaire while physiological health will be evaluated through biomarker analyses in exhaled breath condensate.

My project will contribute several novel aspects to the ARTIS study by assessing exposure to a new indoor air pollutant of interest (PMc) as well as elucidate intervention-based exposure variation. It will also contribute towards a better understanding of indoor coarse fraction and airborne endotoxin exposure, as well as the associated health effects in asthmatic children living in homes with wood stoves. Data collection for this project will occur simultaneously with the established data collection strategy for ARTIS (Figure 1.4). Briefly, four 48-hour sampling episodes occur at each participant home. Two sampling episodes occur during the pre-intervention winter and two sampling episodes occur during the post-intervention winter. The implementation of household interventions (i.e. active air filter or placebo filter) occurs during the summer or fall between the two winters. Each sampling episode includes data collection for both health outcomes and exposure assessment (Noonan, 2012b).

Figure 1.4. ARTIS data collection strategy.
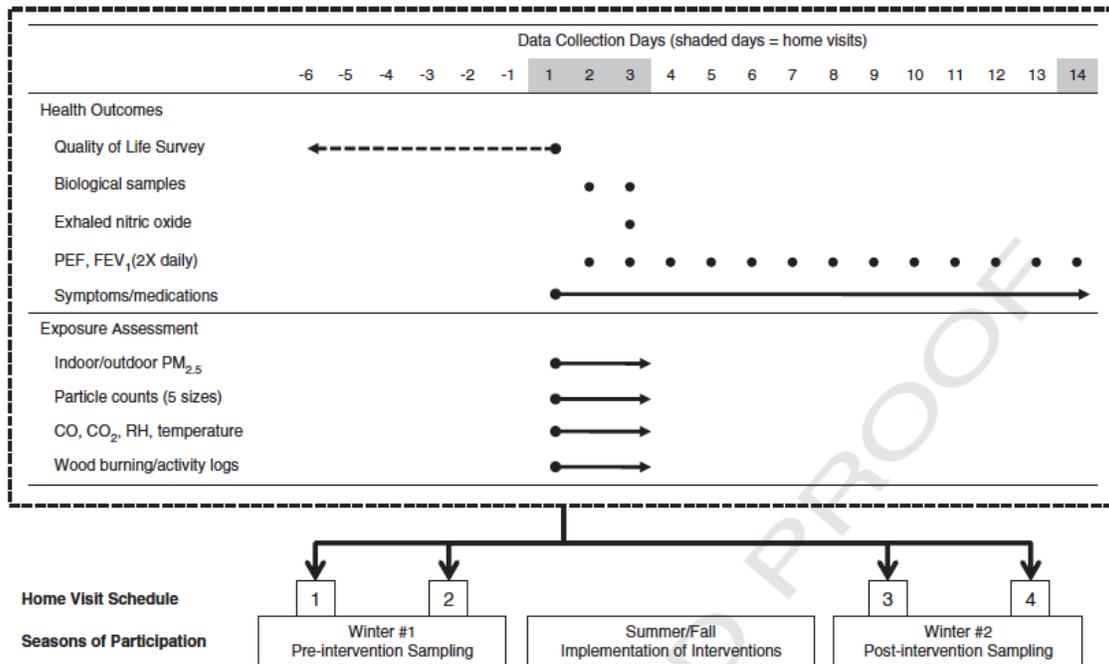
Figure 1.4. ARTIS data collection strategy. Four sampling episodes occur at each participant home. Two sampling episodes occur during the pre-intervention winter, and two sampling episodes occur during the post-intervention winter. The implementation of household interventions (i.e., active air filter or placebo filter) occurs during the summer or fall between the two winters. Each sampling episode, illustrated in the table surrounded by the dashed line box, includes data collection for both health outcomes and exposure assessment. The dashed arrow for the pediatric asthma quality of life survey indicates that the data reflect the child's symptoms, limitations and other quality of life indicators during the week prior to the corresponding home visit. Solid lines indicate periods during which data are collected on a continuous basis. Solid dots indicate measures that are assessed at specific time points (e.g., mornings for biological sample collections or mornings and evenings for pulmonary function measures). Not listed is co-sampling with a CPEM which occurrs simultaneously to other exposure assessment measurements (Noonan, 2012b).

REFERENCES

Alexis, N.E., Lay, J.C., Zeman, K., Bennett, W.E., Peden, D.B., Soukup, J.M., Devlin, R.B. and Becker, S. (2006) "Biological material on inhaled coarse fraction particulate matter activates airway phagocytes in vivo in healthy volunteers", *Journal of Allergy and Clinical Immunology*, **117**, 1396-1403.

Allen, R.W., Carlsten, C., Karlen, B., Leckie, S., Van Eeden, S., Vedal, S., Wong, I. and Brauer, M. (2009) "An air filter intervention study of endothelial function among healthy adults in a woodsmoke-impacted community", *Am J Respir Crit Care Med*, **183**, 1222-1230.

Barnig, C., Reboux, G., Roussel, S., Casset, A., Sohy, C., Dalphin, J.C. and De Blay, F. (2012) "Indoor dust and air concentrations of endotoxin in urban and rural environments", *Lett Appl Microbiol*, **56**, 161-167.

Bloom, B., Cohen, R.A. and Freeman, G. (2012) Summary health statistics for U.S. children: National Health Interview Survey, 2011, Vol. 10, Vital Health Stat, National Center for Health Statistics, 88.

Booze, T.F., Reinhardt, T.E., Quiring, S.J. and Ottmar, R.D. (2004) "A screening-level assessment of the health risks of chronic smoke exposure for wildland firefighters", *J Occup Environ Hyg*, **1**, 296-305.

Boy, E., Bruce, N. and Delgado, H. (2002) "Birth weight and exposure to kitchen wood smoke during pregnancy in rural Guatemala", *Environ Health Perspect*, **110**, 109-114.

Brooks, C., Pearce, N. and Douwes, J. (2013) "The hygiene hypothesis in allergy and asthma: an update", *Curr Opin Allergy Clin Immunol*, **13**, 70-77.

Brunekreef, B. and Forsberg, B. (2005) "Epidemiological evidence of effects of coarse airborne particles on health", *Eur Respir J*, **26**, 309-318.

Chung, F. (2001) "Anti-inflammatory cytokines inasthma and allergy: interleukin-10, interleukin-12, interferon-g", *Mediators Inflamm.*, **10**, 51-59.

Cohen, J. (2002) "The immunopathogenesis of sepsis", *Nature*, **420**, 885-891.

Corrigan, C. (2004) "Mechanisms of intrinsic asthma", *Curr Opin Allergy Clin Immunol*, **4**, 53-56.

Crinnion, W.J. (2012) "Do environmental toxicants contribute to allergy and asthma?", *Altern Med Rev*, **17**, 6-18.

Dale, V.H., Joyce, L.A., Mcnulty, S., Neilson, R.P., Ayres, M.P., Flannigan, M.D., Hanson, P.J., Irland, L.C., Lugo, A.E., Peterson, C.J., Simberloff, D., Swanson,

F.J., Stocks, B.J. and Michael Wotton, B. (2001) "Climate Change and Forest Disturbances", *BioScience*, **51**, 723-734.

Douwes, J., Pearce, N. and Heederik, D. (2002) "Does environmental endotoxin exposure prevent asthma?", *Thorax*, **57**, 86-90.

Duquenne, P., Marchand, G. and Duchaine, C. (2012) "Measurement of Endotoxins in Bioaerosols at Workplace: A Critical Review of Literature and a Standardization Issue", *Ann Occup Hyg*.

Eick, S.A. and Richardson, G. (2011) "Investigation of different approaches to reduce allergens in asthmatic children's homes--the Breath of Fresh Air Project, Cornwall, United Kingdom", *Sci Total Environ*, **409**, 3628-3633.

Ellegard, A. (1996) "Cooking fuel smoke and respiratory symptoms among women in low-income areas in Maputo", *Environ Health Perspect*, **104**, 980-985.

Ezzati, M. and Kammen, D.M. (2002) "The health impacts of exposure to indoor air pollution from solid fuels in developing countries: knowledge, gaps, and data needs", *Environ Health Perspect*, **110**, 1057-1068.

FDA. (1984) 1983 code of Federal Regulations, In: Service, P. H. (ed), Vol. 48, Food and Drug Administration, 13096.

Fisk, W. (2013) "Health benefits of particle filtration", *Indoor Air*, **ePub ahead of print**.

Fullerton, D.G., Bruce, N. and Gordon, S.B. (2008) "Indoor air pollution from biomass fuel smoke is a major health concern in the developing world", *Trans R Soc Trop Med Hyg*, **102**, 843-851.

Gualtieri, M., Ovrevik, J., Holme, J.A., Perrone, M.G., Bolzacchini, E., Schwarze, P.E. and Camatini, M. (2010) "Differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells", *Toxicology in Vitro*, **24**, 29-39.

Gustafson, P., Ostman, C. and Sallsten, G. (2008) "Indoor levels of polycyclic aromatic hydrocarbons in homes with or without wood burning for heating", *Environ Sci Technol*, **42**, 5074-5080.

Hart, J.F., Ward, T.J., Spear, T.M., Rossi, R.J., Holland, N.N. and Loushin, B.G. (2011) "Evaluating the effectiveness of a commercial portable air purifier in homes with wood burning stoves: a preliminary study", *J Environ Public Health*, **2011**, 27.

Hecker, W., Witthauer, D. and Staerk, A. (1994) "Validation of Dry Heat Inactivation of Bacterial Endotoxins", *PDA Journal of Pharmaceutical Science and Technology*, **48**, 197-204.

Heederik, D. and Douwes, J. (1997) "Towards an occupational exposure limit for endotoxins? ", *Ann Agric Environ Med.*, **4**, 17-19.

Heinrich, J., Pitz, M., Bischof, W., Krug, N. and Borm, P.J.A. (2003) "Endotoxin in fine (PM2.5) and coarse (PM2.5-10) particle mass of ambient aerosols. A temporo-spatial analysis", *Atmospheric Environment*, **37**, 3659-3667.

Hoffmann, B., Moebus, S., Mohlenkamp, S., Stang, A., Lehmann, N., Dragano, N., Schmermund, A., Memmesheimer, M., Mann, K., Erbel, R. and Jockel, K.H. (2007) "Residential exposure to traffic is associated with coronary atherosclerosis", *Circulation*, **116**, 489-496.

Horick, N., Weller, E., Milton, D.K., Gold, D.R., Li, R. and Spiegelman, D. (2006) "Home endotoxin exposure and wheeze in infants: correction for bias due to exposure measurement error", *Environ Health Perspect*, **114**, 135-140.

Johnson, L., Ciaccio, C., Barnes, C.S., Kennedy, K., Forrest, E., Gard, L.C., Pacheco, F., Dowling, P. and Portnoy, J.M. (2009) "Low-cost interventions improve indoor air quality and children's health", *Allergy Asthma Proc*, **30**, 377-385.

Johnston, F.H., Hanigan, I.C., Henderson, S.B. and Morgan, G.G. (2013) "Evaluation of interventions to reduce air pollution from biomass smoke on mortality in Launceston, Australia: retrospective analysis of daily mortality, 1994-2007", *BMJ*, **346**.

Kay, A.B. (2001) "Allergy and allergic diseases. First of two parts", *N Engl J Med*, **344**, 30-37.

Klepeis, N.E., Nelson, W.C., Ott, W.R., Robinson, J.P., Tsang, A.M., Switzer, P., Behar, J.V., Hern, S.C., Engelmann, W.H. (2001) "The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants", *J Expo Anal Environ Epidemiol*, **11**, 231-252.

Kramer, A., Bekeschus, S., Broker, B.M., Schleibinger, H., Razavi, B. and Assadian, O. (2013) "Maintaining health by balancing microbial exposure and prevention of infection: the hygiene hypothesis versus the hypothesis of early immune challenge", *J Hosp Infect*, **6701**, 60007-60009.

Kurmi, O.P., Lam, K.B. and Ayres, J.G. (2012) "Indoor air pollution and the lung in low- and medium-income countries", *Eur Respir J*, **40**, 239-254.

Landrigan, P.J., Schechter, C.B., Lipton, J.M., Fahs, M.C. and Schwartz, J. (2002) "Environmental pollutants and disease in American children: estimates of

morbidity, mortality, and costs for lead poisoning, asthma, cancer, and developmental disabilities", *Environ Health Perspect*, **110**, 721-728.

Lawson, J.A., Dosman, J.A., Rennie, D.C., Beach, J., Newman, S.C. and Senthilselvan, A. (2011) "The association between endotoxin and lung function among children and adolescents living in a rural area", *Can Respir J*, **18**, e89-94.

Levesque, B., Allaire, S., Gauvin, D., Koutrakis, P., Gingras, S., Rhainds, M., Prud'homme, H. and Duchesne, J.F. (2001) "Wood-burning appliances and indoor air quality", *Sci Total Environ*, **281**, 47-62.

Liu, A.H. (2004) "Something old, something new: Indoor endotoxin, allergens and asthma", *Paediatric Respiratory Reviews*, **5**, S65-S71.

Martin, W.J., 2nd, Glass, R.I., Balbus, J.M. and Collins, F.S. (2011) "A major environmental cause of death", *Science*, **334**, 180-181.

Mathiesen, M., Pedersen, E.K., Bjorseth, O., Egeberg, K.W. and Syversen, T. (2004) "Heating of indoor dust causes reduction in its ability to stimulate release of IL-8 and TNFalpha in vitro compared to non-heated dust", *Indoor Air*, **14**, 226-234.

Mccracken, J., Smith, K.R., Stone, P., Diaz, A., Arana, B. and Schwartz, J. (2011) "Intervention to lower household wood smoke exposure in Guatemala reduces ST-segment depression on electrocardiograms", *Environ Health Perspect*, **119**, 1562-1568.

Mccracken, J.P., Smith, K.R., Diaz, A., Mittleman, M.A. and Schwartz, J. (2007) "Chimney stove intervention to reduce long-term wood smoke exposure lowers blood pressure among Guatemalan women", *Environ Health Perspect*, **115**, 996-1001.

McDonald, E., Cook, D., Newman, T., Griffith, L., Cox, G. and Guyatt, G. (2002) "Effect of air filtration systems on asthma: a systematic review of randomized trials", *Chest*, **122**, 1535-1542.

McGowan, J.A., Hider, R.N., Chacko, E. and Town, G.I. (2002) "Particulate air pollution and hospital admissions in Christchurch, New Zealand", *Aust N Z J Public Health*, **26**, 23-29.

McNamara, M.L., Semmens, E.O., Gaskill, S., Palmer, C., Noonan, C.W. and Ward, T.J. (2011) "Base camp personnel exposure to particulate matter during wildland fire suppression activities", *J Occup Environ Hyg*, **9**, 149-156.

Medicine, I.O. (2010) *Clearing the Air: Asthma and Indoor Air Exposures,* Washington, D.C., National Academy Press.

Menetrez, M.Y., Foarde, K.K., Esch, R.K., Schwartz, T.D., Dean, T.R., Hays, M.D., Cho, S.H., Betancourt, D.A. and Moore, S.A. (2009) "An evaluation of indoor and outdoor biological particulate matter", *Atmospheric Environment*, **43**, 5476-5483.

Mishra, V., Dai, X., Smith, K.R. and Mika, L. (2004) "Maternal exposure to biomass smoke and reduced birth weight in Zimbabwe", *Ann Epidemiol*, **14**, 740-747.

Miyata, R. and Van Eeden, S.F. (2011) "The innate and adaptive immune response induced by alveolar macrophages exposed to ambient particulate matter", *Toxicol Appl Pharmacol*, **257**, 209-226.

Moen, B.E., Sakwari, G., Mamuya, S.H., Kayumba, A.V., Larsson, L., Pehrson, C., Mashalla, Y.J. and Bratveit, M. (2012) "Respiratory inflammation among workers exposed to airborne dust with endotoxins in a coffee curing factory", *J Occup Environ Med*, **54**, 847-850.

Molnar, P., Gustafson, P., Johannesson, S., Boman, J., Barregard, L. and Sallsten, G. (2005) "Domestic wood burning and PM2.5 trace elements: Personal exposures, indoor and outdoor levels", *Atmospheric Environment*, **39**, 2643-2653.

Mortimer, K., Gordon, S.B., Jindal, S.K., Accinelli, R.A., Balmes, J. and Martin, W.J., 2nd. (2012) "Household air pollution is a major avoidable risk factor for cardiorespiratory disease", *Chest*, **142**, 1308-1315.

Naeher, L.P., Brauer, M., Lipsett, M., Zelikoff, J.T., Simpson, C.D., Koenig, J.Q. and Smith, K.R. (2007) "Woodsmoke health effects: a review", *Inhal Toxicol*, **19**, 67-106.

Noonan, C.W., Navidi, W., Sheppard, L., Palmer, C.P., Bergauff, M., Hooper, K. and Ward, T.J. (2012a) "Residential indoor PM2.5 in wood stove homes: follow-up of the Libby changeout program", *Indoor Air*, **22**, 492-500.

Noonan, C.W. and Ward, T.J. (2012b) "Asthma randomized trial of indoor wood smoke (ARTIS): Rationale and methods", *Contemp Clin Trials*, **33**, 1080-1087.

Nurmagambetov, T.A., Barnett, S.B., Jacob, V., Chattopadhyay, S.K., Hopkins, D.P., Crocker, D.D., Dumitru, G.G. and Kinyota, S. (2011) "Economic value of home-based, multi-trigger, multicomponent interventions with an environmental focus for reducing asthma morbidity a community guide systematic review", *Am J Prev Med*, **41**, 011.

Oeder, S., Jorres, R.A., Weichenmeier, I., Pusch, G., Schober, W., Pfab, F., Behrendt, H., Schierl, R., Kronseder, A., Nowak, D., Dietrich, S., Fernandez-Caldas, E., Lintelmann, J., Zimmermann, R., Lang, R., Mages, J., Fromme, H. and Buters,

J.T. (2012) "Airborne Indoor Particles from Schools are More Toxic than Outdoor Particles", *Am J Respir Cell Mol Biol*, **47**, 575-582.

Park, J.H., Spiegelman, D.L., Gold, D.R., Burge, H.A. and Milton, D.K. (2001) "Predictors of airborne endotoxin in the home", *Environ Health Perspect*, **109**, 859-864.

Pearce, N., Pekkanen, J. and Beasley, R. (1999) "How much asthma is really attributable to atopy?", *Thorax*, **54**, 268-272.

Peden, D.B. (2000) "Development of atopy and asthma: candidate environmental influences and important periods of exposure", *Environ Health Perspect*, **3**, 475-482.

Pipinic, I.S., Varnai, V.M., Lucic, R.B., Cavlovic, A., Prester, L., Orct, T. and Macan, J. (2010) "Endotoxin exposure assessment in wood-processing industry: airborne versus settled dust levels", *Arh Hig Rada Toksikol*, **61**, 161-166.

Pope, C.A., Dockery, D.W. and Schwartz, J. (1995) "Review of Epidemiological Evidence of Health Effects of Particulate Air Pollution", *Inhalation Toxicology*, **7**, 1-18.

Pope, D.P., Mishra, V., Thompson, L., Siddiqui, A.R., Rehfuess, E.A., Weber, M. and Bruce, N.G. (2010) "Risk of Low Birth Weight and Stillbirth Associated With Indoor Air Pollution From Solid Fuel Use in Developing Countries", *Epidemiol Rev*, **32**, 70-81.

Reinhardt, T.E. and Ottmar, R.D. (2004) "Baseline Measurements of Smoke Exposure Among Wildland Firefighters", *Journal of Occupational and Environmental Hygiene*, **1**, 593 - 606.

Robinson, D.S., Hamid, Q., Ying, S., Tsicopoulos, A., Barkans, J., Bentley, A.M., Corrigan, C., Durham, S.R. and Kay, A.B. (1992) "Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma", *N Engl J Med*, **326**, 298-304.

Ryan, P., Bernstein, D., Lockey, J., Reponen, T., Levin, L., Grinshpun, S., Villareal, M., Hershey, G., Burkle, J. and Lemasters, G. (2009) "Exposure to traffic-related particles and endotoxin during infancy is associated with wheezing at age 3 years", *American journal of respiratory and cricial care medicine*, **180**, 1068-1075.

Semple, S., Devakumar, D., Fullerton, D.G., Thorne, P.S., Metwali, N., Costello, A., Gordon, S.B., Manandhar, D.S. and Ayres, J.G. (2010) "Airborne Endotoxin

Concentrations in Homes Burning Biomass Fuel", *Environ Health Perspect*, **118**, 988-991.

Semple, S., Garden, C., Coggins, M., Galea, K.S., Whelan, P., Cowie, H., Sanchez-Jimenez, A., Thorne, P.S., Hurley, J.F. and Ayres, J.G. (2011) "Contribution of solid fuel, gas combustion, or tobacco smoke to indoor air pollutant concentrations in Irish and Scottish homes", *Indoor Air*, **22**, 212-223.

Siddiqui, A.R., Gold, E.B., Yang, X., Lee, K., Brown, K.H. and Bhutta, Z.A. (2008) "Prenatal exposure to wood fuel smoke and low birth weight", *Environ Health Perspect*, **116**, 543-549.

Singh, U., Levin, L., Grinshpun, A., Schaffer, C., Adhikari, A. and Reponen, T. (2011) "Influence of home characteristics on airborne and dustborne endotoxin and B-D-glucan", *J. Environ. Monit.*, **13**, 3246-3253.

Smith, K.R., Bruce, N., Arana, B., Jenny, A., Khalakdina, A., Mccracken, J., Diaz, A., Schei, M. and Gove, S. (2006) "Conducting The First Randomized Control Trial of Acute Lower Respiratory Infections and Indoor Air Pollution: Description of Process and Methods", *Epidemiology*, **17**, S44.

Smith, K.R., Mccracken, J.P., Thompson, L., Edwards, R., Shields, K.N., Canuz, E. and Bruce, N. (2009) "Personal child and mother carbon monoxide exposures and kitchen levels: methods and results from a randomized trial of woodfired chimney cookstoves in Guatemala (RESPIRE)", *J Expo Sci Environ Epidemiol*, **20**, 406-416.

Smith, K.R., Mccracken, J.P., Weber, M.W., Hubbard, A., Jenny, A., Thompson, L.M., Balmes, J., Diaz, A., Arana, B. and Bruce, N. (2011) "Effect of reduction in household air pollution on childhood pneumonia in Guatemala (RESPIRE): a randomised controlled trial", *The Lancet*, **378**, 1717-1726.

Song, D.H. and Lee, J.O. (2012) "Sensing of microbial molecular patterns by Toll-like receptors", *Immunol Rev*, **250**, 216-229.

Sood, A. (2012) "Indoor fuel exposure and the lung in both developing and developed countries: an update", *Clin Chest Med*, **33**, 649-665.

Sordillo, J.E., Alwis, U.K., Hoffman, E., Gold, D.R. and Milton, D.K. (2011) "Home Characteristics as Predictors of Bacterial and Fungal Microbial Biomarkers in House Dust", *Environ Health Perspect*, **119**, 189-195.

Sordillo, J.E., Hoffman, E.B., Celedon, J.C., Litonjua, A.A., Milton, D.K. and Gold, D.R. (2010) "Multiple microbial exposures in the home may protect against asthma or allergy in childhood", *Clin Exp Allergy*, **40**, 902-910.

Tager, I.B., Lurmann, F.W., Haight, T., Alcorn, S., Penfold, B. and Hammond, S.K. (2010) "Temporal and Spatial Patterns of Ambient Endotoxin Concentrations in Fresno, California", *Environ Health Perspect*, **118**, 1490-1496.

Tang, M.L., Coleman, J. and Kemp, A.S. (1995) "Interleukin-4 and interferon-gamma production in atopic and non-atopic children with asthma", *Clin Exp Allergy*, **25**, 515-521.

Thompson, L.M., Bruce, N., Eskenazi, B., Diaz, A., Pope, D. and Smith, K.R. (2011) "Impact of reduced maternal exposures to wood smoke from an introduced chimney stove on newborn birth weight in rural Guatemala", *Environ Health Perspect*, **119**, 1489-1494.

Thorne, P.S., Cohn, R.D., Mav, D., Arbes, S.J. and Zeldin, D.C. (2009) "Predictors of endotoxin levels in U.S. housing", *Environ Health Perspect*, **117**, 763-771.

Thorne, P.S. and Duchaine, C. (2007) Airborne bacteria and endotoxin, In: Cj, H., RI, C., JI, G., Da, L., AI, M. and Ld, S. (eds) *Manual of Environmental Microbiology*, Washington, DC, ASM Press, 989-1004.

Thorne, P.S., Kulhankova, K., Yin, M., Cohn, R., Arbes, S.J., Jr. and Zeldin, D.C. (2005) "Endotoxin Exposure Is a Risk Factor for Asthma: The National Survey of Endotoxin in United States Housing", *Am. J. Respir. Crit. Care Med.*, **172**, 1371-1377.

Tielsch, J.M., Katz, J., Thulasiraj, R.D., Coles, C.L., Sheeladevi, S., Yanik, E.L. and Rahmathullah, L. (2009) "Exposure to indoor biomass fuel and tobacco smoke and risk of adverse reproductive outcomes, mortality, respiratory morbidity and growth among newborn infants in south India", *Int. J. Epidemiol.*, **38**, 1351-1363.

Tong, H., Cheng, W.Y., Samet, J.M., Gilmour, M.I. and Devlin, R.B. (2010) "Differential Cardiopulmonary Effects of Size-Fractioned Ambient Particulate Matter in Mice", *Cardiovasc Toxicol*, **10**, 259-267.

Van Berlo, D., Hullmann, M. and Schins, R.P. (2012) "Toxicology of ambient particulate matter", *Exs*, **101**, 165-217.

Van Der Heide, S., Van Aalderen, W.M., Kauffman, H.F., Dubois, A.E. and De Monchy, J.G. (1999) "Clinical effects of air cleaners in homes of asthmatic children sensitized to pet allergens", *J Allergy Clin Immunol*, **104**, 447-451.

Walker, C., Bode, E., Boer, L., Hansel, T.T., Blaser, K. and Virchow, J.C., Jr. (1992) "Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage", *Am Rev Respir Dis*, **146**, 109-115.

Ward, T. and Lange, T. (2010) "The impact of wood smoke on ambient PM2.5 in northern Rocky Mountain valley communities", *Environmental Pollution*, **158**, 723-729.

Ward, T., Palmer, C., Bergauff, M., Hooper, K. and Noonan, C. (2008) "Results of a residential indoor PM2.5 sampling program before and after a woodstove changeout", *Indoor Air*, **18**, 408-415.

Wegesser, T.C., Franzi, L.M., Mitloehner, F.M., Eiguren-Fernandez, A. and Last, J.A. (2010) "Lung antioxidant and cytokine responses to coarse and fine particulate matter from the great California wildfires of 2008", *Inhal Toxicol*, **22**, 561-570.

Williams, D.W. and Liebhold, A.M. (2002) "Climate change and the outbreak ranges of two North American bark beetles", *Agricultural & Forest Entomology*, **4**, 87-99.

World Health Organization. (2009) Global health risks. Mortality and burden of disease attributable to selected major risks., Geneva, Switzerland, World Health Organization.

Zhu, Z., Oh, S.Y., Zheng, T. and Kim, Y.K. (2010) "Immunomodulating effects of endotoxin in mouse models of allergic asthma", *Clin Exp Allergy*, **40**, 536-546.

CHAPTER 2

Coarse Particulate Matter and Airborne Endotoxin Within Wood Stove Homes

ABSTRACT

Emissions from indoor biomass burning are a major public health concern in developing areas of the world. Less is known about indoor air quality, particularly airborne endotoxin, in homes burning biomass fuel in residential wood stoves in higher income countries. A filter-based sampler was used to evaluate wintertime indoor coarse particulate matter (PMc) and airborne endotoxin (EU/m^3, EU/mg) concentrations in 50 homes using wood stoves as their primary source of heat in western Montana. We investigated number of residents, number of pets, dampness (humidity), and frequency of wood stove usage as potential predictors of indoor airborne endotoxin concentrations. Two 48-hour sampling events per home revealed a mean winter PMc concentration (± sd) of 12.9 (± 8.6) $\mu g/m^3$, while $PM_{2.5}$ concentrations averaged 32.3 (± 32.6) $\mu g/m^3$. Endotoxin concentrations measured from PMc filter samples were 9.2 (± 12.4) EU/m^3 and 1,010 (± 1,524) EU/mg. PMc and $PM_{2.5}$ were significantly correlated in wood stove homes (r=0.36, p<0.05). The presence of pets in the homes was associated with PMc but not with endotoxin concentrations. Importantly, none of the other measured home characteristics was a strong predictor of airborne endotoxin, including frequency of residential wood stove usage.

INTRODUCTION

It is well known that cookstoves are a significant source of indoor air pollution in many developing areas throughout the world. Studies have shown that particles <10 µm (PM_{10}) can be elevated in homes that use biomass fuel, with indoor concentrations ~10 to 70 times above ambient concentrations observed in some of the world's most polluted cities (Salvi and Barnes, 2010). Globally, less focus has been placed on understanding the biomass combustion exposures associated with domestic heating. Wood burning has been found to be a significant source of ambient particulate matter (PM) in rural areas of Scandinavia, New Zealand, Canada and the Northwest United States, including the northern Rocky Mountains of western Montana (NERI, 2005; McGowan et al., 2002; Larson and Koenig, 1994; Maykut et al., 2003; Ward and Lange, 2010; Ward et al., 2006; Schumpert et al., 2006; Noonan and Ward, 2007; Noonan et al., 2012c), in some cases accounting for up to 90% of ambient fine particles ($PM_{2.5}$) (McGowan et al., 2002). Importantly, indoor air quality can be impacted by wood stove use. Elevated short- and long-term $PM_{2.5}$ concentrations generated from wood burning are frequently much higher than those observed in the ambient environment (Ward et al., 2011; Ward et al., 2008; Noonan et al., 2012a) and often higher than the 24-hour ambient standard established by the U.S. Environmental Protection Agency (EPA) of 35 µg/m^3.

As people spend the majority of their time indoors (Klepeis et al., 2001), it is important to understand indoor exposures to hazardous pollutants such as PM_{10} and $PM_{2.5}$. PM_{10} and $PM_{2.5}$ can penetrate into the thoracic and lower airways, respectively, and are known to cause a variety of adverse health effects (Brunekreef and Forsberg, 2005). The coarse size fraction, consisting of particles with an average aerodynamic diameter of <10 µm and >2.5 µm (PMc), has arisen as a new class of interest because, unlike PM_{10}, it is not as strongly correlated with $PM_{2.5}$ (Tager et al., 2010). PMc has been

shown to cause a more significant pulmonary inflammatory response than $PM_{2.5}$ in an animal model (Tong et al., 2010) as well as increased inflammatory potential in multiple respiratory cell lines (Gualtieri et al., 2010; Oeder et al., 2012). Coarse particles captured during wildfire activity have shown more proinflammatory and oxidative stress potential on an equal-dose basis than fine particles (Wegesser et al., 2010). This inflammatory potential may be linked to the biological components of PMc, as biological inactivation of the coarse fraction significantly attenuates inflammatory effects in healthy humans (Alexis et al., 2006).

A major biological component of PMc is endotoxin (Liu, 2004). Endotoxins, lipopolysaccharides derived from the outer membrane of Gram-negative bacteria, have long been recognized to cause both immediate and sustained airflow obstruction in asthmatics (Liu, 2004). A temporo-spatial analysis detected ambient endotoxin in the coarse fraction at concentrations 10-fold higher than found in $PM_{2.5}$ (Heinrich et al., 2003), and this relationship exists in both indoor and outdoor environments (Menetrez et al., 2009). A recent study conducted in Nepal and Malawi measured airborne endotoxin concentrations in homes burning biomass fuel at orders of magnitude higher than those found in homes in developed countries (Semple et al., 2010). A similar study found wood-burning homes in Scotland and Ireland had higher airborne endotoxin concentrations than homes burning coal, peat, or using gas cooking (Semple et al., 2011). However, the frequency of stove loading and stoking was not accounted for in that study.

Here we present results from a two-winter study that measured indoor $PM_{2.5}$, PMc, and airborne endotoxin concentrations within 50 wood stove homes throughout western Montana, evaluating home characteristics previously identified as predictors of endotoxin. These characteristics include dampness/moisture sources (Sordillo et al., 2011; Park et al., 2001), presence of pets, (Thorne et al., 2009; Park et al., 2001;

Sordillo et al., 2011), and number of people living in the home (Thorne et al., 2009;

Singh et al., 2011). We also collected novel data relevant to wood stove usage and

ambient weather conditions, both of which could potentially influence indoor biomass

smoke exposures.

METHODS

Description of Parent Study

Asthma Randomized Trial of Indoor Wood Smoke (ARTIS) is an intervention-based study aimed at improving the quality of life of asthmatic children living in wood stove homes by reducing in-home $PM_{2.5}$ exposures. The ARTIS study involves four separate 48-hour sampling visits per household over two consecutive winters (a baseline winter and post-intervention winter) (Noonan and Ward, 2012b). The use of a non-EPA certified (older model) wood stove as the primary heating source in the home was required for inclusion in the study. In this manuscript, we present baseline (i.e. pre-intervention) indoor air quality data collected over two winters (7 homes during November 2010-March 2011 and 43 homes during November 2011-March 2012) for homes within a 200-mile radius of Missoula, Montana.

Home Characteristics Data

At each sampling visit, homeowners reported descriptive characteristics of their residence, including number of pets (furry animals only, i.e. dogs and cats) and square footage. Throughout the 48-hour sampling event the homeowners were asked to track wood stove usage, recording each time the wood stove was loaded or stoked. Ambient meteorological data including temperature, humidity, wind speed, and precipitation were collected for all sampling days from the weather station nearest to the respective homes. Mean distance (range) from homes to the nearest weather station was 16.8 miles (0.6-44.4 miles). Meteorological data were averaged across visits for each home.

Indoor Sampling

Samplers were collocated in each home in the common living area on a table 3-5 feet off the ground and across the room from the wood stove, if possible. In each sampling

event, $PM_{2.5}$ was continuously measured with a DustTrak (TSI Inc., Shoreview, MN, USA). The DustTrak is an optical scattering instrument that measures PM in the airflow by measuring the extent of forward scattering of an infrared diode laser beam. The device is factory calibrated to the respirable fraction of standard ISO 12103-1, A1 test dust (formerly Arizona Test Dust). DustTraks were zeroed and the impaction plate was cleaned and greased/oiled as necessary prior to each sampling event. During each sampling event, a DustTrak 8530 recorded 60-second averages of $PM_{2.5}$.

Optical mass measurements (such as those made by the DustTrak) are dependent upon particle size and material properties, therefore custom calibrations are needed to improve the measurement accuracy when evaluating specific sources of combustion. In an effort to accurately present wood smoke-related $PM_{2.5}$ concentrations, all DustTrak $PM_{2.5}$ measurements presented in this manuscript were corrected to an indoor wood smoke-specific correction factor of 1.65 developed by our research group (McNamara et al., 2011). In addition to the DustTraks, co-located Q-traks (TSI Inc., Shoreview, MN, USA) were used to record 60-second averages of indoor temperature and humidity (%rH). Data were downloaded from the DustTraks and Q-traks at the conclusion of each sampling event.

Coarse fraction concentrations commonly are calculated from separate PM_{10} and $PM_{2.5}$ measurements. In this study, direct measurements of PMc and $PM_{2.5}$ were collected using a filter-based CPEM developed by RTI International (Research Triangle Park, NC, USA). As previously described (Williams et al., 2009; Thornburg et al., 2009), the CPEM is a series of three separation stages designed to be inserted into the MSP Model 200 PM_{10} PEM (MSP Corp., Shoreview, MN). PMc is collected on two sequential 25 mm PTFE filters with polymethylpentene (PMP) support rings (thickness 3.0 μm; Zefon International, Inc., Ocala, FL, USA) while $PM_{2.5}$ is collected on a final 37 mm PTFE filter with PMP support rings (thickness 2.0 μm; Zefon International, Inc., Ocala,

FL, USA). The CPEM utilizes a battery-operated pump to achieve a flow rate of 2 Lpm. Flow rates measured with a Drycal DC-Lite (BIOS International, Butler, NJ, USA) prior to and following each sampling event were averaged to calculate the sample volume. If necessary, pump flow was adjusted at deployment to achieve 2 Lpm. All data from multiple sampling events per home were averaged to produce one winter average per home.

Gravimetric Analyses

The gravimetric analyses of PMc and PM$_{2.5}$ CPEM filters were conducted according to previously described guidelines (Lawless and Rodes, 1999) and modified for optimal conditions for teflon filters (Menetrez et al., 2009). Prior to sampling, each filter was placed into individually labeled sterile polystyrene Analyslide containers (Pall Corp., Ann Arbor, MI, USA) and allowed to equilibrate for 24-48 hours in an environmentally controlled weighing facility at The University of Montana. The facility was maintained at a temperature of 20-23 °C ± 2 °C with relative humidity of 30-40% ± 5%. Humidity was maintained using an 8-gallon Kenmore Whole House humidifier (model 758.15408). Static was controlled using a grounded anti-static floor mat (ComfortKing USA, Inc., Fairfield, NJ, USA) as well as a radioactive neutralizer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Filters were weighed prior to and after each sampling event with a calibrated MT5 microbalance (Mettler-Toledo LLC, Columbus, OH, USA). Multiple laboratory blanks were within the repeatability of the scale (0.8 µg). A minimum of 10% field blanks was included in all analyses.

Endotoxin Extraction and Analyses

Filter extraction methods were optimized for endotoxin analyses using methods adapted from Thorne (Thorne, 2000) and Spaan *et al.* (Spaan et al., 2007). For PMc the two

sequential 25 mm teflon filters were combined and extracted in 5 mL sterile, pyrogen-free water containing 0.05% Tween-20 for one hour at room temperature with vigorous shaking. The extracts were then centrifuged at 1,000-x g for 15 minutes at room temperature and supernatants were transferred into sterile, endotoxin-free 15 mL conical tubes (Greiner Bio-One North America Inc., Monroe, NC, USA). Extracts were diluted 50-fold with pyrogen-free water prior to endotoxin analysis to counteract enhanced yields due to Tween interference (Spaan et al., 2008). Samples were analyzed for endotoxin using a kinetic chromogenic *Limulus* amebocyte lysate (LAL) assay (Endosafe Endochrome-K; Charles River Laboratories Inc., Charleston, SC, USA). Lyophilized standard endotoxin, chromogenic substrate, and LAL preparations were reconstituted using pyrogen-free water. A 12-point standard curve was generated, ranging from 50 to 0.005 endotoxin units (EU)/ml. The absorbance in each well was measured at 405 nm every 30 seconds for 90 minutes (Thorne, 2000). Samples with nondetectable endotoxin levels were assigned a value of two-thirds the limit of detection (LOD) of the assay (Spaan et al., 2008). Final endotoxin results are presented as both EU/m^3 and EU/mg.

Statistical Analyses

SAS v9.2 (Cary, NC, USA) was used to perform all statistical analyses. Pearson's correlation coefficients were estimated to investigate relationships between log-transformed indoor air quality (IAQ) measurements assessed using different sampling instruments. In regression analyses, we used the natural log of PMc and airborne endotoxin concentrations to satisfy model assumptions, which we assessed in plots of residuals. Results, therefore, are reported as the percent change in geometric mean PMc and airborne endotoxin concentrations associated with various home characteristics. Multiple linear regression was used to examine these relationships in models adjusted for other home characteristics. Sensitivity analyses were performed to

examine the impact on results after excluding a home with an average $PM_{2.5}$ concentration ~63% higher than the next highest $PM_{2.5}$ concentration (163.08 $\mu g/m^3$ compared to next highest 101.94 $\mu g/m^3$). In addition, we examined the relationship between home characteristics and IAQ concentrations first using only visit 1 measurements and then using only visit 2 measurements to compare the results by visit with those obtained in the primary analysis using averaging across visits.

RESULTS

In-Home Sampling

During the winters (November-March) of 2010-2011 and 2011-2012, 50 homes with wood stoves were sampled for a total of 100, 48-hour events. PM and endotoxin sampling results are summarized in Table 2.1, along with home characteristic and indoor/ambient meteorological information. Throughout the sampling program, the average (± sd) PMc concentration in the homes was 12.9 (± 8.6) µg/m^3. Endotoxin in PM$_{2.5}$ was not detectable in 32% (n=16) of the homes. Maximum endotoxin concentrations in PM$_{2.5}$ were 2.9 EU/m^3 and 73.8 EU/mg. The average (± sd) corrected PM$_{2.5}$ concentration in the homes was 32.3 (± 32.6) µg/m^3. The average (± sd) endotoxin concentration in PMc was 9.2 (± 12.4) EU/m^3 and 1,010 (± 1,524) EU/mg of PMc. During the winter sampling months, the ambient temperature was -0.38 (± 3.02) °C, wind speed was 4.79 (± 2.04) mph and precipitation was 0.12 (± 0.21) inches. Homes had an average of approximately 4 (± 1) residents and an average size of 1,881 (± 986) square feet. Indoor humidity was on average less than half of ambient humidity (28.1 vs. 69.7 %rH, respectively).

Table 2.1. Home characteristics, air sampling, and meteorological results from all homes (n=50). Abbreviations: SD: standard deviation, %rH: percent relative humidity, mph: miles per hour, in.: inches.

	Mean	SD	Minimum	Maximum
Home Characteristics				
Number of Residents	4.2	1.4	2	8
Number of Pets	1.5	0.89	0	4
Times Loaded/Stoked	9.6	6.5	0	36
Square Footage	1881	986	420	4500
Indoor Air Measurements				
PMc ($\mu g/m^3$)	12.9	8.6	5.1	46.2
EU/m^3	9.2	12.4	0.002	52.0
EU/mg	1010	1524	0.35	6918
$PM_{2.5}$ ($\mu g/m^3$)	32.3	32.6	6.0	163
Temperature (°C)	22.4	2.5	16.6	28.5
Humidity (%rH)	28.1	6.4	17.6	48.1
Ambient Air Measurements				
Temperature (°C)	-0.38	3.02	-5.95	4.71
Humidity (%rH)	69.7	8.5	44.8	83.2
Windspeed (mph)	4.79	2.04	1.77	11.0
Total Precip. (in.)	0.12	0.21	0	0.81

Note: EU/m^3 and EU/mg in PMc.

IAQ Correlations

Table 2.2 presents Pearson's correlation coefficients between log-transformed concentrations of $PM_{2.5}$, PMc, and endotoxin within the wood stove homes (n=50). DustTrak-measured $PM_{2.5}$ and CPEM-measured $PM_{2.5}$ were significantly, though only modestly, correlated (r=0.29, p<0.05), possibly due to the loss of volatile and semi-volatile compounds present in $PM_{2.5}$ from the CPEM filters. The CPEM filters also collected very small masses of $PM_{2.5}$ due to the low flow rate (2 L/min) and short sampling period (48 hours), resulting in reduced precision in the measurements as homes with low $PM_{2.5}$ concentrations were within the sensitivity of the scale. Therefore, due to the DustTrak's optimized design for $PM_{2.5}$ sampling and the development of our wood smoke-specific DustTrak correction factor (McNamara et al., 2011), only corrected DustTrak values were used for further $PM_{2.5}$ analyses. As presented in Table 2.2, $PM_{2.5}$ (as measured by the DustTrak) was significantly correlated with PMc (r=0.36, p<0.05). Measures of airborne endotoxin concentrations in the coarse fraction (EU/m^3 and EU/mg) were correlated strongly with each other (r=0.76, p<0.0001) and endotoxin as measured in EU/m^3 but not EU/mg was significantly correlated with PMc mass concentrations.

Table 2.2. Pearson's correlation coefficients between log-transformed concentrations of $PM_{2.5}$, PMc, and endotoxin within the wood stove homes (n=50). * $p < 0.05$. ** $p < 0.0001$.

	$PM_{2.5}$ (DustTrak)	$PM_{2.5}$ (CPEM)	PMc (CPEM)	EU/m^3	EU/mg
$PM_{2.5}$ (DustTrak)	1	0.29*	0.36*	0.10	0.01
$PM_{2.5}$ (CPEM)	---	1	-0.10	0.11	0.32
PMc (CPEM)	---	---	1	0.31*	-0.04
EU/m^3	---	---	---	1	0.76**
EU/mg	---	---	---	---	1

Note: EU/m^3 and EU/mg in PMc.

Home Characteristics and IAQ Concentrations

Table 2.3 presents linear regression results (95% confidence interval) describing the

relationship between home characteristics and PMc and airborne endotoxin

concentrations in crude analyses and analyses adjusted for number of residents,

number of pets, times loaded/stoked, square footage, ambient temperature, indoor

humidity, and DustTrak $PM_{2.5}$. Number of pets was significantly associated with PMc

concentrations with each additional pet in the home associated with an estimated ~20%

increase in PMc concentrations in crude (95% CI: 3%, 44%) and adjusted (95% CI: 0%,

42%) analyses. A 500 ft^2 increase in home size was significantly associated with an 8%

(95% CI: 15%, 1%) decrease in PMc concentration. The point estimate was similar in

analyses adjusted for other home characteristics, but the association did not remain

statistically significant. A 10 $\mu g/m^3$ elevation in $PM_{2.5}$ was significantly associated with a

3% increase in PMc although this association did not persist after adjustment for other

home characteristics. The number of times the stove was loaded/stoked was not

associated with PMc concentrations or either measurement of airborne endotoxin. The

magnitude of each association was weakened slightly in adjusted analyses. Wood

stoves were in use in the vast majority of homes during our sampling events. However

we should note that one home reported no burning activity during the sampling periods

and had an average of 6.7 $\mu g/m^3$ PMc, 0.18 EU/m^3 and 53.2 EU/mg of PMc (results not

shown).

Table 2.3. Linear regression results (95% confidence intervals)[a] describing the relationship between home characteristics and PMc and airborne endotoxin concentrations (n=50). * p<0.05.

	PMc		EU/m^3		EU/mg	
	Unadjusted	Adjusted[d]	Unadjusted	Adjusted[d]	Unadjusted	Adjusted[d]
Number of Residents	0.98 (0.91, 1.14)	1.03 (0.92, 1.15)	1.16 (0.77, 1.73)	1.33 (0.85, 2.08)	1.11 (0.75, 1.64)	1.26 (0.81, 1.97)
Number of Pets	1.22 (1.03, 1.44)*	1.19 (1.00, 1.42)*	1.28 (0.69, 2.32)	1.27 (0.64, 2.53)	1.09 (0.59, 2.01)	1.12 (0.56, 2.22)
Times Loaded/Stoked	1.01 (0.99, 1.04)	1.00 (0.98, 1.02)	1.00 (0.92, 1.10)	0.99 (0.90, 1.09)	0.99 (0.91, 1.07)	0.98 (0.90, 1.08)
Square Footage[b]	0.92 (0.85, 0.99)*	0.95 (0.88, 1.04)	0.83 (0.63, 1.10)	0.83 (0.60, 1.16)	0.91 (0.69, 1.20)	0.89 (0.64, 1.23)
Ambient Temperature (°F)	1.03 (0.97, 1.08)	1.00 (0.95, 1.06)	1.03 (0.85, 1.24)	1.03 (0.84, 1.26)	1.00 (0.83, 1.19)	1.01 (0.83, 1.24)
Indoor Humidity (%rH)	1.02 (0.99, 1.04)	1.01 (.99, 1.04)	0.96 (0.88, 1.05)	0.94 (0.85, 1.04)	0.95 (0.87, 1.03)	0.94 (0.85, 1.03)
DustTrak PM$_{2.5}$ (µg/m^3)[c]	1.03 (1.00, 1.06)*	1.02 (0.99, 1.05)	1.03 (0.93, 1.14)	1.01 (0.89, 1.14)	1.00 (0.90, 1.10)	0.99 (0.88, 1.12)

a- regression results and confidence intervals are reported as percent change in geometric mean PMc, EU/m^3, or EU/mg. For example, 0.96 (0.91, 1.14) signifies a 4% reduction in geometric mean (95% CI: -9%, 14%).

b- results per 500 ft^2 increase

c- results per 10 µg/m^3 increase

d- analyses adjusted for number of residents, number of pets, times loaded/stoked, square footage, ambient temperature, indoor humidity, and DustTrak PM$_{2.5}$

Note: All coefficients correspond to one unit increase in predictor variable unless otherwise noted. EU/m^3 and EU/mg in PMc.

Findings from sensitivity analyses were consistent with results from our primary analyses. Excluding the home with the highest $PM_{2.5}$ concentration from our analyses had little impact on the results. To determine if averaging across visits affected results, all variables were investigated for associations with air sampling data using only the first visit and again using only the second visit of each winter, and results were similar (data not shown).

DISCUSSION

Several factors have been identified as predictors of indoor endotoxin concentrations. Larger numbers of residents and/or pets in the home may stir up, or resuspend, particulate matter due to increased activity. Higher number of residents has also been found to be significantly associated with increased endotoxin concentrations in dust samples in two large studies sampling 184 and 2,552 homes, respectively (Singh et al., 2011; Thorne et al., 2009). An increase in residents in our study homes suggested an increase in the mean EU/m^3 although these results were not statistically significant. Our smaller sample size compared to the larger studies described above limited our precision in describing associations. Another important distinction between this study and prior studies was the choice of sample media for assessing endotoxin levels. Both of the larger studies used dust endotoxin as a surrogate of the airborne endotoxin directly evaluated in our study. Airborne endotoxin measured over 1.5 days has been determined to be a more direct measure of exposure than the use of dust endotoxin (Horick et al., 2006). This is primarily due to dust endotoxin typically correlating poorly with airborne endotoxin levels (Park et al., 2001; Mazique et al., 2011). Despite the differences in sample media, our study suggests a similar relationship between number of residents and airborne endotoxin.

Number of pets has also been identified as a predictor of in-home endotoxin levels (Thorne et al., 2009; Park et al., 2001; Sordillo et al., 2011). Park *et al*. (Park et al., 2001) investigated presence of dogs in homes and found a 96% difference in airborne endotoxin in total suspended particulate between homes currently having a dog and homes that had never had a dog. Thorne *et al.* (Thorne et al., 2009) and Sordillo *et al.* (Sordillo et al., 2011) evaluated number of pets and endotoxin in dust using presence/absence of dogs and cats. Our study collected data on all 'furry' animals currently living in the home (dogs, cats, guinea pigs, etc). Number of pets showed no

relationship with both measurements of endotoxin in our study but was associated with PMc concentrations.

A unique aspect of our study was the focus on homes using biomass combustion for heating and the inclusion of wood stove usage data. Loading and stoking of a wood stove are important events to document because a plume of smoke can enter the indoor air when the stove's door is opened. The number of times the wood stove was loaded/stoked was not associated with PMc concentrations or either measurement of airborne endotoxin. Residents of homes in this study opened their stove doors an average of ~10 times during the 48-hour visits, with a range of 0-36 times (Table 2.1). In two similar studies, homes that contained non-EPA certified wood stoves had an average of five loading/stoking events over their sampling period of 24-48 hours (Ward et al., 2011; Noonan et al., 2012a).

Evaluating indoor air quality based on square footage of the home gave us some intriguing results. Our homes ranged from 420 to 4500 square feet with a mean (± sd) of 1,881 (± 986) square feet. A 500 ft^2 increase in home size was significantly associated with an 8% decrease in PMc concentration in unadjusted analyses, although this relationship was not significant when adjusted for other home characteristics. This finding is likely influenced by many factors. It is possible that the smaller homes, typically mobile homes in our study, may not be as well ventilated or insulated as the larger, newer homes in our study. Portable classrooms, constructed similar to mobile homes, have shown low ventilation rates (Shendell et al., 2004). As a result, the smaller homes may be experiencing greater re-infiltration of emitted particles. Conversely, particles in smaller volumes (smaller homes) are not dispersed as efficiently as larger volume spaces (such as larger homes) leading to higher concentrations in smaller homes. The discrepancy in insulation may also necessitate less wood burning per volume to maintain

a comfortable indoor temperature in the larger homes. Further studies are needed to validate these theories.

Ambient temperature and indoor humidity were not significantly correlated with any of the indoor air quality values reported here. Dampness/moisture sources have been identified as predictors of in-home endotoxin (Sordillo et al., 2011; Park et al., 2001), but these studies investigated dampness qualitatively by survey data on humidifier use, report of water damage, visible mold, use of central air, and living in an apartment. A recent review emphasized the lack of standardized and validated exposure assessment methods for microbial components in indoor air, with most studies using either trained fieldworkers or dust sampling to investigate moisture sources (Tischer and Heinrich, 2013). However, the continuous measurement of relative humidity in homes has been used in health studies. One study with indoor continuous relative humidity levels similar to ours (mean: 29.1% (± 5.0)) concluded that a 1% increase in relative humidity was associated with an increased risk of lower respiratory tract infections among young Inuit children in Canada (Kovesi et al., 2007). We evaluated continuous relative humidity data and did not find indoor humidity to be a significant predictor of airborne endotoxin. We are unaware of any studies that have investigated the relationship between the field identification of dampness/moisture sources and real-time indoor relative humidity in wood stove homes. Until this relationship is established, we are not confident that our data goes against the paradigm of dampness/moisture sources being a large predictor of indoor endotoxin.

Our sampling regimen allowed for simultaneous measurements of indoor temperature, humidity, $PM_{2.5}$ and PMc. Although our study focuses on reporting coarse fraction concentrations, it is notable that overall 48-hour average (*corrected*) $PM_{2.5}$ levels were similar to the EPA's health-based 24-hour ambient air quality standard of 35 $\mu g/m^3$. In addition, 16 (32%) sampled homes had winter $PM_{2.5}$ averages above this

concentration. These concentrations were consistent with previous studies conducted by our team in Libby, Montana. Two residential studies focused on 16 and 21 homes with older model wood stoves measured average (± sd) $PM_{2.5}$ concentrations of 51.2 (± 32.0) $\mu g/m^3$ and 45.0 (± 33.0) $\mu g/m^3$, respectively (Ward et al., 2008; Noonan et al., 2012a). If the correction factor was applied to the Libby residential studies as it was in this study, the average $PM_{2.5}$ concentrations measured in our study homes would be very similar (31.03 and 27.27 $\mu g/m^3$ in Libby vs. 32.3 $\mu g/m^3$ in our study) (Noonan et al., 2012a; Ward et al., 2008).

With its unique setting (wood stove homes), the use of a filter-based sampler to measure airborne endotoxin directly as opposed to the use of surrogate dust endotoxin, and the analysis of home characteristics as well as meteorological data, our study has a number of strengths. Several study limitations should be considered when attempting to generalize our findings. The lack of a standardized protocol for endotoxin sampling and analysis is the main challenge in comparing our quantitative results to the limited literature on indoor air quality in homes burning biomass fuel in developed countries. Many methodological factors can influence the measured concentration of endotoxin in a sample, such as filter type, extraction solution, extract storage, and assay solution (Spaan et al., 2007; Spaan et al., 2008). A study in Ireland and Scotland monitored several airborne pollutants in homes using biomass fuel and found EU/m^3 concentrations similar to those reported here, although they were investigating endotoxin in $PM_{2.5}$ only (Semple et al., 2011). Endotoxin concentrations in EU/m^3 as well as EU/mg of particle collected were reported within the ranges of our results in a sampling methods comparison study, but particle size selection and presence of biomass burning in the homes was not indicated (Frankel et al., 2012). We could not identify the partial contribution of spike events due to wood stove loading/stoking activity to the 48-hour averages (as can be done with continuous data) because the CPEMs are filter-based

samplers. Finally, although our study is the first to use CPEMs in wood stove homes, our in-home coarse fraction concentrations were comparable to those reported by the other published studies to-date that deployed the CPEM (our study: 12.9 (± 8.6 µg/m^3) vs. 12.8 (± 18.5 µg/m^3) in homes (Williams et al., 2009) vs. 11.6 (± 8.5 µg/m^3) during personal monitoring (Williams et al., 2012)).

CONCLUSION

This study is the first to describe coarse fraction particulate matter and airborne

endotoxin in wood stove homes using a state-of-the-art filter-based sampler. Home

characteristics and ambient weather conditions were evaluated for their influence on

pollutant levels although only number of pets was associated with PMc concentrations in

the homes. Our study shows a relationship between number of residents and airborne

endotoxin, consistent with previous literature establishing this relationship with dust

endotoxin. After application of an indoor wood smoke-specific correction factor,

concentrations of $PM_{2.5}$ were slightly below the EPA 24-hour standard of 35 μg/m^3. PMc

and $PM_{2.5}$ were significantly correlated in the homes, presenting a challenge for future

health effects research in distinguishing the separate health effects of PMc and $PM_{2.5}$ in

wood stove homes. Airborne endotoxin in the coarse fraction may be a unique exposure

of interest in health effects studies with people living in wood stove homes as both

measurements of airborne endotoxin (EU/m^3 and EU/mg) showed no correlation with

$PM_{2.5}$.

REFERENCES

Alexis, N.E., Lay, J.C., Zeman, K., Bennett, W.E., Peden, D.B., Soukup, J.M., Devlin, R.B. and Becker, S. (2006) "Biological material on inhaled coarse fraction particulate matter activates airway phagocytes in vivo in healthy volunteers", *Journal of Allergy and Clinical Immunology*, **117**, 1396-1403.

Brunekreef, B. and Forsberg, B. (2005) "Epidemiological evidence of effects of coarse airborne particles on health", *Eur Respir J*, **26**, 309-318.

Frankel, M., Timm, M., Hansen, E.W. and Madsen, A.M. (2012) "Comparison of sampling methods for the assessment of indoor microbial exposure", *Indoor Air*, **22**, 405-414.

Gualtieri, M., Ovrevik, J., Holme, J.A., Perrone, M.G., Bolzacchini, E., Schwarze, P.E. and Camatini, M. (2010) "Differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells", *Toxicology in Vitro*, **24**, 29-39.

Heinrich, J., Pitz, M., Bischof, W., Krug, N. and Borm, P.J.A. (2003) "Endotoxin in fine (PM2.5) and coarse (PM2.5-10) particle mass of ambient aerosols. A temporo-spatial analysis", *Atmospheric Environment*, **37**, 3659-3667.

Horick, N., Weller, E., Milton, D.K., Gold, D.R., Li, R. and Spiegelman, D. (2006) "Home endotoxin exposure and wheeze in infants: correction for bias due to exposure measurement error", *Environ Health Perspect*, **114**, 135-140.

Institute, N.E.R. (2005) Luftforurening med partikler i Danmark, Vol. 2005, Roskilde, Denmark.

Klepeis, N.E., Nelson, W.C., Ott, W.R., Robinson, J.P., Tsang, A.M., Switzer, P., Behar, J.V., Hern, S.C., Engelmann, W.H. (2001) "The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants", *J Expo Anal Environ Epidemiol*, **11**, 231-252.

Kovesi, T., Gilbert, N.L., Stocco, C., Fugler, D., Dales, R.E., Guay, M. and Miller, J.D. (2007) "Indoor air quality and the risk of lower respiratory tract infections in young Canadian Inuit children", *Cmaj*, **177**, 155-160.

Larson, T.V. and Koenig, J.Q. (1994) "Wood smoke: emissions and noncancer respiratory effects", *Annu Rev Public Health*, **15**, 133-156.

Lawless, P.A. and Rodes, C.E. (1999) "Maximizing data quality in the gravimetric analysis of personal exposure sample filters", *J Air Waste Manag Assoc*, **49**, 1039-1049.

Liu, A.H. (2004) "Something old, something new: Indoor endotoxin, allergens and asthma", *Paediatric Respiratory Reviews*, **5**, S65-S71.

Maykut, N.N., Lewtas, J., Kim, E. and Larson, T.V. (2003) "Source Apportionment of PM2.5 at an Urban IMPROVE Site in Seattle, Washington", *Environmental Science & Technology*, **37**, 5135-5142.

Mazique, D., Diette, G.B., Breysse, P.N., Matsui, E.C., Mccormack, M.C., Curtin-Brosnan, J., Williams, D.L., Peng, R.D. and Hansel, N.N. (2011) "Predictors of airborne endotoxin concentrations in inner city homes", *Environ Res*, **111**, 614-617.

McGowan, J.A., Hider, R.N., Chacko, E. and Town, G.I. (2002) "Particulate air pollution and hospital admissions in Christchurch, New Zealand", *Aust N Z J Public Health*, **26**, 23-29.

McNamara, M.L., Noonan, C.W. and Ward, T.J. (2011) "Correction factor for continuous monitoring of wood smoke fine particulate matter", *Aerosol and Air Quality Research*, **11**, 315-322.

Menetrez, M.Y., Foarde, K.K., Esch, R.K., Schwartz, T.D., Dean, T.R., Hays, M.D., Cho, S.H., Betancourt, D.A. and Moore, S.A. (2009) "An evaluation of indoor and outdoor biological particulate matter", *Atmospheric Environment*, **43**, 5476-5483.

Noonan, C.W., Navidi, W., Sheppard, L., Palmer, C.P., Bergauff, M., Hooper, K. and Ward, T.J. (2012a) "Residential indoor PM2.5 in wood stove homes: follow-up of the Libby changeout program", *Indoor Air*, **22**, 492-500.

Noonan, C.W. and Ward, T.J. (2007) "Environmental tobacco smoke, woodstove heating and risk of asthma symptoms", *J Asthma*, **44**, 735-738.

Noonan, C.W. and Ward, T.J. (2012b) "Asthma randomized trial of indoor wood smoke (ARTIS): Rationale and methods", *Contemp Clin Trials*, **33**, 1080-1087.

Noonan, C.W., Ward, T.J., Navidi, W. and Spehhard, L. (2012c) "A rural community intervention targeting biomass combustion sources: effects on air quality and reporting of children's respiratory outcomes", *Occup Environ Med*, **69**, 354-360.

Oeder, S., Jorres, R.A., Weichenmeier, I., Pusch, G., Schober, W., Pfab, F., Behrendt, H., Schierl, R., Kronseder, A., Nowak, D., Dietrich, S., Fernandez-Caldas, E., Lintelmann, J., Zimmermann, R., Lang, R., Mages, J., Fromme, H. and Buters,

J.T. (2012) "Airborne Indoor Particles from Schools are More Toxic than Outdoor Particles", *Am J Respir Cell Mol Biol*, **47**, 575-582.

Park, J.H., Spiegelman, D.L., Gold, D.R., Burge, H.A. and Milton, D.K. (2001) "Predictors of airborne endotoxin in the home", *Environ Health Perspect*, **109**, 859-864.

Salvi, S. and Barnes, P.J. (2010) "Is exposure to biomass smoke the biggest risk factor for COPD globally?", *Chest*, **138**, 3-6.

Schumpert, J.C., Noonan, C.W., Sylvester, J., Vanek, D., Ward, T. and Holian, A. (2006) "Patterns of asthma symptoms and perceptions of harm from seasonal atmospheric events in rural Western Montana", *Int J Occup Environ Health*, **12**, 52-58.

Semple, S., Devakumar, D., Fullerton, D.G., Thorne, P.S., Metwali, N., Costello, A., Gordon, S.B., Manandhar, D.S. and Ayres, J.G. (2010) "Airborne Endotoxin Concentrations in Homes Burning Biomass Fuel", *Environ Health Perspect*, **118**, 988-991.

Semple, S., Garden, C., Coggins, M., Galea, K.S., Whelan, P., Cowie, H., Sanchez-Jimenez, A., Thorne, P.S., Hurley, J.F. and Ayres, J.G. (2011) "Contribution of solid fuel, gas combustion, or tobacco smoke to indoor air pollutant concentrations in Irish and Scottish homes", *Indoor Air*, **22**, 212-223.

Shendell, D.G., Winer, A.M., Weker, R. and Colome, S.D. (2004) "Evidence of inadequate ventilation in portable classrooms: results of a pilot study in Los Angeles County", *Indoor Air*, **14**, 154-158.

Singh, U., Levin, L., Grinshpun, A., Schaffer, C., Adhikari, A. and Reponen, T. (2011) "Influence of home characteristics on airborne and dustborne endotoxin and B-D-glucan", *J. Environ. Monit.*, **13**, 3246-3253.

Sordillo, J.E., Alwis, U.K., Hoffman, E., Gold, D.R. and Milton, D.K. (2011) "Home Characteristics as Predictors of Bacterial and Fungal Microbial Biomarkers in House Dust", *Environ Health Perspect*, **119**.

Spaan, S., Doekes, G., Heederik, D., Thorne, P.S. and Wouters, I.M. (2008) "Effect of extraction and assay media on analysis of airborne endotoxin", *Appl Environ Microbiol*, **74**, 3804-3811.

Spaan, S., Heederik, D.J.J., Thorne, P.S. and Wouters, I.M. (2007) "Optimization of Airborne Endotoxin Exposure Assessment: Effects of Filter Type, Transport Conditions, Extraction Solutions, and Storage of Samples and Extracts", *Appl. Environ. Microbiol.*, **73**, 6134-6143.

Tager, I.B., Lurmann, F.W., Haight, T., Alcorn, S., Penfold, B. and Hammond, S.K. (2010) "Temporal and Spatial Patterns of Ambient Endotoxin Concentrations in Fresno, California", *Environ Health Perspect*, **118**.

Thornburg, J., Rodes, C.E., Lawless, P.A. and Williams, R. (2009) "Spatial and temporal variability of outdoor coarse particulate matter mass concentrations measured with a new coarse particle sampler during the Detroit Exposure and Aerosol Research Study", *Atmospheric Environment*, **43**, 4251-4258.

Thorne, P.S. (2000) "Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation", *Toxicology*, **152**, 13-23.

Thorne, P.S., Cohn, R.D., Mav, D., Arbes, S.J. and Zeldin, D.C. (2009) "Predictors of endotoxin levels in U.S. housing", *Environ Health Perspect*, **117**, 763-771.

Tischer, C.G. and Heinrich, J. (2013) "Exposure assessment of residential mould, fungi and microbial components in relation to children's health: Achievements and challenges", *Int J Hyg Environ Health*, **216**, 109-114.

Tong, H., Cheng, W.Y., Samet, J.M., Gilmour, M.I. and Devlin, R.B. (2010) "Differential Cardiopulmonary Effects of Size-Fractionated Ambient Particulate Matter in Mice", *Cardiovasc Toxicol*, **10**, 259-267.

Ward, T., Boulafentis, J., Simpson, J., Hester, C., Moliga, T., Warden, K. and Noonan, C. (2011) "Lessons learned from a woodstove changeout on the Nez Perce Reservation", *Sci Total Environ*, **409**, 664-670.

Ward, T. and Lange, T. (2010) "The impact of wood smoke on ambient PM2.5 in northern Rocky Mountain valley communities", *Environmental Pollution*, **158**, 723-729.

Ward, T., Palmer, C., Bergauff, M., Hooper, K. and Noonan, C. (2008) "Results of a residential indoor PM2.5 sampling program before and after a woodstove changeout", *Indoor Air*, **18**, 408-415.

Ward, T.J., Rinehart, L.R. and Lange, T. (2006) "The 2003/2004 Libby, Montana PM$_{2.5}$ Source Apportionment Research Study", *Aerosol Science and Technology*, **40**, 166 - 177.

Wegesser, T.C., Franzi, L.M., Mitloehner, F.M., Eiguren-Fernandez, A. and Last, J.A. (2010) "Lung antioxidant and cytokine responses to coarse and fine particulate matter from the great California wildfires of 2008", *Inhal Toxicol*, **22**, 561-570.

Williams, R., Rappold, A.G., Case, M., Schmitt, M., Stone, S., Jones, P., Thornburg, J. and Devlin, R.B. (2012) "Multi-pollutant exposures in an asthmatic cohort", *Atmospheric Environment*, **61**, 244-252.

Williams, R.O.N., Rea, A., Vette, A., Croghan, C., Whitaker, D., Stevens, C., Mcdow, S., Fortmann, R.O.Y., Sheldon, L., Wilson, H., Thornburg, J., Phillips, M., Lawless, P., Rodes, C. and Daughtrey, H. (2009) "The design and field implementation of the Detroit Exposure and Aerosol Research Study", *Journal of Exposure Science & Environmental Epidemiology*, **19**, 643-659.

CHAPTER 3

Efficacy of Filtration Unit Intervention on Reduction of Coarse Fraction and

Airborne Endotoxin Concentrations in Wood Stove Homes

ABSTRACT

Biomass burning has been shown to produce poor indoor air quality (IAQ) in developing countries as well as in rural areas of the United States. Forty-three (43) wood stove homes in western Montana were randomized to receive air filtration units with either an active filter or a placebo alternative. A filter-based sampler was used to evaluate changes in wintertime indoor coarse particulate matter (PMc) and airborne endotoxin (EU/m^3 and EU/mg) pre-and post-intervention. Continuous concentrations of fine particulate matter (PM$_{2.5}$) and particle concentrations in multiple size fractions (0.30-0.49, 0.50-0.99, 1.0-2.49, 2.50-4.99, 5.0-10.0 µm) were also collected. The active filter intervention was associated with a 27.4 (95% CI: -50.0, -4.84) µg/m^3 greater reduction in PM$_{2.5}$ relative to the placebo intervention with a treatment effect of ~90%. These effects were also significant after excluding homes that did not demonstrate at least 50% filter-use compliance. The placebo filters unexpectedly resulted in significantly reduced PMc and airborne endotoxin concentrations. The active filter intervention showed no enhanced ability to reduce PMc and airborne endotoxin relative to the placebo intervention. These findings validate the use of active air filters in reducing PM$_{2.5}$ and may provide a low-cost alternative for reducing PMc and airborne endotoxin in homes burning biomass fuel.

INTRODUCTION

Particulate matter is known to exacerbate asthma symptoms (van Berlo, 2012) with size

fraction playing an important role in the toxicity of the particles (Tong, 2010).

Epidemiological evidence indicates that coarse particulate matter (particles with a

aerodynamic diameter <10 μm and >2.5 μm; PMc) has an acute effect on respiratory

health, particularly among susceptible populations such as asthmatics (Brunekreef,

2005). The coarse fraction has been shown to cause a more significant pulmonary

inflammatory response than the fine fraction ($PM_{2.5}$) in an animal model (Tong, 2010) as

well as increased inflammatory potential in multiple respiratory cell lines (Gualtieri, 2010,

Oeder, 2012). Coarse particles captured during wildfire activity have shown more

proinflammatory and oxidative stress potential on an equal-dose basis than fine particles

(Wegesser, 2010). This inflammatory potential may be linked to the biological

components of PMc, as biological inactivation of the coarse fraction significantly

attenuates inflammatory effects in healthy humans (Alexis, 2006). Bioaerosols such as

airborne endotoxin, a component of the cell wall of Gram-negative bacteria, have long

been known to be asthma triggers (van Berlo, 2012) and in-home levels of bioaerosols

have been associated with asthma symptoms and severity in children (Liu, 2004).

Indoor asthma triggers have been the targets of many intervention studies as

people spend a majority of their time indoors, averaging 86-87% of time for the general

population and 89-90% for children (Klepeis, 2001). The home environment is ideal for

health-based interventions because it can be altered with fewer resources and fewer

barriers than the outdoor environment. A recent review determined the economic value

of home-based multicomponent interventions for reducing asthma morbidity to have a

benefit-cost ratio ranging from 5.3-14.0, implying that for every dollar spent on the

intervention, the monetary value of the resulting benefits, such as averted medical costs,

was $5.30 - $14.00 (Nurmagambetov, 2011). Indoor air filtration interventions aimed at

preventing exposure to asthma exacerbating agents are popular due to demonstrated efficacy as well as their ease of installation and continued use.

Multiple reviews have established the effectiveness of indoor air filtration systems on reducing asthma symptoms (McDonald, 2002), including a review of recent intervention studies which determined air filtration to be responsible for ~7-25% health improvement in adverse allergy and asthma outcomes (Fisk, 2013). These air filters can reduce exposure to asthma triggers such as airborne pet allergen (van der Heide, 1999) and particulate matter. Air filters have been shown to significantly reduce breathing problems, allergy attacks (Johnson, 2009), and asthma symptoms scores (Eick, 2011) in asthmatic children. Air filtration has also been shown to be effective in adults, reducing biomarkers of inflammation in healthy adults (Allen, 2009) and medication requirement in asthmatic adults (McDonald, 2002). Therefore, low-cost air filtration interventions have the potential to greatly improve indoor air quality (IAQ) while reducing health costs for the family of an asthmatic child.

Presented here are IAQ results from a randomized intervention study investigating the efficacy of stand-alone air filtration units on the reduction of several asthma triggers within 43 homes with wood stoves in western Montana. Established asthma triggers PMc and airborne endotoxin were quantified before and after an intervention of an active air filter unit or a unit with a placebo filter. Twenty-one (21) homes received an air filtration unit with a high efficiency rated air filter and 22 homes received an air filtration unit with a low efficiency rated (placebo) air filter. Mass-based concentrations as well as particle counts were analyzed to evaluate PMc in these homes pre- and post-intervention.

METHODS

Description of Parent Study

Asthma Randomized Trial of Indoor Wood Smoke (ARTIS) is an intervention-based study aimed at improving the quality of life of asthmatic children living in wood stove homes by reducing in-home levels of $PM_{2.5}$. The ARTIS study involves four separate 48-hour sampling visits per household over two consecutive winters (a baseline winter and post-intervention winter) (Noonan, 2012b). The use of a non-EPA certified (older model) wood stove as the primary heating source in the home was required for inclusion in the study. After baseline sampling was completed, homes were randomized into two treatment arms: 1) homes receiving an active filtration unit and 2) homes receiving a placebo air filtration unit. In this manuscript, we present pre- and post-intervention IAQ measures from a subset of 43 homes within the ARTIS study. These homes are unique in that they were co-sampled with a filter-based coarse particle environmental monitor (CPEM) designed to collect coarse particulate matter for both baseline and post-intervention winters. At each sampling visit, homeowners reported descriptive characteristics of their residence, including number of full-time residents (adults and children), number of pets (furry animals only, i.e., dogs and cats) and square footage.

Intervention

As previously described (Noonan, 2012b), following the baseline winter all homes received two individual filtration units. One large (20ftX18ft) Filtrete air filtration unit (Ultra Clean Air Purifiers, 3M, St. Paul, MN, USA) was placed in the same room as the wood stove, typically the main living room. Within the child's bedroom, a smaller Filtrete (17ftX10ft) unit was installed. These units have been shown to reduce indoor $PM_{2.5}$ in a wood stove home by 76% (Hart, 2011). Participants were instructed to operate the units on the "high" setting throughout the duration of the winter with filters changed out

monthly. Homes randomly assigned to the placebo group received filters with a Minimum

Efficiency Reporting Value (MERV) of 2 while homes assigned to the active filter group

received filters with a MERV of 13. A higher MERV indicates better performance. To

ensure study participants were blinded to their intervention, the placebo filters were real

filters that only met specifications for removing particles >10 μm with typical application

in residential air conditioners. The active filters met specifications for capturing >90% of

particles 1-3 μm with a dust-spot efficiency of 80-90% with typical application in hospital

laboratories (EPA, 2012).

To assess compliance with continuous usage each Filtrete unit was fitted with a

data logging (voltage measuring) device (kilowatt meter; KWM) which monitored on/off

status and amount of kilowatt-hours used. Laboratory tests of the Filtrete units

determined a predicted kilowatt-hour usage of 3.13 per day for the large units and 1.90

per day for the small units while run continuously on "high". KWM values were recorded

at each sampling visit. Two homes had no associated KWM values. Filter use

compliance was set at three levels: 1) average (large and small unit) >50% predicted

KWM value (n=28), 2) at least one unit >50% predicted KWM value (n=33), 3) field notes

validating at least one KWM was dysfunctional or the home ran both units continuously

on the Medium setting (n=36). Sensitivity analyses were performed at all levels.

Indoor Sampling

Samplers were collocated in each home in the common living area on a table 3-5 feet off

the ground and across the room from the wood stove, if possible. In each sampling

event, $PM_{2.5}$ was continuously measured with a DustTrak (TSI Inc., Shoreview, MN,

USA). The DustTrak is an optical scattering instrument that measures PM in the airflow

by measuring the extent of forward scattering of an infrared diode laser beam. The

device is factory calibrated to the respirable fraction of standard ISO 12103-1, A1 test

dust (formerly Arizona Test Dust). DustTraks were zeroed and the impaction plate was cleaned and greased/oiled as necessary prior to each sampling event. During each sampling event, a DustTrak 8530 recorded 60-second averages of $PM_{2.5}$.

Optical mass measurements (such as those made by the DustTrak) are dependent upon particle size and material properties, therefore custom calibrations are needed to improve the measurement accuracy when evaluating specific sources of combustion. In an effort to accurately present wood smoke-related $PM_{2.5}$ concentrations, all DustTrak $PM_{2.5}$ measurements presented in this manuscript were corrected to an indoor wood smoke-specific correction factor of 1.65 developed by our research group (McNamara, 2011). This correction factor has been used in previous indoor air studies derived from the ARTIS program (McNamara, 2013).

A co-located 3016IAQ particle counter (Lighthouse Worldwide Solutions, Fremont, CA, USA) continuously recorded simultaneous counts of particles in multiple size ranges (0.3-0.49, 0.5-0.99, 1.0-2.49, 2.5-4.9 and 5.0-10.0 µm). Air was sampled at 2.83 LPM and data was recorded on 60-second averages. Particle counts for the fine fraction were calculated to be the sum of the 0.3-0.49, 0.5-0.99, and 1.0-2.49 µm particle counts while the coarse fraction was calculated to be the sum of the 2.5-4.9 and 5.0-10.0 µm particle counts. Continuous sampling of $PM_{2.5}$ as well as particle counts was required to be at least 80% complete to be included in the analyses.

Coarse fraction concentrations commonly are calculated from separate PM_{10} (particles less than 10 µm) and $PM_{2.5}$ measurements. In this study, direct measurements of PMc and $PM_{2.5}$ were collected using a Teflon filter-based CPEM developed by RTI International (Research Triangle Park, NC, USA). As previously described (Williams, 2009, Thornburg, 2009, McNamara, 2013) the CPEM is a series of three separation stages designed to be inserted into the MSP Model 200 PM10 PEM air sampler (MSP Corp., Shoreview, MN, USA). PMc is collected on two sequential 25 mm PTFE filters

with polymethylpentene (PMP) support rings (thickness 3.0 µm; Zefon International, Inc., Ocala, FL, USA) while $PM_{2.5}$ is collected on a final 37 mm PTFE filter with PMP support rings (thickness 2.0 µm; Zefon International, Inc., Ocala, FL, USA). The CPEM utilizes a battery-operated pump to achieve a flow rate of 2 Lpm. Flow rates measured with a Drycal DC-Lite (BIOS International, Butler, NJ, USA) prior to and following each sampling event were averaged to calculate the sample volume. If necessary, pump flow was adjusted at deployment to achieve 2 Lpm. In addition, all data from multiple sampling events per home were averaged to produce one winter average per home.

Gravimetric Analyses

The gravimetric analyses of PMc and $PM_{2.5}$ CPEM filters were conducted according to previously described guidelines (Lawless, 1999) and modified for optimal conditions for teflon filters (Menetrez, 2009). Prior to sampling, each filter was placed into individually labeled sterile polystyrene Analyslide containers (Pall Corp., Ann Arbor, MI, USA) and allowed to equilibrate for 24-48 hours in an environmentally controlled weighing facility at The University of Montana. The facility was maintained at a temperature of 20-23 °C ± 2 °C with relative humidity of 30-40% ± 5%. Humidity was maintained using an 8-gallon Kenmore Whole House humidifier (model 758.15408). Static was controlled using a grounded anti-static floor mat (ComfortKing USA, Inc., Fairfield, NJ, USA) as well as a radioactive neutralizer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Filters were weighed prior to and after each sampling event with a calibrated MT5 microbalance (Mettler-Toledo LLC, Columbus, OH, USA). Multiple laboratory blanks were within the repeatability of the scale (0.8 µg). A minimum of 10% field blanks was included in all analyses.

Endotoxin Extraction and Analyses

Filter extraction methods were optimized for endotoxin analyses using methods adapted

from Thorne *et al.* (Thorne, 2009) and Spaan *et al.* (Spaan, 2007), as previously

described (McNamara, 2013). For PMc the two sequential 25 mm teflon filters were

combined and extracted in 5 mL sterile, pyrogen-free water containing 0.05% Tween-20

for one hour at room temperature with vigorous shaking. The extracts were then

centrifuged at 1,000-x g for 15 minutes at room temperature and supernatants were

transferred into sterile, endotoxin-free conicals (Greiner Bio-One North America Inc.,

Monroe, NC, USA). Extracts were diluted 50-fold with pyrogen-free water prior to

endotoxin analysis to counteract enhanced yields due to Tween interference (Spaan,

2008). Samples were analyzed for endotoxin using a kinetic chromogenic Limulus

amebocyte lysate (LAL) assay (Endosafe Endochrome-K; Charles River Laboratories

Inc., Charleston, SC, USA). Lyophilized standard endotoxin, chromogenic substrate, and

LAL preparations were reconstituted using pyrogen-free water. A 12-point standard

curve was generated, ranging from 0.005 to 50 endotoxin units (EU)/ml. The absorbance

in each well was measured at 405 nm every 30 seconds for 90 minutes (Thorne, 2000).

Samples with nondetectable endotoxin levels were assigned a value of two-thirds the

limit of detection (LOD) of the assay (Spaan, 2008). Final endotoxin results are

presented as both EU/m^3 and EU/mg.

Statistical Analysis

SAS v9.3 (SAS, Cary, NC, USA) was used to perform all statistical analyses. Student t-

tests were used to ensure home characteristics were not significantly different between

the placebo filter group and the active filter group following randomization. To be

included in the analyses the homes had to be sampled with a CPEM at least once for

both baseline and post-intervention winters. To better characterize long-term changes in

IAQ measures, air sampling data from homes that were successfully sampled twice during either winter were averaged to produce one winter-wide average for each IAQ measure per home per winter. Previous analyses indicated that averaging across visits is appropriate for IAQ measures (McNamara, 2013). All analyses presented here were repeated using only first visit values and again using only second visit values to ensure that averaging visits did not skew results or change their interpretation.

The absolute change from pre- to post-intervention for all IAQ measures was calculated as (Post-Intervention value – Pre-intervention value) to indicate a reduction in the pollutant of interest following the intervention. The percent change for all IAQ measures was calculated as ((Post-intervention value – Pre-intervention value)/ Pre-intervention value)*100. Similar to absolute change, a negative percent change value indicates a percent reduction in the pollutant of interest following the intervention. Non-parametric tests were used to evaluate statistical significance. The Wilcoxon Signed Rank test was used to evaluate the mean absolute change in paired data and the One-Sample Wilcoxon Signed Rank test was used to determine if mean percent change was significantly different than zero ($H_0=0$). Treatment effect of the active intervention relative to the placebo intervention was evaluated using linear regression, with results presented as parameter estimate (β–coefficient) with 95% confidence intervals. For continuously collected data ($PM_{2.5}$ and particle counts), sampling was required to be at least 80% complete to be included in the analyses.

RESULTS

In-home Sampling

Overall, 22 placebo homes and 21 active filter homes were sampled with a CPEM in two consecutive winters (November-March). Entry to the study was staggered across two winters. Baseline (pre-intervention) sampling occurred during the winter of 2010-2011 for five homes and during the winter of 2011-2012 for 38 homes. There was no difference in square footage, number of adults, number of kids, total common residents (number of people living full-time in the home), dog ownership, cat ownership, or total number of furry pets between homes assigned to the placebo group and active filter group (Table 3.1). The difference in number of adults and total common residents in placebo compared to active filter homes approached significance but sensitivity analyses revealed these differences did not influence any further results.

Baseline concentrations of all IAQ measures were similar between homes that received the placebo or active filter intervention. Results of Student t-tests revealed there were no significant differences between baseline concentrations of all IAQ measures (data not shown). Particle counter data was not analyzed in seven of the 22 placebo homes due to less than 80% completion of continuous sampling (Table 3.2). Particle counter data was also not analyzed in seven of the 21 active filter homes for the same reason. One active filter home did not achieve at least 80% complete data with the DustTrak for $PM_{2.5}$, therefore the $PM_{2.5}$ data from that home was not included in the analyses (Table 3.3).

Table 3.1. Descriptive characteristics for homes receiving a placebo filter (n=22) and homes receiving an active air filter (n=21).

	Placebo Homes	Active Filter Homes	p-value
Square footage	1843 (1032)	2087 (981)	0.74
Number of adults	1.9 (0.5)	1.7 (0.5)	0.11
Number of kids	2.6 (1.3)	2.0 (0.9)	0.30
Common residents	4.5 (1.6)	3.8 (1.1)	0.15
Dog, % of homes (n)	68 (15)	71 (15)	0.63
Cat, % of homes (n)	59 (13)	48 (10)	0.38
Total pets	1.4 (0.9)	1.4 (0.8)	0.63

Notes: Presented as mean (standard deviation) except for dog and cat, presented as percentage of homes with dog or cat (n).

Efficacy of Filters

Placebo Filters

Placebo filters reduced both measures of airborne endotoxin (EU/m^3 and EU/mg of PMc) as well as concentrations of PMc. The mean absolute change and mean percent change were significant for EU/m^3 (p<0.05) and approached significance for EU/mg (p<0.10) (Table 3.2). There were no significant changes across winters in PM$_{2.5}$ or the particle counts of the fine fraction (0.3-0.49, 0.50-0.99, 1.0-2.49 µm) in placebo homes. The placebo filters were effective in significantly reducing the mean of both individual size fractions constituting the coarse fraction (2.5-4.9 and 5.0-10.0 µm) as well the sum of the particle counts constituting the coarse fraction (Table 3.2).

Table 3.2. Effect of placebo intervention on IAQ measures (n=22). *: p<0.10, **: p<0.05, ***p<0.01.

		Placebo			
	n	Pre-intervention	Post-intervention	Mean Absolute Change	Mean Percent Change
Endotoxin					
EU/m^3	22	10.1 (10.8)	2.05 (2.20)	-8.08 (10.9)***	-153 (774)**
EU/mg	22	1187 (1675)	387 (543)	-801 (1838)*	-242 (690)*
Particle Concentrations (µg/m^3)					
PMc	22	12.8 (7.75)	7.62 (3.77)	-5.17 (7.75)**	-26.0 (52.3)**
PM$_{2.5}$	22	29.5 (24.8)	38.3 (43.6)	8.81 (37.7)	36.4 (102)
Particle Counts§					
0.3-0.49 (x10^7)	15	5.78 (4.34)	6.23 (9.24)	0.45 (7.21)	9.54 (126)
0.50-0.99 (x10^7)	15	0.87 (0.69)	0.90 (1.15)	0.03 (1.20)	27.6 (156)
1.0-2.49 (x10^5)	15	8.00 (7.14)	4.37 (3.14)	-3.63 (8.55)	-7.77 (85.5)
2.5-4.9 (x10^5)	15	3.14 (2.34)	1.37 (0.86)	-1.77 (2.64)**	-32.2 (57.5)
5.0-10.0 (x10^4)	15	5.11 (3.41)	2.27 (1.50)	-2.84 (3.73)**	-17.7 (123)**
Fine (x10^7)	15	6.73 (5.01)	7.17 (10.3)	0.44 (8.38)	10.8 (127)
Coarse (x10^5)	15	3.65 (2.64)	1.59 (0.98)	-2.06 (2.97)**	-31.8 (62.0)

Notes: Presented as mean (standard deviation). EU/m^3 and EU/mg in PMc.
§7 particle counter files less than 80% complete

Active Filters

In the 21 homes that received the active filter, airborne endotoxin and PMc were significantly reduced as they were in the placebo homes. Although the percent change in EU/mg was not significant (18%) the absolute change was significant (-438 EU/mg; $p<0.05$). Additionally the active filters significantly reduced $PM_{2.5}$ as well as each size fraction less than 2.5 microns (Table 3.3). $PM_{2.5}$ was the IAQ measure most effectively reduced with a mean percent reduction of 54.3% ($p<0.01$). Unlike in the placebo filter homes, the largest size fraction constituting PMc (5.0-10.0 microns) was not significantly reduced in the active filter homes (Table 3.3).

Table 3.3. Effect of active filter intervention on IAQ measures (n=21). *: $p<0.10$, **: $p<0.05$, ***$p<0.01$.

		Active Filter			
	n	Pre-intervention	Post-intervention	Mean Absolute Change	Mean Percent Change
Endotoxin					
EU/m^3	21	5.68 (8.96)	1.23 (3.00)	-4.45 (9.58)***	-24.4 (195)***
EU/mg	21	612 (950)	174 (306)	-438 (1036)**	18.0 (208)
Particle Concentrations ($\mu g/m^3$)[§]					
PMc	21	10.1 (4.78)	5.65 (3.88)	-4.45 (5.90)***	-33.4 (60.3)**
$PM_{2.5}$	20	31.0 (41.1)	12.4 (17.7)	-18.6 (34.3)***	-54.3 (44.1)***
Particle Counts[§]					
0.3-0.49 ($\times 10^7$)	13	3.80 (3.86)	1.68 (2.55)	-2.12 (2.21)***	-55.1 (39.2)***
0.50-0.99 ($\times 10^7$)	13	0.44 (0.33)	0.27 (0.42)	-0.17 (0.31)	-42.3 (54.5)*
1.0-2.49 ($\times 10^5$)	13	5.02 (2.74)	3.60 (5.44)	-1.41 (4.61)	-32.8 (56.7)*
2.5-4.9 ($\times 10^5$)	13	2.21 (1.09)	1.31 (1.45)	-0.90 (1.22)	-32.8 (66.3)*
5.0-10.0 ($\times 10^4$)	13	3.28 (1.22)	2.39 (2.50)	-0.89 (2.44)	-14.7 (191)
Fine ($\times 10^7$)	13	4.29 (4.18)	1.98 (3.02)	-2.31 (2.37)***	-53.9 (40.3)***
Coarse ($\times 10^5$)	13	3.65 (2.64)	1.59 (0.98)	-0.99 (1.44)**	-28.5 (75.4)**

Notes: Presented as mean (standard deviation). EU/m^3 and EU/mg in PMc.
[§]One DustTrak ($PM_{2.5}$) and 7 particle counter files less than 80% complete

Treatment Effect

Relative to the placebo intervention, the active filter intervention is associated with a 27.4 µg/m^3 greater reduction in $PM_{2.5}$ ($p<0.05$). This corresponds to an approximately 91% greater reduction in $PM_{2.5}$ in active filter homes relative to placebo homes ($p<0.01$) (Table 3.4). The active filters also reduced the particle count of the fine fraction by approximately 65% relative to the placebo homes ($p<0.10$). No other IAQ measures had a significant treatment effect with the active filter relative to the placebo filter (Table 3.4). Investigation of the residuals for absolute change and percent change revealed heteroscedasticity, but the use of a Wilcoxon two-sample test showed findings similar to the linear regression results (data not shown).

Table 3.4. Treatment effect of active filter intervention (n=21) relative to placebo intervention (n=22) on absolute change and percent change of IAQ measures. Abbreviations: CI: Confidence Intervals, *: p<0.10, **: p<0.05, ***p<0.01.

	Absolute Change β (95% CI)	Percent Change β (95% CI)
Endotoxin		
EU/m^3	3.63 (-2.71, 9.96)	-177 (-529, 174)
EU/mg	362 (-563, 1287)	-234 (541, 93.1)
Particle Concentrations		
PMc	0.73 (-3.53, 4.98)	-7.43 (-42.15, 27.30)
PM$_{2.5}$	-27.4 (-50.0, -4.84)**	-90.7 (-141, -40.8)***
Particle Counts		
0.3-0.49 (x10^7)	-2.57 (-6.95, 1.71)	-64.6 (-139, 10.2)*
0.50-0.99 (x10^7)	-0.19 (-0.90, 0.51)	-69.9 (-164, 23.9)
1.0-2.49 (x10^5)	2.22 (-3.25, 7.68)	-25.0 (-85.0, 34.9)
2.5-4.9 (x10^5)	0.87 (-0.77, 2.51)	-0.62 (-48.7, 47.4)
5.0-10.0 (x10^4)	1.95 (-0.54, 4.45)	32.4 (-90.7, 155)
Fine (x10^7)	-2.75 (-7.70, 2.21)	-64.7 (-140, 10.9)*
Coarse (x10^5)	1.06 (-7.07, 2.92)	3.29 (-50.1, 56.7)

Notes: Presented as linear regression β–coefficient and 95% confidence intervals. EU/m^3 and EU/mg in PMc. PMc and PM$_{2.5}$ reported in μg/m^3.

Sensitivity Analyses

The treatment effect was evaluated while adjusting for each characteristic in Table 3.1. We found no change in treatment effect for any home characteristic (data not shown). There were no differences in level of filter compliance between placebo and active filter homes. Sensitivity analyses were performed at each level of filter use compliance. Findings were consistent with results from our primary analyses. Including only homes with at least 50% average compliance for both filter units (Level 1, n=28) the active filter intervention is associated with a 19.1 µg/m^3 greater reduction in $PM_{2.5}$ relative to the placebo intervention (p<0.05). This corresponds to an 87% greater reduction in $PM_{2.5}$ in active filter homes relative to placebo homes (p<0.01) (data not shown).

DISCUSSION

The active filters used in this study were extremely efficient in reducing in-home concentrations of $PM_{2.5}$ relative to the placebo filters. The active filters produced no additional reduction in PMc or airborne endotoxin relative to the placebo filters. This is due to the placebo filters performing surprisingly well in capturing larger particles as well as reducing airborne endotoxin concentrations. Both measures of endotoxin within the coarse fraction were reduced in absolute concentration (EU/m^3 $p<0.01$, EU/mg $p<0.10$) and percent change (EU/m^3 $p<0.05$, EU/mg $p<0.10$) following the implementation of the placebo filter unit (Table 3.2). PMc collected with the CPEM, as well as characterized by particle counts (2.5-4.9 and 5.0-10.0 µm fractions), was also significantly reduced following the placebo intervention (mean absolute change $p<0.05$). This unexpected finding is likely due to the nature of the placebo filter. To ensure the study participants were blinded to their treatment arm, a realistic-looking yet sub-optimal filter material was used in place of the active filters in the placebo homes. The specifications of the placebo fiberglass filter product include that it filters particle >10 µm but does not have the capacity to remove bacteria, mold, smoke, or odor from the air (EPA, 2012). As intended, the placebo filters did not affect $PM_{2.5}$ concentrations in the placebo homes.

The placebo filter's ability to significantly reduce PMc and airborne endotoxin is an important finding in several ways. Principally, the health effects of $PM_{2.5}$ reduction will be easier to elucidate in the ARTIS program as $PM_{2.5}$ is reduced in the active filter homes but not in the placebo homes. On a larger scale this finding demonstrates that known respiratory irritants (including PMc and airborne endotoxin) can be efficiently reduced in wood stove homes without the use of an expensive, high-efficiency filter unit. Homes burning other types of biomass fuel, such as dung and charcoal, have airborne endotoxin concentrations orders of magnitude higher than have been found in developed countries (Semple, 2010). Reducing this exposure in a cost-effective manner could

impact the global disease burden of household biomass smoke exposure, which was recognized as one of the top 10 major preventable risk factors for lost healthy years in 2000 (Naeher, 2007).

We are not aware of any previous studies that have investigated the effect of air filtration on in-home levels of airborne endotoxin. Dampness/moisture sources have been identified as predictors of in-home endotoxin (Sordillo, 2011, Park, 2001) and a moisture-damage intervention study effectively reduced airborne microbes in a school environment (Roponen, 2013). Intensive repair of the moisture damage resulted in a reduced capacity of indoor particulate matter to induce inflammatory responses *in vitro* (Roponen, 2013). The use of a cost-effective and convenient intervention (such as the use of the placebo filter material) to reduce airborne endotoxin in wood stove homes could have a lasting impact for asthmatic children and adults living in these homes.

The majority of global biomass-smoke mediated interventions take place in developing countries where open burning is common (Barnes 1994, McCracken 2007, Thompson 2011). However, wood stove biomass combustion is a common source of residential heating in the United States and has been identified as a major source of ambient $PM_{2.5}$ in the Northern Rocky Mountains of western Montana (Ward, 2010, Ward, 2006, Schumpert, 2006, Noonan, 2007). Our research group has previously attempted to reduce indoor $PM_{2.5}$ concentrations in wood stove homes by replacing older model wood stoves with cleaner burning EPA-certified wood stoves (Ward, 2008, Noonan, 2012a). Crude overall indoor $PM_{2.5}$ concentrations were reduced by 56% following the wood stove changeouts but there was substantial variability in efficacy. For example, five of the 21 homes investigated in the Noonan *et al.* study demonstrated no average reduction in indoor $PM_{2.5}$, and seven homes had at least one post-changeout sampling with higher indoor $PM_{2.5}$ than the concentrations observed during pre-changeout sampling (Noonan, 2012a). The lack of consistent indoor air quality improvements

following wood stove changeouts supports the use of an air filtration intervention to reduce in-home particulate levels in wood stove homes.

In non-wood stove homes, several air filtration studies have been performed with the goal of reducing in-home exposure to particulate matter, primarily from cigarette smoke. Both fine and coarse particulate matter were significantly reduced in a randomized trial investigating the use of air cleaners and health coaches in reducing secondhand smoke exposure in inner-city homes of asthmatic children (Butz, 2011). The health coaches provided no additional reduction in PM concentrations. This study had similar baseline concentrations of PMc and $PM_{2.5}$ compared to our study (~19 and ~40 $\mu g/m^3$, respectively, versus 12.8 and 29.5 $\mu g/m^3$, respectively, in our placebo homes and 10.1 and 31.0 $\mu g/m^3$, respectively, in our active filter homes), despite our study including only non-smoking homes. Another study involving a stand-alone air filter intervention found that filter use was variable across the study population and declined over the study duration, emphasizing the use of a mechanism to track actual filter usage (Batterman, 2012). We found 28 homes had at least 50% average compliance with no differences between placebo compliance and active filter compliance.

Sensitivity analyses at three levels of filter use compliance revealed that 50% average compliance was sufficient to reduce $PM_{2.5}$ by approximately 90% in active filter homes relative to placebo homes. The significant reduction of airborne endotoxin and PMc by the placebo filters was seen in homes with greater than 50% filter-use compliance. The significant treatment effect of the active filter intervention on $PM_{2.5}$ reduction relative to the placebo filter intervention was also seen in homes with greater than 50% compliance. This finding can have a lasting impact, as participants in the study commonly acknowledged the electricity expense of continuous filter use. These findings confirm that PMc and airborne endotoxin is significantly reduced with the placebo filter

material when run at least 50% of the time and that $PM_{2.5}$ is significantly reduced by the active filter relative to the placebo filter when run at least 50% of the time.

As anticipated following randomization, several home characteristics that have been identified as predictors of endotoxin were not significantly different between treatment groups (Table 3.1). These characteristics include number of people living in the home (Thorne, 2009, Singh, 2011) and presence of pets (Thorne, 2009, Park, 2001, Sordillo, 2011). Differences in number of adults (p=0.11) and total number of full-time residents (p=0.15) approached significance between placebo and filter treatment randomized groups. To ensure these potential predictors of in-home endotoxin concentrations did not influence the results presented here, the treatment effect was evaluated while adjusting for each characteristic in Table 3.1. We found no change in treatment effect (data not shown). This is in agreement with a previous study that found no relationship between number of residents, number of pets, and square footage with airborne endotoxin content in wood stove homes (McNamara, 2011).

There are several limitations of this study. First and foremost we have investigated the efficacy of an air filtration intervention in only wood stove homes during the winter months. While these homes make excellent candidates for PM reduction due to elevated baseline levels of PM (Noonan, 2012a) it will be important to validate our findings in non-wood stove homes and across seasons. A limitation that makes it difficult to compare our airborne endotoxin results to other studies is the sample media used. Previous studies typically use dust endotoxin as a surrogate of the airborne endotoxin directly evaluated in our study (Singh, 2011, Thorne, 2009). Airborne endotoxin measured over 1.5 days has been determined to be a more direct measure of exposure than the use of dust endotoxin (Horick, 2006). This is primarily due to dust endotoxin typically correlating poorly with airborne endotoxin levels (Park, 2001, Mazique, 2011). Finally, we did not achieve complete compliance with filter usage. Although all primary

analyses are done based on intention to treat, all results were consistent after excluding

homes that did not demonstrate at least 50% filter-use compliance.

CONCLUSION

For the first time, we investigated the efficacy of an active air filtration unit in reducing PMc and airborne endotoxin in wood stove homes with a placebo controlled randomized trial. Forty-three (43) homes with wood stoves were sampled with a CPEM during two consecutive winters in western Montana. The active filter intervention was associated with a 27.4 (95% CI: -50.0, -4.84) μg/m^3 greater reduction in $PM_{2.5}$ (p<0.05) relative to the placebo intervention, a treatment effect of ~90%. The placebo filters, intended to blind the participants to their treatment group, significantly reduced PMc as well as airborne endotoxin in the wood stove homes. Sensitivity analyses revealed that 50% filter use compliance is sufficient to observe these significant reductions. The results of this study are limited to a specific subset of homes (wood stove homes in winter months) but can potentially offer a low-cost option for reducing in-home PMc and endotoxin where biomass fuel is burned.

REFERENCES

Alexis, N.E., Lay, J.C., Zeman, K., Bennett, W.E., Peden, D.B., Soukup, J.M., Devlin, R.B. and Becker, S. (2006) "Biological material on inhaled coarse fraction particulate matter activates airway phagocytes in vivo in healthy volunteers", *Journal of Allergy and Clinical Immunology*, 117, 1396-1403.

Allen, R.W., Carlsten, C., Karlen, B., Leckie, S., Van Eeden, S., Vedal, S., Wong, I. and Brauer, M. (2009) "An air filter intervention study of endothelial function among healthy adults in a woodsmoke-impacted community", *Am J Respir Crit Care Med*, 183, 1222-1230.

Batterman, S., Du, L., Mentz, G., Mukherjee, B., Parker, E., Godwin, C., Chin, J.Y., O'toole, A., Robins, T., Rowe, Z. and Lewis, T. (2012) "Particulate matter concentrations in residences: an intervention study evaluating stand-alone filters and air conditioners", *Indoor Air*, 22, 235-252.

Brunekreef, B. and Forsberg, B. (2005) "Epidemiological evidence of effects of coarse airborne particles on health", *Eur Respir J*, 26, 309-318.

Butz, A.M., Matsui, E.C., Breysse, P., Curtin-Brosnan, J., Eggleston, P., Diette, G., Williams, D., Yuan, J., Bernert, J.T. and Rand, C. (2011) "A randomized trial of air cleaners and a health coach to improve indoor air quality for inner-city children with asthma and secondhand smoke exposure", *Arch Pediatr Adolesc Med*, 165, 741-748.

Eick, S.A. and Richardson, G. (2011) "Investigation of different approaches to reduce allergens in asthmatic children's homes--the Breath of Fresh Air Project, Cornwall, United Kingdom", *Sci Total Environ*, 409, 3628-3633.

Epa, U. (2012) Residential Air Cleaners (Second Edition): A Summary of Available Information, Vol. 2013, US EPA.

Fisk, W. (2013) "Health benefits of particle filtration", *Indoor Air*, ePub ahead of print.

Gualtieri, M., Ovrevik, J., Holme, J.A., Perrone, M.G., Bolzacchini, E., Schwarze, P.E. and Camatini, M. (2010) "Differences in cytotoxicity versus pro-inflammatory potency of different PM fractions in human epithelial lung cells", *Toxicology in Vitro*, 24, 29-39.

Hart, J.F., Ward, T.J., Spear, T.M., Rossi, R.J., Holland, N.N. and Loushin, B.G. (2011) "Evaluating the effectiveness of a commercial portable air purifier in homes with wood burning stoves: a preliminary study", *J Environ Public Health*, 2011, 27.

Horick, N., Weller, E., Milton, D.K., Gold, D.R., Li, R. and Spiegelman, D. (2006) "Home endotoxin exposure and wheeze in infants: correction for bias due to exposure measurement error", *Environ Health Perspect*, 114, 135-140.

Johnson, L., Ciaccio, C., Barnes, C.S., Kennedy, K., Forrest, E., Gard, L.C., Pacheco, F., Dowling, P. and Portnoy, J.M. (2009) "Low-cost interventions improve indoor air quality and children's health", *Allergy Asthma Proc*, 30, 377-385.

Klepeis, N.E., Nelson, W.C., Ott, W.R., Robinson, J.P., Tsang, A.M., Switzer, P., Behar, J.V., Hern, S.C., Engelmann, W.H. (2001) "The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants", *J Expo Anal Environ Epidemiol*, 11, 231-252.

Lawless, P.A. and Rodes, C.E. (1999) "Maximizing data quality in the gravimetric analysis of personal exposure sample filters", *J Air Waste Manag Assoc*, 49, 1039-1049.

Liu, A.H. (2004) "Something old, something new: Indoor endotoxin, allergens and asthma", *Paediatric Respiratory Reviews*, 5, S65-S71.

Mazique, D., Diette, G.B., Breysse, P.N., Matsui, E.C., Mccormack, M.C., Curtin-Brosnan, J., Williams, D.L., Peng, R.D. and Hansel, N.N. (2011) "Predictors of airborne endotoxin concentrations in inner city homes", *Environ Res*, 111, 614-617.

McDonald, E., Cook, D., Newman, T., Griffith, L., Cox, G. and Guyatt, G. (2002) "Effect of air filtration systems on asthma: a systematic review of randomized trials", *Chest*, 122, 1535-1542.

McNamara, M., Thornburg, J., Semmens, E., Ward, T. and Noonan, C. (2013) "Coarse particulate matter and airborne endotoxin within wood stove homes", *Indoor Air*, 2013.

McNamara, M.L., Noonan, C.W. and Ward, T.J. (2011) "Correction factor for continuous monitoring of wood smoke fine particulate matter", *Aerosol and Air Quality Research*, 11, 315-322.

Menetrez, M.Y., Foarde, K.K., Esch, R.K., Schwartz, T.D., Dean, T.R., Hays, M.D., Cho, S.H., Betancourt, D.A. and Moore, S.A. (2009) "An evaluation of indoor and outdoor biological particulate matter", *Atmospheric Environment*, 43, 5476-5483.

Naeher, L.P., Brauer, M., Lipsett, M., Zelikoff, J.T., Simpson, C.D., Koenig, J.Q. and Smith, K.R. (2007) "Woodsmoke health effects: a review", *Inhal Toxicol*, 19, 67-106.

Noonan, C.W., Navidi, W., Sheppard, L., Palmer, C.P., Bergauff, M., Hooper, K. and Ward, T.J. (2012a) "Residential indoor PM2.5 in wood stove homes: follow-up of the Libby changeout program", *Indoor Air*, 22, 492-500.

Noonan, C.W. and Ward, T.J. (2007) "Environmental tobacco smoke, woodstove heating and risk of asthma symptoms", *J Asthma*, 44, 735-738.

Noonan, C.W. and Ward, T.J. (2012b) "Asthma randomized trial of indoor wood smoke (ARTIS): Rationale and methods", *Contemp Clin Trials*, 33, 1080-1087.

Nurmagambetov, T.A., Barnett, S.B., Jacob, V., Chattopadhyay, S.K., Hopkins, D.P., Crocker, D.D., Dumitru, G.G. and Kinyota, S. (2011) "Economic value of home-based, multi-trigger, multicomponent interventions with an environmental focus for reducing asthma morbidity a community guide systematic review", *Am J Prev Med*, 41, 011.

Oeder, S., Jorres, R.A., Weichenmeier, I., Pusch, G., Schober, W., Pfab, F., Behrendt, H., Schierl, R., Kronseder, A., Nowak, D., Dietrich, S., Fernandez-Caldas, E., Lintelmann, J., Zimmermann, R., Lang, R., Mages, J., Fromme, H. and Buters, J.T. (2012) "Airborne Indoor Particles from Schools are More Toxic than Outdoor Particles", *Am J Respir Cell Mol Biol*, 47, 575-582.

Park, J.H., Spiegelman, D.L., Gold, D.R., Burge, H.A. and Milton, D.K. (2001) "Predictors of airborne endotoxin in the home", *Environ Health Perspect*, 109, 859-864.

Roponen, M., Meklin, T., Rintala, H., Hyvarinen, A. and Hirvonen, M.R. (2013) "Effect of moisture-damage intervention on the immunotoxic potential and microbial content of airborne particles and on occupants' upper airway inflammatory responses", *Indoor Air*.

Schumpert, J.C., Noonan, C.W., Sylvester, J., Vanek, D., Ward, T. and Holian, A. (2006) "Patterns of asthma symptoms and perceptions of harm from seasonal atmospheric events in rural Western Montana", *Int J Occup Environ Health*, 12, 52-58.

Semple, S., Devakumar, D., Fullerton, D.G., Thorne, P.S., Metwali, N., Costello, A., Gordon, S.B., Manandhar, D.S. and Ayres, J.G. (2010) "Airborne Endotoxin Concentrations in Homes Burning Biomass Fuel", *Environ Health Perspect*, 118, 988-991.

Singh, U., Levin, L., Grinshpun, A., Schaffer, C., Adhikari, A. and Reponen, T. (2011) "Influence of home characteristics on airborne and dustborne endotoxin and B-D-glucan", *J. Environ. Monit.*, 13, 3246-3253.

Sordillo, J.E., Alwis, U.K., Hoffman, E., Gold, D.R. and Milton, D.K. (2011) "Home Characteristics as Predictors of Bacterial and Fungal Microbial Biomarkers in House Dust", *Environ Health Perspect*, 119, 189-195.

Spaan, S., Doekes, G., Heederik, D., Thorne, P.S. and Wouters, I.M. (2008) "Effect of extraction and assay media on analysis of airborne endotoxin", *Appl Environ Microbiol*, 74, 3804-3811.

Spaan, S., Heederik, D.J.J., Thorne, P.S. and Wouters, I.M. (2007) "Optimization of Airborne Endotoxin Exposure Assessment: Effects of Filter Type, Transport Conditions, Extraction Solutions, and Storage of Samples and Extracts", *Appl. Environ. Microbiol.*, 73, 6134-6143.

Thornburg, J., Rodes, C.E., Lawless, P.A. and Williams, R. (2009) "Spatial and temporal variability of outdoor coarse particulate matter mass concentrations measured with a new coarse particle sampler during the Detroit Exposure and Aerosol Research Study", *Atmospheric Environment*, 43, 4251-4258.

Thorne, P.S. (2000) "Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation", *Toxicology*, 152, 13-23.

Thorne, P.S., Cohn, R.D., Mav, D., Arbes, S.J. and Zeldin, D.C. (2009) "Predictors of endotoxin levels in U.S. housing", *Environ Health Perspect*, 117, 763-771.

Tong, H., Cheng, W.Y., Samet, J.M., Gilmour, M.I. and Devlin, R.B. (2010) "Differential Cardiopulmonary Effects of Size-Fractionated Ambient Particulate Matter in Mice", *Cardiovasc Toxicol*, 10, 259-267.

Van Berlo, D., Hullmann, M. and Schins, R.P. (2012) "Toxicology of ambient particulate matter", *Exs*, 101, 165-217.

Van Der Heide, S., Van Aalderen, W.M., Kauffman, H.F., Dubois, A.E. and De Monchy, J.G. (1999) "Clinical effects of air cleaners in homes of asthmatic children sensitized to pet allergens", *J Allergy Clin Immunol*, 104, 447-451.

Ward, T. and Lange, T. (2010) "The impact of wood smoke on ambient PM2.5 in northern Rocky Mountain valley communities", *Environmental Pollution*, 158, 723-729.

Ward, T., Palmer, C., Bergauff, M., Hooper, K. and Noonan, C. (2008) "Results of a residential indoor PM2.5 sampling program before and after a woodstove changeout", *Indoor Air*, 18, 408-415.

Ward, T.J., Rinehart, L.R. and Lange, T. (2006) "The 2003/2004 Libby, Montana PM$_{2.5}$ Source Apportionment Research Study", *Aerosol Science and Technology*, 40, 166 - 177.

Wegesser, T.C., Franzi, L.M., Mitloehner, F.M., Eiguren-Fernandez, A. and Last, J.A. (2010) "Lung antioxidant and cytokine responses to coarse and fine particulate matter from the great California wildfires of 2008", *Inhal Toxicol*, 22, 561-570.

Williams, R.O.N., Rea, A., Vette, A., Croghan, C., Whitaker, D., Stevens, C., Mcdow, S., Fortmann, R.O.Y., Sheldon, L., Wilson, H., Thornburg, J., Phillips, M., Lawless, P., Rodes, C. and Daughtrey, H. (2009) "The design and field implementation of the Detroit Exposure and Aerosol Research Study", *Journal of Exposure Science & Environmental Epidemiology*, 19, 643-659.

CHAPTER 4

Health Effects of Coarse Fraction and Endotoxin Exposure in Asthmatic Children

Living in Wood Stove Homes

ABSTRACT

Prevalence of childhood asthma is increasing in industrialized nations and many efforts have been made to characterize and reduce exposures that exacerbate asthma symptoms. Asthmatic children living in wood stoves homes face unique challenges such as elevated levels of fine particulate matter ($PM_{2.5}$). However, the health effects of other in-home asthma triggers, such as coarse particulate matter (PMc) and airborne endotoxin, have not been characterized in these homes. We performed indoor air sampling over two 48-hour periods during the winter months in 38 wood stove homes of asthmatic children in western Montana. Pediatric Asthma Quality of Life Questionnaires (PAQLQs) were administered and lung function measurements (PEF and FEV_1) and exhaled breath condensate (EBC) samples were collected to fully characterize the overall health of the subjects. EBC was analyzed for 8-isoprostane, a marker of oxidative stress, as well as a multiplex immunoassay for pro-inflammatory (Granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL-)1β, IL-6, IL-8, tumor necrosis factor (TNF)-α) and T-helper Type 1 and 2 (Th1 and Th2, respectively) cytokines (IL-2, interferon (IFN)-γ, IL-4, IL-5, IL-10). We found cross sectional relationships between increased PMc concentrations and increased frequency of PEF daily variability. Increases in airborne endotoxin was associated with adverse impacts on lung function (reduced percent-predicted PEF) when expressed per mg of PMc (EU/mg). Subjects living in homes with higher levels of airborne endotoxin (EU/m^3) had an increased odds ratio of having above median Th1 cytokines, pro-inflammatory IL-8, and 8-isoprostane, with no relationship to Th2 cytokines. Higher concentrations of PMc were associated with reduced odds of having above-median pro-inflammatory cytokines as well as the asthma-associated IL-4. As a whole we found a more general pro-inflammatory and oxidative stress response to increased endotoxin exposure than the phenotypic allergic asthma response described in the literature. These results provide a

unique insight into the differential health effects associated with PMc and airborne

endotoxin concentrations in wood stove homes and provide evidence that negative

health effects of PMc may not be solely due to its endotoxin content.

INTRODUCTION

Childhood asthma is a chronic illness. The estimated number of U.S. children diagnosed with asthma was 10 million in 2011, with 1.8 million emergency room visits listing asthma as the primary diagnosis (Bloom, 2012). Health care for children with asthma consumes >$2.0 billion annually in the U.S. alone (Landrigan, 2002). Prevalence of childhood asthma has drastically increased in the past several decades, especially in industrialized nations (Kay, 2001). Characterizing exposures and relevant health effects to asthma exacerbating agents is crucial to reduce this health care burden.

Epidemiological evidence indicates that coarse particulate matter (particles with an aerodynamic diameter <10 μm and >2.5 μm; PMc) has an acute effect on respiratory health, particularly among susceptible populations such as asthmatics (Brunekreef, 2005). As most people spend the majority of their time indoors (Klepeis, 2001), indoor exposure to PMc may exacerbate asthma symptoms and affect the asthmatic's quality of life. The mechanism of toxicity of the coarse fraction is thought to be derived from its biological content (Miyata, 2011). Endotoxin, a component of the cell wall of Gram-negative bacteria, is found in the coarse fraction at concentrations 10-fold higher than in fine particulate matter ($PM_{2.5}$) (Heinrich, 2003). In-home levels of endotoxin have been shown to be positively associated with the clinical severity of asthma (Michel, 1996). Therefore, the endotoxin present in the coarse fraction has the potential to exacerbate asthma for children living in wood stove homes.

Although multiple biomarkers of asthma severity have been identified, it is important to choose an appropriate sample matrix based on a study's population of interest. Exhaled breath condensate (EBC) is a simple, non-invasive technique that samples the lung environment with a minimum of inconvenience for the subject (Rosias, 2012). This makes EBC an excellent candidate for use in pediatric respiratory health studies. A recent systematic review of the use of exhaled breath condensate in pediatric

asthma concluded that EBC is a matrix in which there are biologically plausible biomarkers indicative of asthma severity (Thomas, 2013). EBC studies have identified increased markers of oxidative stress, such as 8-isoprostane, in asthmatics versus non-asthmatics (Baraldi, 2003, Montuschi, 1999). EBC has also been used to evaluate the Th1/Th2 immune balance in asthmatics through the analysis of cytokines, primarily IL-10 and the IL-4/IFN-g ratio (Shahid, 2002). While biomarkers can elucidate the physiological health of an asthmatic, the Pediatric Asthma Quality of Life Questionnaire (PAQLQ) developed and validated by Juniper *et al.* (Juniper, 1996) reveals areas that are important to children with asthma, including physical and emotional function. In combination, assessing biomarkers and scores and subscores from the PAQLQ give an in-depth look into the physiological and emotional health of an asthmatic child.

In this manuscript we evaluate the health effects of asthmatic children living in wood stove homes exposed to coarse fraction and airborne endotoxin. Overall health is evaluated through PAQLQ scores and subscores while biomarkers in EBC (8-isoprostane and a cytokine panel) offer insight into the lung environment of these asthmatic children. Lung function is evaluated through twice-daily self-administered peak expiratory flow (PEF) and forced expiratory volume in one second (FEV_1) measurements. These results will aide in the better understanding of the impact of indoor coarse particulate matter and airborne endotoxin on the respiratory health of asthmatic children.

METHODS

Data Collection

Description of Parent Study

Asthma Randomized Trial of Indoor Wood Smoke (ARTIS) is an intervention-based study aimed at improving the quality of life of asthmatic children living in wood stove homes by reducing in-home $PM_{2.5}$ exposures. The ARTIS study involves four separate 48-hour sampling visits per household over two consecutive winters (a baseline winter and post-intervention winter) (Noonan, 2012). The use of a non-EPA certified (older model) wood stove as the primary heating source in the home was required for inclusion in the study. At each sampling visit, homeowners reported descriptive characteristics of their residence, including number of full-time residents (adults and children), number of pets (furry animals only, i.e. dogs and cats) and square footage (Noonan, 2012, McNamara, 2013). This manuscript presents baseline (i.e., pre-intervention) indoor air quality (IAQ) and health outcome data collected during the wintertime (November-March) for 38 asthmatic children living in wood stove homes within a 200-mile radius of Missoula, Montana.

Indoor Air Sampling

The indoor air sampling regimen is described elsewhere in detail (McNamara, 2013). Briefly, $PM_{2.5}$ was continuously recorded at 60-second intervals for 48-hours with a DustTrak (TSI Inc., Shoreview, MN, USA). Final 48-hour averages were corrected to an indoor wood smoke-specific correction factor of 1.65 developed by our research group (McNamara, 2011). PMc was collected on Teflon filters using a coarse particulate environmental monitor (CPEM) (Williams, 2009, Thornburg, 2009, Williams, 2012, McNamara, 2013). Endotoxin was extracted and analyzed using a kinetic chromogenic

LAL assay (Charles River, Charleston, SC, USA) as previously described (McNamara, 2013). A minimum of 10% of field blanks was included in all analyses.

Health Measures Collection

Quality of Life

The Pediatric Asthma Quality of Life Questionnaire (PAQLQ) was administered to each subject at the start of each sampling visit. This 23-item validated instrument gives domain scores for symptoms (10 items), emotions (8 items), and activity limitations (5 items). The total PAQLQ score is calculated as the mean score across the three domains. The questionnaire covers a one-week recall period. Subjects use a 1-7 scale to answer each item, with 1 corresponding to most severe and 7 corresponding to no symptoms.

Lung Function Measurements

A PiKo-1 peak flow meter (nSprire, Longmont, CO, USA) was used to collect peak flow (PEF) and forced expiratory volume in the first second (FEV_1) data from the subjects. Prior to sampling, a field worker instructed subjects on proper peak flow meter use. Subjects were instructed to record three PEF and FEV_1 values each morning and night over a two week period. Data was calculated to percent-predicted PEF and percent-predicted FEV_1 based on the height, weight, and age of the subject at the time of sampling as previously described (Hankinson, 1999). All percent predicted PEF and FEV_1 values were averaged within visits to produce one value per subject per visit. Values recorded in the morning were also averaged separately to give one morning-specific average per subject per visit. The frequency of days with evening to morning PEF variability greater than 20% is presented as the number of these days per days of PEF data collected (Morgan, 2004).

Biosample Collection

Both EBC and urine samples were collected from study participants. A field worker instructed subjects on EBC sample collection technique. Subjects used an Rtube (Respiratory Research, Austin, TX, USA) for 10 minutes for two consecutive mornings per visit concurrent with indoor air sampling. Samples were kept in a freezer and transported back to the laboratory on ice. Samples were thawed for aliquotting and kept at -80°C prior to analysis.

Each subject collected their first urinary void on concurrent mornings with EBC collection and indoor air sampling. Samples were kept in the refrigerator and transported back to the laboratory on ice. Samples were aliquotted and kept at -80°C prior to analysis.

Biosample Analysis

One EBC and urine sample per subject per visit was analyzed for the endpoints of interest. When available the second morning's EBC and urine was used to best represent effects of exposure characterized by the sampling equipment. EBC samples were bubbled with ultra-pure argon gas for 3 minutes to achieve maximum deaeration (Accordino, 2008). PH was measured using a Jenco Model 60 digital pH meter with a microelectrode (Jenco Electronics, LTD, San Diego, CA, USA) to ensure samples were within physiological range. 8-isoprostane was measured following manufacturer's instructions using an EIA kit (Cayman Chemical, Ann Arbor, MI, USA). An ultrasensitive human cytokine 10-plex panel (Life Technologies, Grand Island, NY, USA) was used to simultaneously quantify pro-inflammatory cytokines (GM-CSF, IL-1β, IL-6, IL-8, TNF-α), Th1 cytokines (IL-2, IFN-γ) and Th2 cytokines (IL-4, IL-5, IL-10) in the EBC. The panel was run on a Luminex LX100 using STarStation software (Applied Cytometry Systems,

Sheffield, UK). Urine was analyzed for cotinine and creatinine concentrations using ELISA kits following manufacturer's instructions (Calbiotech, Spring Valley, CA, USA and Cayman Chemical, Ann Arbor, MI, USA, respectively).

Data Analysis

Subjects and their paired IAQ measures were included in analyses if IAQ data and health data were collected for both sampling visits (n=38). $PM_{2.5}$ continuous data were required to be >80% complete (at least 2,304 60-second averages during 48 hour period). IAQ and associated health data from 19 females (12.6 ± 2.5 years old) and 19 males (ages 12.3 ± 2.7 years old) were eligible for inclusion in analysis. If the mass deposited on the CPEM filters during sampling was within the sensitivity of the scale the home was assigned a PMc concentration half the detectable value (0.24 µg/m^3) (McNamara, 2013). Homes with samples below the limit of detection (LOD) for endotoxin (0.005 EU/ml extract) were assigned half of the detectable concentrations of the extract (0.002 EU/m^3 and 0.16 EU/mg PMc) (Thorne, 2000). EBC samples below the LOD for 8-isoprostane (0.8 pg/mL) were assigned a value of half the LOD (Piotrowski, 2012). Due to the ultrasensitive nature of the cytokine panel, a more conservative assignment of two-thirds their respective LODs was used for undetectable cytokines (Benjamin, 2010). The BMIs of each subject was calculated as (weight in kg/ (height in cm^2)*10,000) (Himes, 2009). Subjects were identified as having passive exposure to environmental tobacco smoke if their cotinine: creatinine ratio (CCR) exceeded 30 ng/mg, a ratio established for children in community-based studies (Henderson, 1989). Sensitivity analyses were performed excluding subjects with a urinary CCR >30 ng/mg (n=2).

SAS v9.3 (SAS, Cary, NC, USA) was used to perform all statistical analyses. A paired t-test was used to determine if there was a significant difference between visits for all health outcomes of interest. To account for multiple observations per subject, we fit a

general estimating equation (GEE) using exchangeable covariance structure to estimate the parameters of a generalized linear model associating an increase in interquartile range (IQR) of PMc and airborne endotoxin to lung function parameters and quality of life scores. GEE was also performed using the logistic link and exchangeable covariance structure to estimate the parameters associating an increase in IQR of PMc and airborne endotoxin to the odds ratio of being above the median for each EBC analyte, with the exception of TNF-α. Due to many subjects having undetected concentrations of TNF-α in EBC, in substitution for a median split the LOD was used as the cutpoint with 11 samples above and 65 samples below the LOD. GEE models were adjusted for parameters of subject variability (age, gender, and BMI; Model 2) as well as for subject variability in addition to home characteristics (square footage, number of residents, total number of furry pets (i.e. dogs, cats) and $PM_{2.5}$ concentrations; Model 3). GEE was also performed to investigate relationships between all health outcomes gathered at both visits.

RESULTS

Sampling Completion and Variability

Thirty-eight (38) homes were sampled for PMc, airborne endotoxin, and $PM_{2.5}$ twice during one winter with all homes having >80% complete continuous $PM_{2.5}$ data at each visit. Seventy-one percent (n=27) of homes owned a dog and 42% (n=21) of homes owned a cat, with all homes averaging 1.5 "furry" pets (Table 4.1). Each home had a primary asthmatic child (i.e., the recruited child, not an asthmatic sibling) that successfully captured at least one EBC and urine sample per visit. The subjects were 12.4 (± 2.6) years old with BMIs of 21.9 (± 4.8) at the time of sampling (Table 4.1). Five percent of the subjects (n=2) had a CCR >30 ng/mg, indicating potential passive environmental tobacco smoke exposure (Table 4.1).

Table 4.1. Descriptive home, subject, and IAQ characteristics (n=38).

	Mean	SD	Min	Max
Home Characteristics				
Square footage	1950	1029	600	4500
Number of adults	1.8	0.5	1	3
Number of kids	2.2	1.2	1	6
Common residents	4.1	1.4	2	8
Dog (% of homes (n))	71 (27)	---	---	---
Cat (% of homes (n))	42 (21)	---	---	---
Total pets	1.5	1	---	---
Study Subjects				
Age	12.4	2.6	7	17
BMI	21.9	4.8	14	32.6
Cotinine (% >30 ng/mg Creatinine (n))	5 (2)	---	---	---
Indoor Air				
PMc	12.4	7.9	3.3	46.2
EU/m^3	8.2	10.3	0.12	41.4
EU/mg	731	984	11	3781
$PM_{2.5}$	26.7	24.9	6.0	99.9

Note: Dog and Cat categorized as presence/absence, total pets is sum of presence for dog, cat, or other furry animal. Cotinine/Creatinine Ratio from urine reported in ng/mg. EU/m^3 and EU/mg in PMc. PMc and $PM_{2.5}$ reported in $\mu g/m^3$.

Forty-eight-hour average in-home concentrations of PMc ranged from 3.3 to 46.2 μg/m^3, with airborne endotoxin in PMc ranging from 0.12 to 41.4 EU/m^3 and from 11 to 3781 EU/mg (Table 4.1). Following application of an indoor wood smoke-specific correction factor (McNamara, 2011), 48-hour average PM$_{2.5}$ concentrations were 26.7 (± 24.9) μg/m^3 and ranged from 6.0 to 99.9 μg/m^3. IAQ concentrations for all measured parameters did not vary significantly across visits within homes (paired t-test p>0.05, data not shown).

Unlike IAQ parameters, some health outcomes did vary significantly across visits within subjects (paired t-test p<0.05; Table 4.2). Although mean PAQLQ scores did not vary, the mean activities subscore was significantly higher at the second visit than first visit. Several cytokines also varied significantly across visits. Mean IL-2 and IFN-γ concentrations in EBC were significantly different across visits, as well as the mean IL-10/IFN-γ ratio (p<0.05, Table 4.2). IL-1β, IL-6, IL-8, TNF-α, IL-4, and IL-5 approached significance across visits (p≈0.10). Further analyses accounted for multiple visits per subject (GEE models, Tables 4.3, 4.4) therefore these differences across visits were accounted for in final analyses.

Lung function measurements (PEF, FEV$_1$) were not required for subjects to be included in the main analyses. Measurements were collected from 37 subjects at the first visit and 35 subjects at the second visit, with 30 paired collections. Percent predicted PEF, percent predicted FEV$_1$, percent predicted morning-only PEF, percent predicted morning-only FEV$_1$, and the frequency of days with evening to morning PEF variability >20% did not vary significantly across visits (Table 4.2). Subjects performed <85% predicted for PEF and FEV$_1$ at both visits. Morning-only averages were slightly lower at both visits (Table 4.2). On average, subjects had an evening to morning variability in PEF >20% on approximately 20% of the days sampled (Table 4.2).

Table 4.2. Descriptive statistics of health outcomes from all subjects (n=38). Lung function data collected from 37 subjects at Visit 1, 35 subjects at Visit 2, with 30 paired collections.

		Visit 1 Mean (SD)	Visit 2 Mean (SD)	Paired T-test p-value
Lung function	PEF %pred	82.0 (22.9)	77.9 (25.0)	0.39
	FEV$_1$ %pred	84.2 (20.3)	81.3 (18.9)	0.83
	AM PEF%pred	80.0 (23.2)	75.6 (26.7)	0.23
	AM FEV$_1$ %pred	83.9 (20.0)	80.5 (19.4)	0.44
	PEF PM-AM variability >20%	23 (21)	20 (22)	0.73
Quality of Life Scores	Mean score	5.37 (1.17)	5.55 (1.10)	0.24
	Symptoms	5.33 (1.23)	5.45 (1.12)	0.49
	Activities	4.81 (1.47)	5.30 (1.21)	0.01
	Emotions	5.70 (1.16)	5.81 (1.20)	0.46
Exhaled Breath Condensate	8-isoprostane	4.90 (6.37)	5.03 (6.03)	0.89
	Pro-Inflammatory			
	GM-CSF	0.45 (1.25)	0.43 (0.23)	0.92
	IL-1β	0.37 (0.31)	0.28 (0.23)	0.11
	IL-6	0.22 (0.16)	0.17 (0.10)	0.13
	IL-8	1.03 (1.72)	0.59 (0.66)	0.14
	TNF-α	0.29 (0.62)	0.12 (0.29)	0.1
	Th1			
	IL-2	0.36 (0.38)	0.14 (0.21)	0.002
	IFN-γ	0.27 (0.28)	0.13 (0.14)	0.004
	Th2			
	IL-4	1.07 (2.03)	0.58 (1.24)	0.15
	IL-5	0.80 (0.65)	0.61 (0.51)	0.11
	IL-10	0.40 (0.19)	0.41 (0.20)	0.86
	IL-4/IFN-γ	2.32 (3.06)	3.13 (2.50)	0.22
	IL-10/IFN-γ	2.67 (1.94)	4.53 (2.70)	0.001
	IL-4/IL-6	2.56 (3.99)	2.37 (2.70)	0.75

Note: PEF and FEV$_1$ measurements expressed in L/min. Percent-predicted calculated from subject's height, weight, and age at time of sampling. All exhaled breath condensate analytes expressed in pg/mL with exception of cytokine ratios (IL-4/IFN-g, IL-10/IFN-g, IL-4/IL-6). Abbreviations: SD: standard deviation, PEF: peak expiratory flow, FEV$_1$: forced expiratory volume in one second, AM: morning only, %pred: percent-predicted, PEF PM-AM variability>20%: frequency of days where variability between previous night PEF and morning PEF is greater than 20%, GM-CSF: granulocyte macrophage colony-stimulating factor, IL: interleukin, TNF: tumor necrosis factor, IFN: interferon.

Mean PAQLQ score and 8-isoprostane concentration showed no relationships with any other health outcome measured in raw and adjusted GEE models. When adjusted for age, gender, and BMI of the subject as well as square footage of the home, number of residents living in the home, total number of pets, and corrected $PM_{2.5}$ concentrations (Model 3), percent-predicted PEF and FEV_1 values were significantly associated with each other (p<0.05; Table 4.3). Subjects with above median concentrations of pro-inflammatory cytokines were significantly associated with being above median for other pro-inflammatory cytokines as well as above median Th2 cytokines, but were significantly associated with below median Th1 cytokines (Table 4.3). Subjects with above median Th1 cytokines were significantly associated with having below median Th2 cytokines, while having above median Th2 cytokines was significantly associated with having above median concentrations for all other Th2 cytokines (Table 4.3).

Table 4.3. GEE results for adjusted models for within and between cytokine classes. Arrows represent direction of association and level of significance.

	Mean PAQLQ	PEF	FEV	8isoP	GM-CSF	IL-1β	IL-6	IL-8	TNF-α	IL-2	IFN-γ	IL-4	IL-5	IL-10
Mean PAQLQ	1													
PEF	---	1	↑↑								↓			
FEV	---	---	1							↓↓	↓↓	↑		
8isoP	---	---	---	1										
GM-CSF	---	---	---	---	1			↑↑		↓↓↓	↓↓↓	↑↑↑		
IL-1β	---	---	---	---	---	1	↑↑	↑↑↑		↓↓	↓↓	↑	↑↑	↑↑↑
IL-6	---	---	---	---	---	---	1	↑↑↑			↓↓	↑↑↑		
IL-8	---	---	---	---	---	---	---	1		↓↓	↓↓	↑↑↑	↑↑↑	↑↑↑
TNF-α	---	---	---	---	---	---	---	---	1					
IL-2	---	---	---	---	---	---	---	---	---	1		↓↓	↓↓	↓↓↓
IFN-γ	---	---	---	---	---	---	---	---	---	---	1	↓↓	↓↓	↓↓
IL-4	---	---	---	---	---	---	---	---	---	---	---	1	↑↑	↑↑
IL-5	---	---	---	---	---	---	---	---	---	---	---	---	1	↑↑
IL-10	---	---	---	---	---	---	---	---	---	---	---	---	---	1

Health outcomes: Lung function and Quality of Life

Data from GEE models for lung function measurements and quality of life scores are presented as unit change in health outcome with an increase in the interquartile range in the exposure of interest. Interquartile ranges are as follows: 8.85 µg/m^3 PMc, 8.39 EU/m^3, and 721 EU/mg. An increase in PMc was significantly associated with an increase in frequency of days with evening to morning variability in PEF >20% in all GEE models ($p<0.05$, Table 4.4). An increase in EU/mg but not EU/m^3 was significantly associated with lower overall and morning-only PEF percent-predicted values in all GEE models (Table 4.4). Adjusting for age, gender, and BMI of the subjects gave similar results compared to the unadjusted GEE (Table 4.4b). Adjusting for age, gender and BMI of the subjects as well as square footage of the home, number of residents living in the home, total number of pets, and corrected PM$_{2.5}$ concentrations elucidated a relationship approaching statistical significance between an increase EU/mg and a decrease in percent predicted morning-only FEV$_1$ ($p<0.10$, Table 4.4c). All other relationships were consistent across models.

Table 4.4. GEE results for unadjusted (a: Model 1) and adjusted (b: Model 2 and c: Model 3) models for IAQ measure on lung function and quality of life scores/subscores. Presented per IQR change.

a)

Model 1

		PMc	EU/m^3	EU/mg
Lung function	PEF %pred	1.81 (-6.49, 10.12)	-0.70 (-2.30, 0.89)	-1.54 (-2.74, -0.35)**
	FEV$_1$ %pred	-0.91 (-11.26, 9.44)	-0.66 (-2.46, 1.15)	-0.82 (-2.54, 0.90)
	AM PEF %pred	1.56 (-7.47, 10.59)	-0.49 (-2.42, 1.44)	-1.54 (-3.05, -0.04)**
	AM FEV$_1$ %pred	0.26 (-9.68, 10.20)	-0.51 (-2.21, 1.18)	-0.96 (-2.41, 0.50)
	PEF PM-AM variability >20%	5 (1, 9)**	1 (-1, 2)	1 (-1, 2)
Quality of Life Scores	Mean score	-0.03 (-0.20, 0.24)	-0.04 (-0.18, 0.10)	-0.03 (-0.13, 0.08)
	Symptoms	0.02 (-0.29, 0.32)	0.00 (-0.12, 0.13)	-0.02 (-0.13, 0.09)
	Activities	0.00 (-0.35, 0.35)	0.00 (-0.15, 0.15)	0.03 (-0.10, 0.16)
	Emotions	-0.07 (-0.30, 0.16)	-0.11 (-0.30, 0.08)	-0.06 (-0.21, 0.08)

Note: Lung function: n=72. Quality of Life: n=76. Abbreviations: PEF: peak expiratory flow, FEV$_1$: forced expiratory volume in one second, AM: morning only, %pred: percent predicted, PEF PM-AM variability>20%: frequency of days where variability between previous night PEF and morning PEF is greater than 20%, *: p<0.10, **: p<0.05.

b)

Model 2

		PMc	EU/m³	EU/mg
Lung function	PEF %pred	1.33 (-7.67, 9.34)	-0.83 (-2.23, 0.57)	-1.56 (-2.66, -0.46)**
	FEV$_1$ %pred	-2.14 (-12.20, 7.91)	-0.77 (-2.54, 0.99)	-0.87 (-2.60, 0.86)
	AM PEF %pred	0.93 (-7.72 (9.57)	-0.64 (-2.39, 1.11)	-1.59 (-2.99, -0.19)**
	AM FEV$_1$ %pred	-0.74 (-10.42, 8.93)	-0.63 (-2.23, 0.97)	-1.01 (-2.44, 0.42)
	PEF PM-AM variability >20%	5 (1, 10)**	1 (-1, 2)	1 (-1, 2)
Quality of Life Scores	Mean score	-0.04 (-0.28, 0.20)	-0.03 (-0.17, 0.10)	-0.03 (-0.13, 0.07)
	Symptoms	-0.02 (-0.27, 0.24)	0.01 (-0.10, 0.12)	-0.02 (-0.12, 0.09)
	Activities	-0.01 (-0.32, 0.29)	0.01 (-0.12, 0.15)	0.03 (-0.09, 0.14)
	Emotions	-0.09 (-0.31, 0.13)	-0.11 (-0.29, 0.07)	-0.07 (-0.20, 0.07)

Note: Model 2 is adjusted for age, gender, and BMI of the subjects. Lung function: n=72. Quality of Life: n=76. Abbreviations: PEF: peak expiratory flow, FEV$_1$: forced expiratory volume in one second, AM: morning only, %pred: percent predicted, PEF PM-AM variability>20%: frequency of days where variability between previous night PEF and morning PEF is greater than 20%, *: p<0.10, **: p<0.05.

c)

Model 3

		PMc	EU/m^3	EU/mg
Lung function	PEF %pred	3.25 (-4.38, 10.87)	-0.83 (-2.25, 0.58)	-1.69 (-2.70, -0.68)**
	FEV$_1$ %pred	-1.76 (-12.29, 8.77)	-0.79 (-2.45, 0.87)	-0.96 (-2.57, 0.66)
	AM PEF %pred	3.17 (-4.91, 11.26)	-0.64 (-2.39, 1.11)	-1.75 (-3.01, -0.50)**
	AM FEV$_1$ %pred	0.48 (-9.16, 10.12)	-0.74 (-2.29, 0.81)	-1.21 (-2.51, 0.10)*
	PEF PM-AM variability >20%	7 (1, 13)**	1 (0, 3)	1 (0, 3)
Quality of Life Scores	Mean score	-0.05 (-0.29, 0.18)	-0.04 (-0.17, 0.09)	-0.03 (-0.13, 0.07)
	Symptoms	-0.03 (-0.28, 0.23)	0.00 (-0.19, 0.11)	-0.02 (-0.12, 0.08)
	Activities	-0.05 (-0.35, 0.24)	0.00 (-0.13, 0.14)	0.02 (-0.10, 0.14)
	Emotions	-0.09 (-0.32, 0.13)	-0.11 (-0.28, 006)	-0.06 (-0.19, 0.07)

Note: Model 3 is adjusted for age, gender, and BMI of the subjects as well as square footage of their home, number of residents living in the home, total number of pets, and corrected PM$_{2.5}$ values as recorded by the DustTrak. Lung function: n=76. Quality of Life: n=72. Abbreviations: PEF: peak expiratory flow, FEV$_1$: forced expiratory volume in one second, AM: morning only, %pred: percent predicted, PEF PM-AM variability>20%: frequency of days where variability between previous night PEF and morning PEF is greater than 20%, *: p<0.10, **: p<0.05.

Health outcomes: Biomarkers

Data from GEE models for EBC concentrations of 8-isoprostane and all cytokines are presented as odds ratio of being above median for a given analyte with an increase in the interquartile range in the exposure of interest. The exception to this is TNF-α, which, as previously mentioned, had only 11 of 76 samples above the LOD. Therefore these results are presented as the odds ratio of being above the LOD for TNF-α with an increase in the interquartile range in the exposure of interest. As this data is from the same homes and subjects as previously described, the interquartile ranges are the same. The only endotoxin relationship that is consistently significant in all GEE models is an increased odds ratio of having above median 8-isoprostane with an IQR increase in EU/m^3 ($p < 0.05$, Table 4.5a-c). Model 2 and Model 3 show increased EU/m^3 to be associated with a significant increase in odds of having above median IL-8. An increase in PMc approaches significance with a decreased odds ratio of having above median concentrations of cytokines in Model 2 (IL-6, IL-8, IL-4, IL-5, IL-4/IFN-γ, IL-4/IL-6: $p < 0.10$; Table 4.5b) and is significantly associated with a decreased odds ratio of being above median for IL-8, IL-4, and the IL-4/IL-6 ratio in Model 3 ($p < 0.05$; Table 4.5c). An increase in EU/m^3 approaches significance with increased odds ratios of having above median IL-2 and IFN-γ in Model 2 ($p < 0.10$; Table 4.5b) and this relationship is significant in Model 3 ($p < 0.05$, Table 4.5c). TNF-α was unable to be included in Model 3 analyses due to the limiting number of samples above the LOD (n=11). The eleven subjects with samples above the LOD are comprised of 7 girls and 4 boys, therefore attempting to adjust for square footage of the home, number of residents living in the home, total number of furry pets and $PM_{2.5}$ concentrations as well as age, gender, and BMI of the subjects excludes TNF-α from this Model (Table 4.5c).

Table 4.5. Odds ratio results for unadjusted (a: Model 1) and adjusted (b: Model 2 and c: Model 3) models for 8-isoprostane and cytokines measured in exhaled breath condensate. Presented as odds ratio of being above median for given analyte per IQR change[§].

a)

	Model 1		
	PMc	**EU/m^3**	**EU/mg**
8-isoprostane	0.75 (0.51, 1.10)	1.86 (1.08, 3.24)**	1.33 (0.87, 2.06)
Pro-Inflammatory			
GM-CSF	0.92 (0.52, 1.60)	0.95 (0.72, 1.26)	1.00 (0.81, 1.24)
IL-1β	0.88 (0.56, 1.40)	1.04 (0.89, 1.23)	1.00 (0.87, 1.16)
IL-6	0.75 (0.48, 1.19)	1.13 (0.84, 1.52)	1.16 (0.87, 1.43)
IL-8	0.71 (0.43, 1.16)	1.34 (0.98, 1.83)	1.24 (0.87, 1.78)
TNF-α	0.96 (0.62, 1.46)	1.06 (0.80, 1.42)	1.00 (0.81, 1.24)
Th1			
IL-2	1.58 (0.71, 3.58)	1.33 (0.99, 1.78)*	1.24 (0.87, 1.78)
IFN-γ	1.52 (0.61, 1.14)	1.32 (0.97, 1.80)	1.33 (0.93, 1.91)
Th2			
IL-4	0.61 (0.33, 3.36)	0.97 (0.78, 1.20)	1.00 (0.81, 1.24)
IL-5	0.75 (0.47, 1.20)	1.10 (0.85, 1.42)	1.07 (0.87, 1.43)
IL-10	1.07 (0.59, 1.96)	0.91 (0.71, 1.17)	0.93 (0.75, 1.16)
IL-4/IFN-γ	0.61 (0.33, 1.13)	0.97 (0.78, 1.20)	1.00 (0.81, 1.24)
IL-10/IFN-γ	0.91 (0.50, 1.64)	0.88 (0.67, 1.16)	0.93 (0.70, 1.16)
IL-4/IL-6	0.65 (0.36, 1.16)	1.00 (0.80, 1.25)	1.00 (0.81, 1.24)

Note: [§]with exception of TNF-α, presented as odds ratio of being above LOD (n=11 above, 65 below). All other analytes n=38 above median, 38 below. Abbreviations: GM-CSF: granulocyte macrophage colony-stimulating factor, IL: interleukin, TNF: tumor necrosis factor, IFN: interferon. *: p<0.10, **: p<0.05.

b)

	Model 2		
	PMc	**EU/m³**	**EU/mg**
8-isoprostane	0.68 (0.00, 1.08)	1.85 (1.10, 3.11)**	1.31 (0,85, 2.02)
Pro-Inflammatory			
GM-CSF	0.81 (0.45, 1.45)	0.94 (0.73, 1.22)	0.99 (0.79, 1.25)
IL-1β	0.80 (0.44, 1.44)	1.05 (0.88, 1.24)	1.01 (0.85, 1.20)
IL-6	0.69 (0.44, 1.07)*	1.15 (0.80, 1.64)	1.12 (0.82, 1.54)
IL-8	0.59 (0.35, 0.99)*	1.44 (1.03, 2.02)**	1.28 (0.76, 2.16)
TNF-α	0.90 (0.52, 1.56)	1.09 (0.29, 1.35)	1.04 (0.87, 1.25)
Th1			
IL-2	1.54 (0.69, 3.43)	1.38 (0.97, 1.96)*	1.31 (0.81, 2.11)
IFN-γ	1.47 (0.60, 3.62)	1.36 (0.95, 1.94)*	1.37 (0.85, 2.20)
Th2			
IL-4	0.56 (0.30, 1.04)*	0.97 (0.79, 1.20)	1.00 (0.81, 1.22)
IL-5	0.66 (0.42, 1.03)*	1.11 (0.16, 1.48)	1.10 (0.85, 1.43)
IL-10	0.93 (0.52, 1.69)	0.91 (0.69, 1.18)	0.94 (0.74, 1.19)
IL-4/IFN-γ	0.56 (0.30, 1.04)*	0.97 (0.79, 1.20)	1.00 (0.81, 1.22)
IL-10/IFN-γ	0.79 (0.44, 1.42)	0.93 (0.72, 1.19)	0.97 (0.77, 1.23)
IL-4/IL-6	0.60 (0.33, 1.09)*	1.01 (0.81, 1.27)	1.02 (0.81, 1.27)

Note: [§]with exception of TNF-α, presented as odds ratio of being above LOD (n=11 above, 65 below). All other analytes n=38 above median, 38 below. Model 2 is adjusted for age, gender, and BMI of the subjects. Abbreviations: GM-CSF: granulocyte macrophage colony-stimulating factor, IL: interleukin, TNF: tumor necrosis factor, IFN: interferon. *: $p<0.10$, **: $p<0.05$.

c)

	Model 3		
	PMc	EU/m^3	EU/mg
8-isoprostane	0.59 (0.33, 1.04)	1.85 (1.03, 3.33)**	1.30 (0.79, 2.15)
Pro-Inflammatory			
GM-CSF	0.70 (0.34, 1.46)	0.94 (0.70, 1.25)	0.98 (0.77, 1.25)
IL-1β	0.70 (0.35, 1.38)	1.04 (0.89, 1.21)	1.00 (0.84, 1.19)
IL-6	0.60 (0.36, 1.01)*	1.14 (0.81, 1.61)	1.10 (0.81, 1.50)
IL-8	0.53 (0.30, 0.94)**	1.42 (1.02, 1.99)**	1.24 (0.72, 2.14)
TNF-α[x]	---	---	---
Th1			
IL-2	1.56 (0.68, 3.58)	1.47 (1.02, 2.12)**	1.35 (0.79, 2.30)
IFN-γ	1.46 (0.61, 3.48)	1.47 (1.03, 2.10)**	1.45 (0.84, 2.50)
Th2			
IL-4	0.50 (0.25, 0.99)**	1.01 (0.80, 1.26)	1.00 (0.80, 1.24)
IL-5	0.53 (0.31, 0.92)	1.11 (0.83, 1.50)	1.10 (0.84, 1.44)
IL-10	0.91 (0.44, 1.91)	0.83 (0.68, 1.18)	0.93 (0.74, 1.17)
IL-4/IFN-γ	0.50 (0.25, 0.99)	1.01 (0.80, 1.26)	1.00 (0.80, 1.24)
IL-10/IFN-γ	0.76 (0.37, 1.57)	0.90 (0.69, 1.19)	0.96 (0.74, 1.23)
IL-4/IL-6	0.49 (0.25, 0.95)**	1.02 (0.81, 1.29)	1.01 (0.80, 1.28)

[x]: TNF-α not able to be included in Model 3 due to limiting numbers of samples above LOD.
Note: Model 3 is adjusted for age, gender, and BMI of the subjects as well as square footage of their home, number of residents living in the home, total number of pets, and corrected $PM_{2.5}$ values as recorded by the DustTrak. Abbreviations: GM-CSF: granulocyte macrophage colony-stimulating factor, IL: interleukin, TNF: tumor necrosis factor, IFN: interferon. *: $p < 0.10$, **: $p < 0.05$.

DISCUSSION

Health outcomes: Lung function and Quality of Life

Our results showed ~80%-predicted PEF and ~82%-predicted FEV_1 measurements with ~21% of days having >20% variability in PEF (Table 4.2). Similar to our study, a large urban intervention study using almost 1,000 atopic asthmatic children had subjects record PEF and FEV_1 measurements twice daily for two weeks (Morgan, 2004). Their baseline results were largely in agreement with our study despite our limited sample size. Their subjects had approximately 96%-predicted PEF measurements and 88%-predicted FEV_1 measurements with 37% days having >20% variability in PEF (Morgan, 2004). Airborne endotoxin results in our study homes are also in line with published data. Endotoxin concentrations have been reported at 7.63 EU/m^3 in wood stove homes in Ireland and Scotland (Semple, 2011) and at a geometric mean of 5.8 EU/m^3 in rural homes of asthmatic children (Thorne, 2007). For comparison, our homes had an average airborne endotoxin concentration of 8.2 EU/m^3 (Table 4.2).

Daily variability in PEF and frequency of days for which this variability is >20% have been established as measures of bronchial responsiveness in asthma and have been used in a variety of epidemiological studies (Boezen, 1996, Yoo, 2007, Morgan, 2004). The cut-point of 20% change in daily PEF is derived from identifying non-asthmatic variability commonly < 20% (Quackenboss, 1991). We found an increase in PMc but not endotoxin was associated with a significant increase in frequency of days with PEF evening to morning variability >20% in all GEE models (p<0.05; Table 4.4a-c). The evening to morning daily variability best characterizes the acute impacts of the concentrations captured with our air sampling program, as the overnight exposures occur during which time the subject was sleeping. This effect of PMc and not endotoxin on reduced peak flow stability suggests that the respiratory effects of PMc exposure are not primarily dependent on endotoxin content.

113

An increase in EU/mg of PMc was associated with a significant decrease in overall and morning-only percent-predicted PEF values in all GEE models (p<0.05; Table 4.4a-c). Without accounting for mass of particle collected, airborne endotoxin endotoxin (EU/m^3) was not associated with any lung function measures. This finding is counter to a previous study that found house-dust endotoxin to be associated with PEF-variability but not when corrected per gram of sampled dust (Douwes, 2000). Dust endotoxin has been shown to affect lung function of rural children with asthma or wheeze (Lawson, 2011). However, dust endotoxin correlates poorly with airborne endotoxin levels (Park, 2001, Mazique, 2011), so comparing our airborne findings with house-dust endotoxin is problematic. Airborne endotoxin measured over 1.5 days has been determined to be a more direct measure of exposure than the use of dust endotoxin (Horick, 2006) therefore the airborne endotoxin results reported here (measured over 2 days) are likely to best represent exposure.

We found no associations between PMc or either measure of endotoxin with quality of life mean score or subscores (Table 4.4a-c). Mean PAQLQ score also did not correlate with any other health outcome measured. A score change of 0.5 is established as the minimal important difference over time (Juniper, 1994). Although baseline IAQ exposures did not correlate with PAQLQ scores, we will ultimately have 80% power to demonstrate a difference in PAQLQ scores of 1.00 unit or greater at the 95% confidence level with all ARTIS subjects (n=136) (Noonan, 2012). Our ability to interpret this data is also limited by the cross-sectional nature of this study. Our analyses attempted to account for main inter-individual differences (adjusting for age, gender and BMI of the subjects; Tables 4.4, 4.5; Models 2, 3). Future planned analyses to evaluate change in quality of life scores from pre- to post-intervention allow for subjects to function as their own baseline score controls.

EBC is mainly water vapor collected from the saturated exhaled breath, but it also contains a sample of the airway lining fluid (Thomas, 2013). It is postulated that the lining fluid is nebulized by turbulent airflow from the converging branches of the airways but how particles form and change during exhalation before leaving the airways is speculation (Davis, 2012). Micrometer and submicrometer droplets in exhaled breath have been confirmed by laser particle counts (Fairchild, 1987, Papineni, 1997), explaining the presence of non-volatile constituents in EBC such as cytokines and 8-isoprostane. Interpreting EBC biomarkers has unique challenges, as there are multiple areas for variation in sample collection and analysis (Kostikas, 2008, Rosias, 2012, Davis, 2012). It is recommended by the manufacturer of the EBC collection tubes used in our study to analyze biomarker trends in terms of relative change rather than absolute concentration (Davis, 2012, Respiratory Research, 2005). Therefore, we investigated the odds ratios of having above median levels of these markers as opposed to effects on absolute change.

8-isoprostane

8-isoprostane is a biomarker of oxidative stress, with elevated concentrations found in the bronchoalveolar lavage fluid of patients with interstitial lung diseases (Montuschi, 1998). Previous studies have shown oxidative stress is increased in asthmatic subjects as reflected by 8-isoprostane concentrations in EBC (Montuschi, 1999). Increased 8-isoprostane has also been detected in the EBC of severe asthmatics compared to healthy and mildly asthmatic adults, although the differences were not statistically significant (Piotrowski, 2012). Piotrowski *et al.* reported a median (IQR) 8-isoprotane concentration of 4.67 (2.50-27.92) pg/mL in severe asthmatics (Piotrowski, 2012), similar

to the concentrations detected in our subjects (~5.0 pg/mL; Table 4.2). 8-isoprostane was not associated with any other health outcome measured in our study.

Ours is the first study to investigate the relationship between in-home airborne endotoxin and EBC biomarkers of oxidative stress. Previous research has been done in occupational settings with high endotoxin exposures (i.e. grain elevators) and found intensity of endotoxin exposure to be associated with increased markers of oxidative stress (Burch, 2009), including 8-isoprostane in EBC (Do, 2008). We identified a significant association between an increase in airborne endotoxin (EU/m^3 but not EU/mg) with an increased odds ratio of above median EBC 8-isoprostane in all GEE models ($p<0.05$; Table 4.5a-c). The dissimilarity of EU/m^3 and EU/mg results has been reported elsewhere (Douwes, 2000) and emphasizes the inclusion of both measures of endotoxin in health-based studies.

Effects on cytokines

Previous studies utilizing EBC to analyze cytokines in asthmatics have primarily focused on IL-10 and the IL-4/IFN-γ ratio (Shahid, 2002) due to their relevance in the immune status of atopic asthmatics. IL-10 and IL-4 are cytokines derived from CD4+ T-helper type 2 (Th2) cells while IFN-γ is a product of T-helper type 1 (Th1) cells. IL-10 suppresses the activity of Th1 cells and IFN-γ suppresses the differentiation of Th2 cells (Chung, 2001). This feedback typically keeps infection fighting (Th1) and eosinophilic inflammation (Th2) in check. However studies involving bronchalveolar lavage fluid and lung biopsies have confirmed that a Th2-dominated lung environment is seen in atopic asthmatics (Robinson, 1992). As a result, there is elevation of IL-4 and depression of IFN-γ, which can be described with the IL-4/IFN-γ ratio (Walker, 1992, Tang, 1995). We decided to investigate the IL-10/IFN-γ ratio to further describe the Th1/Th2 balance. The IL-4/IL-6 ratio was chosen as a way to investigate Th2 activity relative to inflammation.

IL-6 was selected for this ratio because it is a sensitive marker for endotoxin (Soukup, 2001, Copeland, 2005, Hadina, 2008) and has been shown to be elevated in asthmatics independent of other pro-inflammatory cytokines, such as TNF-α and IL-1β (Neveu, 2010).

The basic role (pro-inflammatory, Th1 or Th2) of all cytokines measured in our subjects is illustrated in Figure 4.1. Subjects with above median Th1 cytokines were significantly associated with having below median Th2 cytokines (Table 4.3). Subjects with above median concentrations of several pro-inflammatory cytokines were significantly associated with having below median Th1 cytokines and above median Th2 cytokines (Table 4.3). Although no significant relationships were found between PMc and endotoxin concentrations with Th1/Th2 ratios (i.e. IL-4/IFN-γ and IL-10/IFN-γ) these associations demonstrate that our multiplex analysis results are consistent with functional categories of cytokines.

Our GEE model adjusting for age, gender, and BMI of the subjects along with square footage of the home, number of residents living in the home, total number of pets, and corrected $PM_{2.5}$ concentrations revealed the strongest relationships between PMc and endotoxin concentrations with cytokine variability (Table 4.5c). Endotoxin concentrations were not associated with above median pro-inflammatory mediators produced by alveolar macrophages (IL-6, IL-1β, GM-CSF and TNF-α) (Table 4.5a-c). Previous studies have not seen differences in TNF-α and IL-1β between asthmatics and non-asthmatics (Neveu, 2010) which may explain the lack of elevation of these cytokines in our subjects.

Figure 4.1. Role of cytokines in development of Th1 and Th2 lymphocytes. As a result of signals from the surrounding microenvironment naïve T cells differentiate into Th1 or Th2 lymphocytes. Adapted from Elias et al. and Ho (Elias, 2003, Ho, 2010).
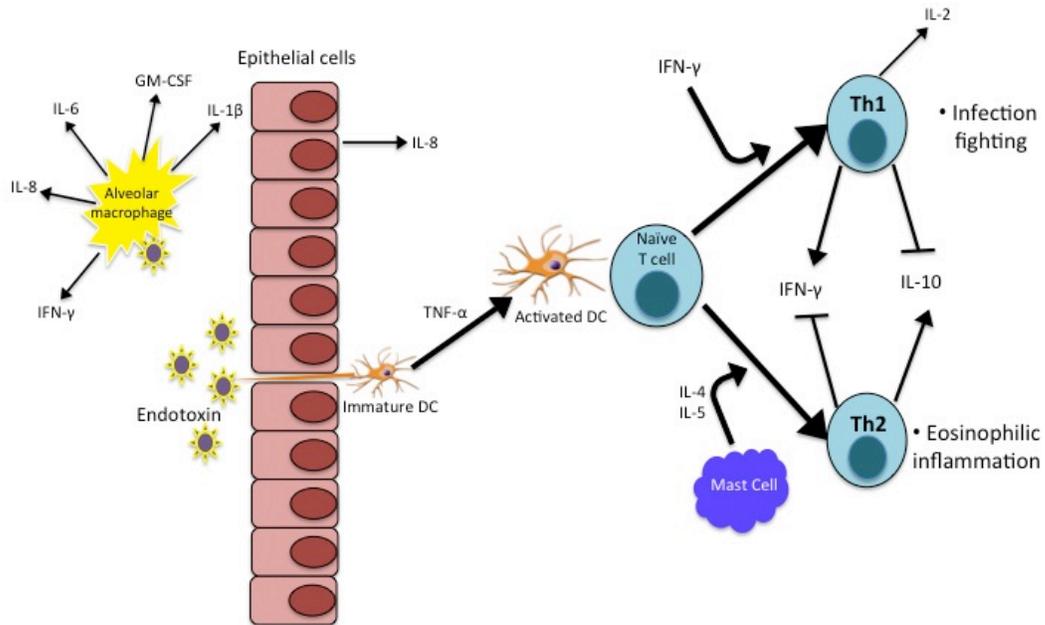
An increase in PMc was associated with a significant odds ratio of having below median IL-8 ($p < 0.05$; Table 4.5c), but an increase in EU/m^3 was associated with a significant odds ratio of having above median IL-8 ($p < 0.05$; Table 4.5c). IL-8 is a pro-inflammatory chemokine produced by alveolar macrophages and lung epithelial cells and is elevated in the bronchoalveolar lavage fluid of asthmatics and those with chronic obstructive pulmonary disease (Nocker, 1996). *In vitro* studies have identified IL-8 release from lung epithelial cells following endotoxin exposure (Cabrera-Benitez, 2012) and heat-treatment (endotoxin removal) of house dust has shown a reduced capacity of human alveolar epithelial cells to produce IL-8 (Mathiesen, 2004). Endotoxin exposures in an agricultural occupational exposure study were also associated with statistically significant increases in IL-8 nasal lavage concentrations (Burch, 2009). Therefore it makes biological sense for endotoxin (EU/m^3) concentrations to be associated with above median IL-8 in asthmatic children. Endotoxin expressed per mg of PMc did not show this relationship, again indicating that the health effects of PMc in asthmatic children living in wood stove homes may be partially due to mechanisms independent of its endotoxin content.

An increase in EU/m^3 was significantly associated with an odds ratio of having above median IL-2 and IFN-γ, both Th1 cytokines ($p < 0.05$, Table 4.5c). This is counter to previous studies that have identified asthmatics as having predominately Th2-mediated lung environments (Robinson, 1992, Walker, 1992, Shahid, 2002). We did not find this to be the case in our subjects, with no relationships found between PMc or airborne endotoxin concentrations and IL-4/IFN-γ and IL-10/IFN-γ ratios. The IL-4/IL-6 ratio was significantly associated with PMc ($p < 0.05$; Table 4.5c) but not endotoxin, indicating that increased Th2 activity may need to be investigated relative to inflammation as well as Th1 activity in asthmatic children.

A Dutch group has recently demonstrated the use of a multiplex immunoassay to detect multiple cytokines at a rate of 95-100% detection in EBC (Rosias, 2010, Robroeks, 2009). They reported TNF-α concentrations to be significantly lower in asthmatics compared to controls, which they attributed to medication use (Rosias, 2010). This may partially explain why the majority (65 of 76) of our samples had below LOD TNF-α. The primary reason for our samples having a lower rate of detection compared to those of the Dutch group is likely due to differences in EBC sample collection equipment (closed glass condenser vs. Rtubes). However, our subjects needed to collect their own EBC in a feasible amount of time in the morning before school and the closed glass condenser could not be used in those circumstances.

CONCLUSION

We investigated, for the first time, the acute health effects of PMc and airborne endotoxin concentrations in asthmatic children living in wood stove homes. We characterized health effects in three ways: 1) with a validated Pediatric Asthma Quality of Life Questionnaire, 2) lung function with PEF and FEV_1 measurements, and 3) biomarker assessment in EBC. The results presented here are cross sectional baseline results from a randomized trial, thus the main limitation of our study was the lack of post-intervention sampling, non-asthmatics or non-wood stove homes for comparison.

Although homes recruited for the ARTIS study are required to be non-smoking homes, we did identify a urinary CCR >30 ng/mg in two of our subjects (Table 4.1). A CCR of 30 ng/mg has been used to identify children exposed to smoking with a high degree of sensitivity (80%) and specificity (100%) (Henderson, 1989) and is appropriate for countries with a low smoking prevalence such as the United States (Keskinoglu, 2007). Sensitivity analyses excluding the subjects with indicators of passive or active tobacco smoke exposure were consistent with results from our primary analyses. This suggests that the health measurements of these two subjects were not unduly biasing the overall results from the subjects with a CCR ratio revealing no passive or active smoke exposure.

The atopic status of these 38 subjects is not yet available. It is specifically atopic asthmatics, not all asthmatics, which have demonstrated a phenotypic Th2-mediated response to endotoxin exposure. Correction for atopic status will clarify whether or not the effects reported here are consistent between atopic and nonatopic asthmatics and/or if atopic status is driving any of these relationships. Overall, these results will be verified by taking into account the atopic status of the subjects.

Our primary findings provide an in-depth perspective into the overall health of these asthmatic children living in a unique environment. Although no relationships were

seen between PMc and airborne endotoxin concentrations with quality of life scores, increased PMc concentrations were associated with increased frequency of PEF daily variability. Increased PMc also showed reduced odds ratios for above-median pro-inflammatory marker IL-8 and asthma-related IL-4. Increases in airborne endotoxin were associated with impacts on lung function (reduced percent-predicted PEF) when expressed per mg of PMc (EU/mg) and on odds ratios of above-median 8-isoprostane, IL-8, IL-2, and IFN-γ when expressed per volume of air sampled (EU/m^3). Perhaps the most important findings from these cross sectional relationships are the differences between PMc and endotoxin concentrations and all categories of health outcomes (lung function, quality of life, and biomarkers). No health outcomes showed the same response with increased concentrations of PMc and endotoxin; in fact, certain outcomes had conflicting responses between the exposures (i.e., IL-8). These findings, although from a small subset of asthmatic children living in a unique environment, indicate that airborne endotoxin and PMc do not have identical impacts on the respiratory health of asthmatics. This is counter to the hypothesized mechanism of PMc toxicity being largely dependent on its endotoxin content (Miyata, 2011).

For the first time, we have established a relationship between in-home concentrations of endotoxin and IL-8, which agrees with published *in vitro* data (Mathiesen, 2004) as well as agriculture occupational studies (Burch, 2009). Subjects living in homes with higher concentrations of airborne endotoxin had higher Th1 cytokines, with airborne endotoxin showing no relationship with increased Th2 cytokines as has previously been described (Robinson, 1992, Walker, 1992, Shahid, 2002). Airborne endotoxin, and not PMc, was also significantly associated with increased 8-isoprostane and IL-8, indicating a more general pro-inflammatory and oxidative stress response to endotoxin exposure than an allergic asthma phenotypic response. Overall these results indicate that airborne endotoxin and PMc have a different profile of adverse

health effects in asthmatic children living in homes with wood stoves. The atopic status of these subjects needs to be verified and included in future analyses to validate these findings.

REFERENCES

Accordino, R., Visentin, A., Bordin, A., Ferrazzoni, S., Marian, E., Rizzato, F., Canova, C., Venturini, R. and Maestrelli, P. (2008) "Long-term repeatability of exhaled breath condensate pH in asthma", *Respir Med*, **102**, 377-381.

Baraldi, E., Ghiro, L., Piovan, V., Carraro, S., Ciabattoni, G., Barnes, P.J. and Montuschi, P. (2003) "Increased exhaled 8-isoprostane in childhood asthma", *Chest*, **124**, 25-31.

Benjamin, S.L. and Belluck, D.A. (2010) A practical guide to understanding, managing, and reviewing environmental risk assessment reports, Boca Raton, FL, CRC Press LLC, 680.

Bloom, B., Cohen, R.A. and Freeman, G. (2012) Summary health statistics for U.S. children: National Health Interview Survey, 2011, Vol. 10, Vital Health Stat, National Center for Health Statistics, 88.

Boezen, H.M., Postma, D.S., Schouten, J.P., Kerstjens, H.A. and Rijcken, B. (1996) "PEF variability, bronchial responsiveness and their relation to allergy markers in a random population (20-70 yr)", *Am J Respir Crit Care Med*, **154**, 30-35.

Brunekreef, B. and Forsberg, B. (2005) "Epidemiological evidence of effects of coarse airborne particles on health", *Eur Respir J*, **26**, 309-318.

Brunetti, L., Francavilla, R., Tesse, R., Fiermonte, P., Fiore, F.P., Lore, M., Margiotta, M. and Armenio, L. (2008) "Exhaled breath condensate cytokines and pH in pediatric asthma and atopic dermatitis", *Allergy Asthma Proc*, **29**, 461-467.

Burch, J.B., Svendsen, E., Siegel, P.D., Wagner, S.E., Von Essen, S., Keefe, T., Mehaffy, J., Martinez, A.S., Bradford, M., Baker, L., Cranmer, B., Saito, R., Tessari, J., Linda, P., Andersen, C., Christensen, O., Koehncke, N. and Reynolds, S.J. (2009) "Endotoxin exposure and inflammation markers among agricultural workers in Colorado and Nebraska", *J Toxicol Environ Health A*, **73**, 5-22.

Cabrera-Benitez, N.E., Perez-Roth, E., Casula, M., Ramos-Nuez, A., Rios-Luci, C., Rodriguez-Gallego, C., Sologuren, I., Jakubkiene, V., Slutsky, A.S., Padron, J.M. and Villar, J. (2012) "Anti-inflammatory activity of a novel family of aryl ureas compounds in an endotoxin-induced airway epithelial cell injury model", *PLoS One*, **7**, 8.

Chung, F. (2001) "Anti-inflammatory cytokines in asthma and allergy: interleukin-10, interleukin-12, interferon-g", *Mediators Inflamm.*, **10**, 51-59.

Copeland, S., Warren, H.S., Lowry, S.F., Calvano, S.E. and Remick, D. (2005) "Acute response to endotoxin in mice and humans", *Clinical and Vaccine Immunology*, **12**, 60-67.

Davis, M.D., Montpetit, A. and Hunt, J. (2012) "Exhaled breath condensate: an overview", *Immunol Allergy Clin North Am*, **32**, 363-375.

Do, R., Bartlett, K.H., Dimich-Ward, H., Chu, W. and Kennedy, S.M. (2008) "Biomarkers of Airway Acidity and Oxidative Stress in Exhaled Breath Condensate from Grain Workers", *American Journal of Respiratory and Critical Care Medicine*, **178**, 1048-1054.

Douwes, J., Zuidhof, A., Doekes, G., Van Der Zee, S.C., Wouters, I., Boezen, M.H. and Brunekreef, B. (2000) "(1-->3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children", *Am J Respir Crit Care Med*, **162**, 1348-1354.

Elias, J.A., Lee, C.G., Zheng, T., Ma, B., Homer, R.J. and Zhu, Z. (2003) "New insights into the pathogenesis of asthma", *The Journal of Clinical Investigation*, **111**, 291-297.

Fairchild, C.I. and Stampfer, J.F. (1987) "Particle concentration in exhaled breath", *Am Ind Hyg Assoc J*, **48**, 948-949.

Hadina, S., Weiss, J.P., Mccray, P.B., Jr., Kulhankova, K. and Thorne, P.S. (2008) "MD-2-dependent pulmonary immune responses to inhaled lipooligosaccharides: effect of acylation state", *Am J Respir Cell Mol Biol*, **38**, 647-654.

Hankinson, J.L., Odencrantz, J.R. and Fedan, K.B. (1999) "Spirometric reference values from a sample of the general U.S. population", *Am J Respir Crit Care Med*, **159**, 179-187.

Heinrich, J., Pitz, M., Bischof, W., Krug, N. and Born, P.J.A. (2003) "Endotoxin in fine (PM2.5) and coarse (PM2.5-10) particle mass of ambient aerosols. A temporo-spatial analysis", *Atmospheric Environment*, **37**, 3659-3667.

Henderson, F.W., Reid, H.F., Morris, R., Wang, O.L., Hu, P.C., Helms, R.W., Forehand, L., Mumford, J., Lewtas, J., Haley, N.J. and Et Al. (1989) "Home air nicotine levels and urinary cotinine excretion in preschool children", *Am Rev Respir Dis*, **140**, 197-201.

Himes, J.H. (2009) "Challenges of Accurately Measuring and Using BMI and Other Indicators of Obesity in Children", *Pediatrics*, **124**, S3-S22.

Ho, S.-M. (2010) "Environmental epigenetics of asthma: An update", *The Journal of allergy and clinical immunology*, **126**, 453-465.

Horick, N., Weller, E., Milton, D.K., Gold, D.R., Li, R. and Spiegelman, D. (2006) "Home endotoxin exposure and wheeze in infants: correction for bias due to exposure measurement error", *Environ Health Perspect*, **114**, 135-140.

Juniper, E.F., Guyatt, G.H., Feeny, D.H., Ferrie, P.J., Griffith, L.E. and Townsend, M. (1996) "Measuring quality of life in children with asthma", *Quality of Life Research*, **5**, 35-46.

Juniper, E.F., Guyatt, G.H., Willan, A. and Griffith, L.E. (1994) "Determining a minimal important change in a disease-specific Quality of Life Questionnaire", *J Clin Epidemiol*, **47**, 81-87.

Kay, A.B. (2001) "Allergy and allergic diseases. First of two parts", *N Engl J Med*, **344**, 30-37.

Keskinoglu, P., Cimrin, D. and Aksakoglu, G. (2007) "Which cut-off level of urine cotinine:creatinine ratio (CCR) should be used to determine passive smoking prevalence in children in community based studies?", *Tobacco Control*, **16**, 358-359.

Klepeis, N.E., Nelson, W.C., Ott, W.R., Robinson, J.P., Tsang, A.M., Switzer, P., Behar, J.V., Hern, S.C., Engelmann, W.H. (2001) "The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants", *J Expo Anal Environ Epidemiol*, **11**, 231-252.

Kostikas, K., Koutsokera, A., Papiris, S., Gourgoulianis, K.I. and Loukides, S. (2008) "Exhaled breath condensate in patients with asthma: implications for application in clinical practice", *Clin Exp Allergy*, **38**, 557-565.

Landrigan, P.J., Schechter, C.B., Lipton, J.M., Fahs, M.C. and Schwartz, J. (2002) "Environmental pollutants and disease in American children: estimates of morbidity, mortality, and costs for lead poisoning, asthma, cancer, and developmental disabilities", *Environ Health Perspect*, **110**, 721-728.

Lawson, J.A., Dosman, J.A., Rennie, D.C., Beach, J., Newman, S.C. and Senthilselvan, A. (2011) "The association between endotoxin and lung function among children and adolescents living in a rural area", *Can Respir J*, **18**, e89-94.

Mathiesen, M., Pedersen, E.K., Bjorseth, O., Egeberg, K.W. and Syversen, T. (2004) "Heating of indoor dust causes reduction in its ability to stimulate release of IL-8 and TNFalpha in vitro compared to non-heated dust", *Indoor Air*, **14**, 226-234.

Mazique, D., Diette, G.B., Breysse, P.N., Matsui, E.C., Mccormack, M.C., Curtin-
Brosnan, J., Williams, D.L., Peng, R.D. and Hansel, N.N. (2011) "Predictors of
airborne endotoxin concentrations in inner city homes", *Environ Res*, **111**, 614-
617.

McNamara, M., Thornburg, J., Semmens, E., Ward, T. and Noonan, C. (2013) "Coarse
particulate matter and airborne endotoxin within wood stove homes", *Indoor Air*,
2013.

McNamara, M.L., Noonan, C.W. and Ward, T.J. (2011) "Correction factor for continuous
monitoring of wood smoke fine particulate matter", *Aerosol and Air Quality
Research*, **11**, 315-322.

Michel, O., Kips, J., Duchateau, J., Vertongen, F., Robert, L., Collet, H., Pauwels, R. and
Sergysels, R. (1996) "Severity of asthma is related to endotoxin in house dust",
Am J Respir Crit Care Med, **154**, 1641-1646.

Miyata, R. and Van Eeden, S.F. (2011) "The innate and adaptive immune response
induced by alveolar macrophages exposed to ambient particulate matter",
Toxicol Appl Pharmacol, **257**, 209-226.

Montuschi, P., Ciabattoni, G., Paredi, P., Pantelidis, P., Du Bois, R.M., Kharitonov, S.A.
and Barnes, P.J. (1998) "8-Isoprostane as a biomarker of oxidative stress in
interstitial lung diseases", *Am J Respir Crit Care Med*, **158**, 1524-1527.

Montuschi, P., Corradi, M., Ciabattoni, G., Nightingale, J., Kharitonov, S.A. and Barnes,
P.J. (1999) "Increased 8-isoprostane, a marker of oxidative stress, in exhaled
condensate of asthma patients", *Am J Respir Crit Care Med*, **160**, 216-220.

Morgan, W.J., Crain, E.F., Gruchalla, R.S., O'connor, G.T., Kattan, M., Evans, R., Stout,
J., Malindzak, G., Smartt, E., Plaut, M., Walter, M., Vaughn, B. and Mitchell, H.
(2004) "Results of a Home-Based Environmental Intervention among Urban
Children with Asthma", *New England Journal of Medicine*, **351**, 1068-1080.

Neveu, W.A., Allard, J.L., Raymond, D.M., Bourassa, L.M., Burns, S.M., Bunn, J.Y.,
Irvin, C.G., Kaminsky, D.A. and Rincon, M. (2010) "Elevation of IL-6 in the
allergic asthmatic airway is independent of inflammation but associates with loss
of central airway function", *Respir Res*, **11**, 1465-9921.

Nocker, R.E., Schoonbrood, D.F., Van De Graaf, E.A., Hack, C.E., Lutter, R., Jansen,
H.M. and Out, T.A. (1996) "Interleukin-8 in airway inflammation in patients with
asthma and chronic obstructive pulmonary disease", *Int Arch Allergy Immunol*,
109, 183-191.

Noonan, C.W. and Ward, T.J. (2012) "Asthma randomized trial of indoor wood smoke (ARTIS): Rationale and methods", *Contemp Clin Trials*, **33**, 1080-1087.

Papineni, R.S. and Rosenthal, F.S. (1997) "The size distribution of droplets in the exhaled breath of healthy human subjects", *J Aerosol Med*, **10**, 105-116.

Park, J.H., Spiegelman, D.L., Gold, D.R., Burge, H.A. and Milton, D.K. (2001) "Predictors of airborne endotoxin in the home", *Environ Health Perspect*, **109**, 859-864.

Piotrowski, W.J., Majewski, S., Marczak, J., Kurmanowska, Z., Gorski, P. and Antczak, A. (2012) "Exhaled breath 8-isoprostane as a marker of asthma severity", *Arch Med Sci*, **8**, 515-520.

Quackenboss, J.J., Lebowitz, M.D. and Krzyzanowski, M. (1991) "The normal range of diurnal changes in peak expiratory flow rates. Relationship to symptoms and respiratory disease", *Am Rev Respir Dis*, **143**, 323-330.

Respiratory Research, I. (2005) Studying cytokines in EBC, In: Respiratory Research, I. (ed) *http://www.rtube.com/library/cytokines-EBC.htm*, Charlottesville, VA, Respiratory Research, Inc., 1.

Robinson, D.S., Hamid, Q., Ying, S., Tsicopoulos, A., Barkans, J., Bentley, A.M., Corrigan, C., Durham, S.R. and Kay, A.B. (1992) "Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma", *N Engl J Med*, **326**, 298-304.

Robroeks, C.M., Rijkers, G.T., Jobsis, Q., Hendriks, H.J., Damoiseaux, J.G., Zimmermann, L.J., Van Schayck, O.P. and Dompeling, E. (2009) "Increased cytokines, chemokines and soluble adhesion molecules in exhaled breath condensate of asthmatic children", *Clin Exp Allergy*, **40**, 77-84.

Rosias, P. (2012) "Methodological aspects of exhaled breath condensate collection and analysis", *J Breath Res*, **6**, 1752-7155.

Rosias, P.P., Robroeks, C.M., Van De Kant, K.D., Rijkers, G.T., Zimmermann, L.J., Van Schayck, C.P., Heynens, J.W., Jobsis, Q. and Dompeling, E. (2010) "Feasibility of a new method to collect exhaled breath condensate in pre-school children", *Pediatr Allergy Immunol*, **21**, 1399-3038.

Semple, S. Devakumar, D., Fullerton, D.G., Thorne, P.S., Metwali, N., Costello, A., Gordon, S.B., Manandhar, D.S. and Ayres, J.G. (2010) "Airborne endotoxin concentrations in homes burning biomass fuel" *Environ Health Perspect*, **118**, 988.991.

Semple, S., Garden, C., Coggins, M., Galea, K.S., Whelan, P., Cowie, H., Sanchez-Jimenez, A., Thorne, P.S., Hurley, J.F. and Ayres, J.G. (2011) "Contribution of solid fuel, gas combustion, or tobacco smoke to indoor air pollutant concentrations in Irish and Scottish homes", *Indoor Air*, **22**, 212-223.

Shahid, S.K., Kharitonov, S.A., Wilson, N.M., Bush, A. and Barnes, P.J. (2002) "Increased Interleukin-4 and Decreased Interferon-Œ≥ in Exhaled Breath Condensate of Children with Asthma", *American Journal of Respiratory and Critical Care Medicine*, **165**, 1290-1293.

Soukup, J.M. and Becker, S. (2001) "Human Alveolar Macrophage Responses to Air Pollution Particulates Are Associated with Insoluble Components of Coarse Material, Including Particulate Endotoxin", *Toxicology and Applied Pharmacology*, **171**, 20-26.

Tang, M.L., Coleman, J. and Kemp, A.S. (1995) "Interleukin-4 and interferon-gamma production in atopic and non-atopic children with asthma", *Clin Exp Allergy*, **25**, 515-521.

Thomas, P.S., Lowe, A.J., Samarasinghe, P., Lodge, C.J., Huang, Y., Abramson, M.J., Dharmage, S.C. and Jaffe, A. (2013) "Exhaled breath condensate in pediatric asthma: Promising new advance or pouring cold water on a lot of hot air? A systematic review", *Pediatr Pulmonol*, **48**, 419-442.

Thornburg, J., Rodes, C.E., Lawless, P.A. and Williams, R. (2009) "Spatial and temporal variability of outdoor coarse particulate matter mass concentrations measured with a new coarse particle sampler during the Detroit Exposure and Aerosol Research Study", *Atmospheric Environment*, **43**, 4251-4258.

Thorne, P.S. (2000) "Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation", *Toxicology*, **152**, 13-23.

Thorne, P.S. and Duchaine, C. (2007) Airborne bacteria and endotoxin, In: Cj, H., Rl, C., Jl, G., Da, L., Al, M. and Ld, S. (eds) *Manual of Environmental Microbiology*, Washington, DC, ASM Press, 989-1004.

Walker, C., Bode, E., Boer, L., Hansel, T.T., Blaser, K. and Virchow, J.C., Jr. (1992) "Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage", *Am Rev Respir Dis*, **146**, 109-115.

Williams, R., Rappold, A.G., Case, M., Schmitt, M., Stone, S., Jones, P., Thornburg, J. and Devlin, R.B. (2012) "Multi-pollutant exposures in an asthmatic cohort", *Atmospheric Environment*, **61**, 244-252.

Williams, R.O.N., Rea, A., Vette, A., Croghan, C., Whitaker, D., Stevens, C., Mcdow, S., Fortmann, R.O.Y., Sheldon, L., Wilson, H., Thornburg, J., Phillips, M., Lawless, P., Rodes, C. and Daughtrey, H. (2009) "The design and field implementation of the Detroit Exposure and Aerosol Research Study", *Journal of Exposure Science & Environmental Epidemiology*, **19**, 643-659.

Yoo, Y., Kim, D.K., Yu, J., Choi, S.H., Kim, C.K. and Koh, Y.Y. (2007) "Relationships of methacholine and AMP responsiveness with peak expiratory flow variability in children with asthma", *Clin Exp Allergy*, **37**, 1158-1164.

CHAPTER 5

Conclusions

CONCLUSIONS

Impact on ARTIS

This project contributed several novel aspects to the ARTIS study and has provided results that will influence future analyses of ARTIS data. This project aimed to characterize airborne coarse fraction (PMc) and airborne endotoxin concentrations in homes with wood stoves, evaluate the efficacy of a filtration unit intervention on reduction of these pollutants, and establish cross sectional relationships between these exposures and a variety of health outcomes. Exposures were characterized in 50 homes with 43 homes successfully sampled pre- and post-intervention to evaluate intervention effectiveness. A total of 38 subjects had all appropriate exposure and health outcome data collected in full during their baseline winter. Overall, several main findings emerge as having the most potential to influence the future data analyses of the ARTIS program.

Endotoxin is found in biomass smoke (Kurmi, 2012) and wood-burning homes have shown higher airborne endotoxin concentrations than homes burning coal, peat, or using gas cooking (Semple, 2011). In addition, several home characteristics have been found to be associated with in-home endotoxin concentrations: rural living (Lawson, 2011, Barnig, 2012), increased number of residents (Singh, 2011, Thorne, 2009), number of pets (Thorne, 2009, Park, 2001, Sordillo, 2011), and dampness/moisture sources (Sordillo, 2011, Park, 2001). Ours is the first study to evaluate frequency of wood stove use (loading/stoking activities over 48 hours) as a potential predictor of airborne endotoxin concentrations. We found that the number of times the wood stove was loaded or stoked had no relationship to airborne endotoxin or PMc concentrations. Counter to our hypothesis, number of residents, number of pets, and humidity were not predictors of airborne endotoxin in these homes. In fact, no measured home characteristics explained the variability in airborne endotoxin concentrations in these homes.

The impact of this finding is augmented by another main conclusion of this project. The material used in the placebo filter was selected to ensure study participants were blinded to their intervention. The material looks to the participant like a real filter but only met specifications for removing particles >10 μm. Unexpectedly, homes receiving a placebo filter had a significant reduction (both overall and percent change) in PMc and airborne endotoxin. The results were so drastic that the active filter intervention, effective in reducing $PM_{2.5}$ by 90% relative to the placebo intervention, showed no enhanced ability to reduce PMc and airborne endotoxin relative to the placebo intervention. This is counter to our original hypothesis that indoor PMc concentrations would be more reduced in active filtration homes than in placebo filtration homes. This finding has a variety of impacts. In regard to the ARTIS program this finding not only validates the choice of active filter material in reducing indoor $PM_{2.5}$ concentrations but will also allow for the determination of $PM_{2.5}$ reduction-specific health effects. The placebo filter unknowingly acted as a control for PMc and endotoxin, reducing these as efficiently as the active filters. Health improvements from the active filter intervention relative to the placebo filter intervention will be primarily and convincingly due to $PM_{2.5}$ reduction. This is additionally important as pre-intervention $PM_{2.5}$ concentrations significantly correlated with PMc concentrations, making the determination of separate health effects difficult.

Another result from this project that will influence future ARTIS analyses is the finding that 50% filter-use compliance (based on kilowatt meter (KWM) readings) is enough to see the effects of the active and placebo filters. Filter compliance was a continual issue in the ARTIS program, with many participants complaining the units were too loud, caused cold air to be distributed around the house, and were too costly to run (despite reimbursements based on calculated energy use). Compliance is commonly an issue in intervention studies, as another study involving a stand-alone air filter intervention found that filter use was variable across the study population and declined

over the study duration (Batterman, 2012). The decision tree that will ultimately be used in the final analyses will be more complex to account for compliance at each visit. With winter averages for all exposure data, one KWM reading could be used for determining compliance versus non-compliance. Future analyses will validate if the sufficiency of 50% compliance found in this subset of homes applies to all active filter and placebo homes in the ARTIS study.

Global Impact

The results presented here have the potential to have implications far beyond the 200-mile radius around Missoula, MT, where sampling took place. First, as it was established that frequency of wood stove use was not a strong predictor of airborne endotoxin and that the placebo filter material effectively reduces PMc and airborne endotoxin, it can be inferred that the placebo filter material may also be effective in reducing PMc and airborne endotoxin in non-wood stove homes. As endotoxin is a potent asthma exacerbating agent (Liu, 2004), a low-cost strategy to reduce in-home exposures can have a lasting impact. Most studies involving in-home strategies to reduce exposure to asthma exacerbating agents take place in urban environments (Vojta, 2002, Morgan, 2004) for several reasons. Multiple asthma exacerbaters such as ozone, nitrogen dioxide and particulate matter are elevated in urban areas (Sarnat, 2007). Population centers are also convenient for human studies as there are more eligible participants and homes visits are more feasible in a small geographical area. Therefore the results of this study are not limited to wood stove homes in the western United States.

Along with the unexpected efficacy of the placebo filters to reduce PMc and airborne endotoxin, we also determined through sensitivity analyses that these significant reductions are seen when participants run their unit on the "high" setting for half of the requested time (i.e., 50% compliant). This finding will impact the ARTIS study, as described above. This finding may also impact the larger research field of indoor biomass smoke. The immense global burden of disease from indoor biomass smoke exposure has been described in previous chapters. Air filtration has been shown to reduce PM in a wood stove home (Hart, 2011) as well as reduce biomarkers of inflammation in healthy adults living in a wood smoke impacted community in Canada (Allen, 2009). However, no studies have investigated the efficacy of air filters in reducing in-home exposure to biomass smoke in developing countries where PM exposures are

135

exponentially higher than in developed countries. An initial hurdle to this research may include the lack of reliable power sources. Poor and rural areas that are dependent on biomass for fuel commonly have no access to cleaner burning gas or electricity (Mortimer, 2012). Approximately 30% of the 534 homes taking part in the RESPIRE study in Guatemala (Smith, 2011) and ~25% of over 3,500 homes taking part in a birth weight study in Zimbabwe (Mishra, 2004) had no electricity. Nevertheless, the impact of an inexpensive filter intervention (such as the placebo filter material) run at least 50% of the time in these homes may have dramatic results on exposures and adverse health effects.

The primary mechanism of PMc is thought to be via the endotoxin-toll-like receptor (TLR) 4 pathway (Miyata, 2011), which is biologically plausible as PMc has endotoxin present at levels 10-fold that of $PM_{2.5}$ in ambient and indoor environments (Heinrich, 2003, Menetrez, 2009). In-home endotoxin concentrations have been linked to an increase in prevalence of wheeze (Horick, 2006, Ryan, 2009) and asthma prevalence (Thorne, 2005). In 38 asthmatic children living in wood stove homes increases in PMc and airborne endotoxin showed different and distinct effects on lung function and biomarkers of inflammation and oxidative stress. This is contrary to our hypothesis that elevated coarse fraction and associated airborne endotoxin exposure will correlate with adverse health outcomes in asthmatic children. Increased PMc was associated with more days of peak expiratory flow (PEF) variability >20% and reduced odds ratios for having above median proinflammatory cytokines in exhaled breath condensate (EBC) (IL-6 and IL-8). Airborne endotoxin (EU/m^3), on the other hand, was associated with an increased odds ratio of having above median 8-isoprostane, a marker of oxidative stress, as well as Th1 cytokines that prime the immune system for elimination of viral and bacterial pathogens (IL-2 and IFN-γ) (Crinnion, 2012). These results, though limited due to their cross-sectional nature, reveal several points of interest.

136

First, as a whole we found a more general pro-inflammatory and oxidative stress response to increased endotoxin exposure than the phenotypic allergic asthma response described in the literature. The interpretation of this finding is most likely not that these subjects living in a unique environment (wood stove homes) have a Th1/Th2 balance different than that of asthmatics documented repeatedly in the literature. The atopic status of these 38 subjects is not yet available and it is specifically atopic asthmatics, not all asthmatics, which have a phenotypic Th2-mediated response to endotoxin exposure. The atopic status of these subjects will clarify the potential bias not accounted for in these results.

These results also provide insight into the unique adverse health effects of PMc and airborne endotoxin exposure. When endotoxin concentrations were presented per mass of PMc collected (EU/mg), there was a significant association with lower percent-predicted PEF values, a relationship that did not occur with PMc concentrations or endotoxin per volume of air sampled (EU/m^3). PMc and airborne endotoxin had differential effects on all health outcomes measured. In the most drastic case, an increase in PMc was associated with a significantly decreased odds ratio of having above median IL-8 while an increase in airborne endotoxin was associated with a significantly increased odds ratio of having above median IL-8. The inclusion of atopic status will eventually clarify whether these effects are consistent between atopic and nonatopic asthmatics or if atopic status may be driving several of these relationships. As of now these findings suggest that in these wood stove homes the mechanism of PMc toxicity is not solely due to endotoxin content, though these results need to be verified by taking into account the atopic status of the subjects.

Taken together these results are the first to characterize PMc and airborne endotoxin concentrations in wood stove homes as well as correlate these exposures with a variety of asthma health outcomes. They also provide the first evidence of the

extreme efficacy of the active filter intervention in reducing indoor PM$_{2.5}$ and the

unexpected efficacy of the placebo filter intervention in reducing indoor PMc and

airborne endotoxin. These results reveal a complete picture of exposure assessment,

exposure reduction, and health effects of PMc and airborne endotoxin in wood stove

homes.

REFERENCES

Allen, R.W., Carlsten, C., Karlen, B., Leckie, S., Van Eeden, S., Vedal, S., Wong, I. and Brauer, M. (2009) "An air filter intervention study of endothelial function among healthy adults in a woodsmoke-impacted community", *Am J Respir Crit Care Med*, **183**, 1222-1230.

Barnig, C., Reboux, G., Roussel, S., Casset, A., Sohy, C., Dalphin, J.C. and De Blay, F. (2012) "Indoor dust and air concentrations of endotoxin in urban and rural environments", *Lett Appl Microbiol*, **56**, 161-167.

Batterman, S., Du, L., Mentz, G., Mukherjee, B., Parker, E., Godwin, C., Chin, J.Y., O'toole, A., Robins, T., Rowe, Z. and Lewis, T. (2012) "Particulate matter concentrations in residences: an intervention study evaluating stand-alone filters and air conditioners", *Indoor Air*, **22**, 235-252.

Crinnion, W.J. (2012) "Do environmental toxicants contribute to allergy and asthma?", *Altern Med Rev*, **17**, 6-18.

Hart, J.F., Ward, T.J., Spear, T.M., Rossi, R.J., Holland, N.N. and Loushin, B.G. (2011) "Evaluating the effectiveness of a commercial portable air purifier in homes with wood burning stoves: a preliminary study", *J Environ Public Health*, **2011**, 27.

Heinrich, J., Pitz, M., Bischof, W., Krug, N. and Borm, P.J.A. (2003) "Endotoxin in fine (PM2.5) and coarse (PM2.5-10) particle mass of ambient aerosols. A temporo-spatial analysis", *Atmospheric Environment*, **37**, 3659-3667.

Horick, N., Weller, E., Milton, D.K., Gold, D.R., Li, R. and Spiegelman, D. (2006) "Home endotoxin exposure and wheeze in infants: correction for bias due to exposure measurement error", *Environ Health Perspect*, **114**, 135-140.

Kurmi, O.P., Lam, K.B. and Ayres, J.G. (2012) "Indoor air pollution and the lung in low- and medium-income countries", *Eur Respir J*, **40**, 239-254.

Lawson, J.A., Dosman, J.A., Rennie, D.C., Beach, J., Newman, S.C. and Senthilselvan, A. (2011) "The association between endotoxin and lung function among children and adolescents living in a rural area", *Can Respir J*, **18**, e89-94.

Liu, A.H. (2004) "Something old, something new: Indoor endotoxin, allergens and asthma", *Paediatric Respiratory Reviews*, **5**, S65-S71.

Menetrez, M.Y., Foarde, K.K., Esch, R.K., Schwartz, T.D., Dean, T.R., Hays, M.D., Cho, S.H., Betancourt, D.A. and Moore, S.A. (2009) "An evaluation of indoor and outdoor biological particulate matter", *Atmospheric Environment*, **43**, 5476-5483.

Mishra, V., Dai, X., Smith, K.R. and Mika, L. (2004) "Maternal exposure to biomass smoke and reduced birth weight in Zimbabwe", *Ann Epidemiol*, **14**, 740-747.

Miyata, R. and Van Eeden, S.F. (2011) "The innate and adaptive immune response induced by alveolar macrophages exposed to ambient particulate matter", *Toxicol Appl Pharmacol*, **257**, 209-226.

Morgan, W.J., Crain, E.F., Gruchalla, R.S., O'connor, G.T., Kattan, M., Evans, R., Stout, J., Malindzak, G., Smartt, E., Plaut, M., Walter, M., Vaughn, B. and Mitchell, H. (2004) "Results of a Home-Based Environmental Intervention among Urban Children with Asthma", *New England Journal of Medicine*, **351**, 1068-1080.

Mortimer, K., Gordon, S.B., Jindal, S.K., Accinelli, R.A., Balmes, J. and Martin, W.J., 2nd. (2012) "Household air pollution is a major avoidable risk factor for cardiorespiratory disease", *Chest*, **142**, 1308-1315.

Park, J.H., Spiegelman, D.L., Gold, D.R., Burge, H.A. and Milton, D.K. (2001) "Predictors of airborne endotoxin in the home", *Environ Health Perspect*, **109**, 859-864.

Ryan, P., Bernstein, D., Lockey, J., Reponen, T., Levin, L., Grinshpun, S., Villareal, M., Hershey, G., Burkle, J. and Lemasters, G. (2009) "Exposure to traffic-related particles and endotoxin during infancy is associated with wheezing at age 3 years", *American journal of respiratory and cricial care medicine*, **180**, 1068-1075.

Sarnat, J.A. and Holguin, F. (2007) "Asthma and air quality", *Curr Opin Pulm Med*, **13**, 63-66.

Semple, S., Garden, C., Coggins, M., Galea, K.S., Whelan, P., Cowie, H., Sanchez-Jimenez, A., Thorne, P.S., Hurley, J.F. and Ayres, J.G. (2011) "Contribution of solid fuel, gas combustion, or tobacco smoke to indoor air pollutant concentrations in Irish and Scottish homes", *Indoor Air*, **22**, 212-223.

Singh, U., Levin, L., Grinshpun, A., Schaffer, C., Adhikari, A. and Reponen, T. (2011) "Influence of home characteristics on airborne and dustborne endotoxin and B-D-glucan", *J. Environ. Monit.*, **13**, 3246-3253.

Smith, K.R., Mccracken, J.P., Weber, M.W., Hubbard, A., Jenny, A., Thompson, L.M., Balmes, J., Diaz, A., Arana, B. and Bruce, N. (2011) "Effect of reduction in household air pollution on childhood pneumonia in Guatemala (RESPIRE): a randomised controlled trial", *The Lancet*, **378**, 1717-1726.

Sordillo, J.E., Alwis, U.K., Hoffman, E., Gold, D.R. and Milton, D.K. (2011) "Home Characteristics as Predictors of Bacterial and Fungal Microbial Biomarkers in House Dust", *Environ Health Perspect*, **119**, 189-195.

Thorne, P.S., Cohn, R.D., Mav, D., Arbes, S.J. and Zeldin, D.C. (2009) "Predictors of endotoxin levels in U.S. housing", *Environ Health Perspect*, **117**, 763-771.

Thorne, P.S., Kulhankova, K., Yin, M., Cohn, R., Arbes, S.J., Jr. and Zeldin, D.C. (2005) "Endotoxin Exposure Is a Risk Factor for Asthma: The National Survey of Endotoxin in United States Housing", *Am. J. Respir. Crit. Care Med.*, **172**, 1371-1377.

Vojta, P.J., Friedman, W., Marker, D.A., Clickner, R., Rogers, J.W., Viet, S.M., Muilenberg, M.L., Thorne, P.S., Arbes, S.J., Jr. and Zeldin, D.C. (2002) "First National Survey of Lead and Allergens in Housing: survey design and methods for the allergen and endotoxin components", *Environ Health Perspect*, **110**, 527-532.

APPENDIX

Comprehensive Methods

COMPREHENSIVE METHODS

The following methods were required for successful completion of my project. Many abbreviated methods are summarized in previous chapters.

CPEM Procurement and Summary

Dr. Jonathan Thornburg *et al.* published a manuscript presenting ambient data from a new coarse particle sampler developed by RTI International (Research Triangle Park, NC, USA) in 2009 (Thornburg, 2009). The coarse particulate environmental monitor (CPEM) is a series of three separation stages designed to be inserted into the MSP Model 200 PM10 PEM (MSP Corp., Shoreview, MN). The CPEM operates on a battery operated pump to collect PMc on two, sequential 25mm Teflon filters. The CPEM's small dimensions (4.7 cm high, 4.2 cm wide), light weight (110g) low flow (2 Lpm) yet rugged construction allow the system to be deployed as a personal exposure monitor as well as a stationary indoor or outdoor monitor (Thornburg, 2009). Subsequent manuscripts from Dr. Thornburg's group at RTI International have demonstrated the usefulness of the CPEM (Williams, 2009, Williams, 2012).

These samplers appeared to have the potential to contribute a great deal of indoor air quality information to the ARTIS program. They were convenient for the small space provided for the sampling equipment in many homes and were financially viable to run and maintain. I contacted Dr. Thornburg in the fall of 2010 and he graciously provided us with nine CPEM units and accessories. THE CPEMs run on D batteries and rechargeable D batteries were tested in the laboratory. The CPEMs could run at the appropriate flow rate (2 Lpm) for up to 5 days with one charge.

<u>CPEM Assembly</u>

The first winter (November-March) of CPEM inclusion in the ARTIS program was 2010-2011. The Butte cohort, which had their baseline sampling that year, was managed by Stacey Harper. It was necessary to train Stacey on how to assemble, sample, disassemble and store CPEM samples. The CPEM Assembly Protocol (Figure A.1) was created to provide step-by-step instructions for the preparation of a field-ready CPEM. The CPEM stages were color-coded to reduce confusion. Stacey was also provided with sufficient 25mm and 37mm filters, rechargeable batteries, a battery charger, Drycal DC-Lite flow monitor (BIOS International, Butler, NJ, USA), environmental chamber and tools (C-ring pliers, screwdriver) to carry out the CPEM sampling.

Figure A.1. CPEM Assembly Protocol.

When the CPEM is fully assembled, the colors from top to bottom will be: red, black, blue. Remember: **blue=bottom**.

1. To disassemble the CPEM, remove the screws and green top.

The top piece you will see is the impactor, which does not need a filter.

2. Remove the impactor plate. You are now looking at the Red stage.

If you are disassembling to remove filters after sampling, you will see a filter instead of the bronze plate.

3. To insert/remove a 25mm Teflon filter into the Red stage, first use the C-clamp pliers to remove the C-ring under the Red stage (be careful that it doesn't fling off).

The bronze plate should come out easily. Using clean tweezers, carefully place the filter into the open space in the Red level. Replace the bronze plate and C-ring to hold it in place.

For reference, the "B" side of the bronze plate stands for "bottom", and should not come into contact with the filter.

4. The Black stage does not require a filter.

Remove this stage to find that the Blue stage is exactly like the Red Stage, and requires a 25mm Teflon filter on top of the bronze plate.

5. Insert a 25mm Teflon filter into the Blue stage following the instructions in step 3. Again, note the "B" on the plate for "bottom".

6. Under the Blue stage there is a metal screen, onto which a 37mm Teflon filter should be placed.

Now reassemble and you are ready to sample!

<u>CPEM Data Sheet</u>

The CPEM Data Sheet (Figure A.2) was designed to capture all pertinent information for each sampling visit. Set-up date, House ID, DryCal ID, and CPEM Pump ID were all recorded. During CPEM assembly the serial number of each filter used was recorded on the data sheet for tracking purposes. At this time the filter was assigned a Filter ID based on the community, date (MMDDYY), House ID, and filter placement within the CPEM. For example, a filter in the top stage of the CPEM sampled at House ID 603 on December 1st, 2010 would receive the filter ID BUT120110603_1. A filter in the second stage from the same CPEM would receive the filter ID BUT120110603_2. This helped track the filters and ensure the appropriate filters were combined for endotoxin analysis at a later time.

Once the CPEM was assembled in the laboratory and the serial numbers of filter IDs were recorded, an initial flow rate was taken using a DryCal flow monitor with the CPEM pump that would accompany the CPEM to the field. The CPEM was placed in a custom environmental chamber during all flow rate measurements. The 20th average from the DryCal was recorded on the CPEM Data Sheet. The duration of sampling was determined using the start date/time and stop date/time. Following sampling, a final flow rate (20th average) reading was taken in the laboratory using the CPEM pump that had accompanied the CPEM to the field. An average flow of initial and final flow rate was used in calculation of volume of air sampled. Volume of air sampled was calculated as: ((Average flow rate (Lpm)*Total sample time (min))/1000). For example, if a CPEM ran for exactly 48 hours (2,880 minutes) at 2 LPM then it would have sampled 5.76 m^3. The appropriate volume for each sampling duration was used to calculate particle (μg/m^3) and endotoxin (EU/m^3) concentrations from each home. All data was entered into a master spreadsheet for analysis.

Figure A.2. CPEM Data Sheet.

Data Sheet for CPEM Sampling

Technician:_____   Set-up Date:_____

House ID:_____

DryCal ID:_____

Pump ID: _____

Start Date:_____(mm/dd/yy)   Stop Date:_____(mm/dd/yy)

Start Time:_____(hh:mm, military) Stop Time:_____(hh:mm, military)

Total Sample Time:_____min

Initial Flow Rate (20th average from DryCal):_____L/min

Adjusted Flow (if applicable):_____L/min

Final Flow Rate (20th average from DryCal):_____L/min

Average Calculated Flow Rate: _____L/min

Total Calculated Volume: _____L

Petri Slide IDs (i.e. MSO111209119 1)

Filter Placement	Serial Number	Filter ID (i.e. MSO111209119 1)
Top (25 mm: 1)		
Middle (25 mm: 2)		
Bottom (37 mm: 3)		

Comments_____

_____

CPEM Field Methods

CPEMs were transported to and from homes in a ziplock bag in a cooler with an ice
pack. This was to reduce any contamination of the filters and also to reduce the loss of
volatiles post-sampling. Unlike all the other instruments in the ARTIS program, the
CPEM does not have a mechanism to program start and stop times, therefore the
precise time that the CPEM ran was determined by when the field worker plugged in the
batteries and when they took them out. These times were recorded on the CPEM data
sheet.

Filters used to collect particulate matter are sensitive to the weighing environment. Humidity, temperature, drafts, vibration, and electrostatic charges on the filters can influence gravimetric microbalance operations and efforts should be made to minimize or eliminate them (Lawless, 1999). This is particularly critical for samples with small mass collections. Without a controlled weighing environment at the University of Montana, teflon filters used in the CPEMs would have to be pre- and post-weighed at a contracted facility, drastically increasing costs. Under the guidance of Dr. Maria Morandi, I established an environmentally controlled weighing facility in the Inhalation and Pulmonary Physiology Core.

The weighing room, located in Skaggs 061C, has temperature controls that allow the temperature to be maintained at 20-23 °C ± 2 °C. Several attempts at finding a humidifier that could keep the relative humidity (rH) at 30-40% ± 5% over 48-hours were performed, with the final result being the use of an 8-gallon Kenmore Whole House humidifier (model 758.15408). This humidifier has an rH sensor that will run the unit only when necessary to maintain a pre-set humidity. The accuracy and sensitivity of this built-in sensor was tested against a Q-trak (TSI Inc., Shoreview, MN, USA), used in the ARTIS program, as well as a HOBO UX100 humidity data logger (Onset Corp., Cape Cod, MA, USA) for multiple 48-hour trials. 24-hour averages were consistent between instruments with relative humidity maintained between 30-40% with a standard deviation less than five (Green, 2003).

Static interference was minimized in the weighing room in two ways. First, the scale operator's chair was kept on a grounded anti-static floor mat (ComfortKing USA, Inc., Fairfield, NJ, USA). Static charge on filters is one of the most difficult external influence to address (Lawless, 1999). We addressed this issue by using a radioactive neutralizer (Thermo Fisher Scientific Inc., Waltham, MA, USA), the most common

method of static elimination. All filters were held next to the neutralizer for 30 seconds prior to weighing. These neutralizers use a small amount of polonium-210, an alpha particle emitter, to provide sufficient numbers of bipolar ions to bring static charges into near-neutral equilibrium (Lawless, 1999).

Vibration was also minimized in two ways. The weighing facility is located in the basement, reducing the effect of building vibrations. A heavy, damped balance table with rubber isolators was placed under the microbalance to further reduce any effects of vibration.

Following validation of temperature and humidity stability and removal of static and vibration interferences, the weighing facility was ready to be used. Prior to sampling, each filter was placed into individually labeled sterile polystyrene Analyslide containers (Pall Corp., Ann Arbor, MI, USA) labeled with its unique serial number and allowed to equilibrate for 24-48 hours in the weighing facility. RTI International provided their Standard Operating Procedures for Gravimetric Analysis, which included a 27-step protocol to be performed each time filters were weighed (Green, 2003). Of note is that every tenth filter is reweighed with replicate filter weights required to vary by no more than 15 μg. A set of filters (one 25 mm and one 37 mm) was weighed at each weighing event and were also required to vary by no more than 15 μg. Filters were weighed prior to and after each sampling event with a calibrated MT5 microbalance (Mettler-Toledo LLC, Columbus, OH, USA). All of these conditions were met with the teflon filters used in the CPEMs.

Predetermined filters that were not sent to the field (laboratory blanks) were weighed at every weighing event and were within the repeatability of the scale (0.8 µg). Field blanks were prepared the same as other CPEMS and were brought to a home, taken out of their bag, placed back into their bag and brought back to the laboratory for disassembly. Pre- and post-flow rates were also recorded for field blanks. This scenario allows for all potential background contamination to occur to the filters independent of in-home sampling. A minimum of 10% field blanks were used each winter and were weighed and analyzed the same as all other filters. All field blanks from all winters varied by no more than 15 µg, the standard for laboratory blanks. All field blank filter extractions were below the limit of detection for endotoxin. Therefore no adjustments were necessary in the data analyses from the CPEM filters.

Endotoxin Extraction & Analysis

Endotoxin extraction and analysis does not have a standardized protocol. To optimize

extraction from teflon filters (Spaan, 2007), I created a protocol for endotoxin extraction

that takes place in pyrogen-free water (PFW) containing 0.05% Tween20 (Figure A.3).

PFW is created by autoclaving ultrapure water in glassware that has been sterilized in a

muffle furnace by heating at 250°C for 60 minutes (Hecker, 1994). This method is also

accepted for removal of endotoxin from pharmaceutical agents by the Food and Drug

Administration (FDA, 1984). My endotoxin analysis protocol using a kinetic chromogenic

Limulus amebocyte lysate (LAL) assay (Endosafe Endochrome-K; Charles River

Laboratories Inc., Charleston, SC, USA) was optimized for extracts from Teflon filters

(Thorne, 2000). All samples were diluted 1:50 in PFW prior to analysis to account for

Tween inhibiting the reactivity of the lipopolysaccharide (LPS) standard, thus shifting the

calibration curve to higher values (Spaan, 2008) (Figure A.4)

Figure A.3. Endotoxin extraction protocol.

Adapted from Spaan et al. 2007, 2008   Modified on 4/2/12 by MM

Endotoxin Extraction Protocol: Teflon Filters

1. Sterilize glassware in muffle furnace for 30 min. at 270°C.

2. Obtain enough ultrapure water for extractions, autoclave water in sterilized glassware from Step 1 to produce pyrogen-free water (PFW).

3. Put coarse fraction filters (2) and fine fraction filters (1) into labeled sterile conicals.

4. Add 5 ml PFW containing 0.05% Tween 20*[i] to conicals.

5. Agitate conical vigorously for 1 hour to remove all endotoxin from filters.

5. Centrifuge conicals at 1,000 x g for 15 min.

6. Transfer extract liquid into new sterile conical.

Use extract for analysis. Store any remaining extract in freezer after use.

---

[i] Easiest to add 0.05% Tween 20 to larger volume of PFW, but make sure to save some PFW without Tween 20 for background measurements and assay medium.

Figure A.4. Endotoxin analysis protocol.

Adapted from Thorne (2000) and Spaan (2008)   4/4/12 by MM

<u>Endosafe Endochrome-K LAL Protocol</u>

1. Reconstitute E. coli Control Standard Endotoxin (CSE) according to Certificate of Analysis to achieve desired stock solution.
      -Vortex vigorously for at least 5 minutes after rehydration, or 1 minute immediately prior to use

2. Follow steps for serial dilution for standard curve (made with CSE stock and LAL reageant water; bottom of page)

3. Rehydrate LAL with 3.2-3.4 ml LAL Reagent Water
      - 4 vials needed for full 96-well plate
      - allow to achieve room temperature before use

4. Prepare plate-reader program

4. Transfer 100 ul of each sample to microplate
- samples should be diluted 1:50 in PFW (Spaan 2008) (20 ul sample + 980 ul PFW)

5. Quickly add 100 ul of LAL (ambient temperature) to each well using multi-dispenser, beginning with negative control and ending with highest endotoxin concentration

6. Insert plate in plate-reader (set to 37°C). Mix.

7. Begin program (read at 405 nm every 30 seconds for 90 minutes).
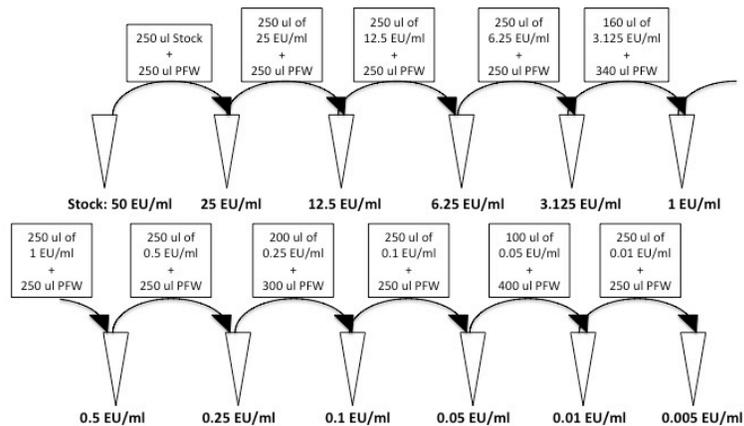
Total Protein Issues

As part of my original research proposal I had intended to quantify total protein within the coarse fraction as well as endotoxin. However, initial total protein analysis of the PMc filter extracts using a NanoOrange Protein Quantitation Assay (Life Technologies, Grand Island, NY, USA) gave unexpected results, with field blank extracts consistently having higher total protein content than regular samples. All field blanks were below the limit of detection in endotoxin analyses. I performed a series of filter spike experiments and determined, through communication of my results with the kit manufacturer, that the Tween in the extracts was interfering with the standard curve. Therefore total protein can not be quantified in the CPEM samples without removal of the Tween. This issue has the potential to be resolved at a future time.

Filter Use Compliance

Several *a priori* decisions needed to be made in order to evaluate filter use compliance

via kilowatt meter (KWM) readings. Predicted daily KWM values were calculated for the

large and small filter units through laboratory trials. KWM readings were available from

41 out of 43 homes included in my analyses. KWM readings were used from installation

through the second filter change, capturing all relevant in-home sampling. Two homes

did not have KWM readings until the third filter change; in these cases the third filter

change value was used. A schematic of the workflow is provided (Figure A.5). The

lowest level of compliance was deemed appropriate if there were notes from the field

validating that at least one KWM was dysfunctional (n=2) or the home ran both units

continuously on the Medium setting (n=1; n=36 total). The next level of compliance

included home with at least one unit >50% of the predicted KWM value (n=33). The most

strict level of compliance occurred where the average (large and small) KWM reading

was >50% of the predicted KWM value (n=28). Sensitivity analyses were performed at

all levels.

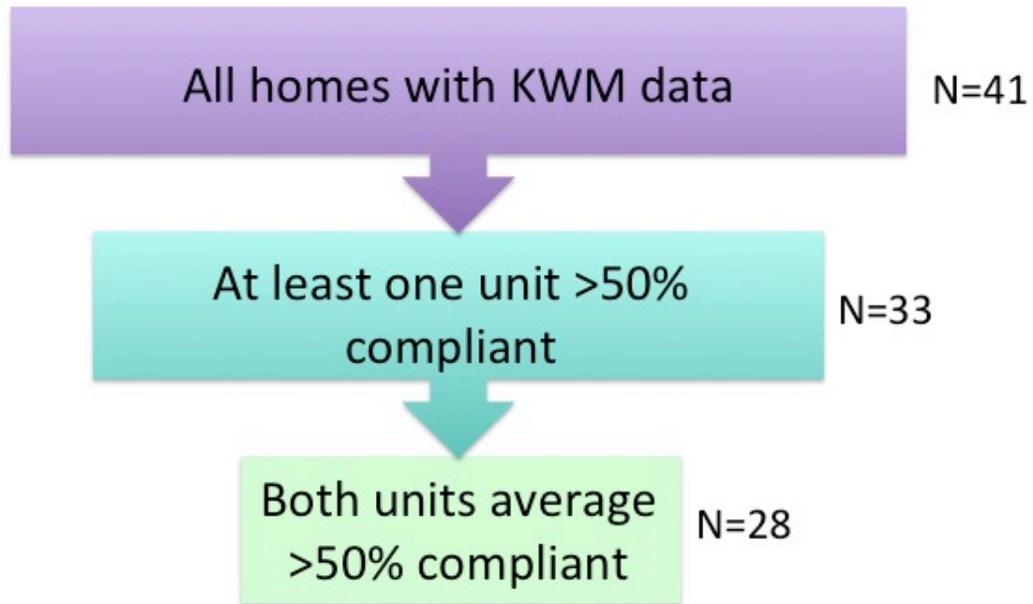**Figure A.5.** KWH compliance workflow.

Figure A.5. KWH compliance workflow.

REFERENCES

FDA. (1984) 1983 code of Federal Regulations, In: Service, P. H. (ed), Vol. 48, Food and Drug Administration, 13096.

Green, L.C. and Jayanty, R.K.M. (2003) Standard Operating Procedure for PM2.5 Gravimetric Analysis, In: Montana, T. U. O. (ed), Research Triangle Park, Research Triangle Institute, 24.

Hecker, W., Witthauer, D. and Staerk, A. (1994) "Validation of Dry Heat Inactivation of Bacterial Endotoxins", *PDA Journal of Pharmaceutical Science and Technology*, **48**, 197-204.

Lawless, P.A. and Rodes, C.E. (1999) "Maximizing data quality in the gravimetric analysis of personal exposure sample filters", *J Air Waste Manag Assoc*, **49**, 1039-1049.

Spaan, S., Doekes, G., Heederik, D., Thorne, P.S. and Wouters, I.M. (2008) "Effect of extraction and assay media on analysis of airborne endotoxin", *Appl Environ Microbiol*, **74**, 3804-3811.

Spaan, S., Heederik, D.J.J., Thorne, P.S. and Wouters, I.M. (2007) "Optimization of Airborne Endotoxin Exposure Assessment: Effects of Filter Type, Transport Conditions, Extraction Solutions, and Storage of Samples and Extracts", *Appl. Environ. Microbiol.*, **73**, 6134-6143.

Thornburg, J., Rodes, C.E., Lawless, P.A. and Williams, R. (2009) "Spatial and temporal variability of outdoor coarse particulate matter mass concentrations measured with a new coarse particle sampler during the Detroit Exposure and Aerosol Research Study", *Atmospheric Environment*, **43**, 4251-4258.

Thorne, P.S. (2000) "Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation", *Toxicology*, **152**, 13-23.

Williams, R., Rappold, A.G., Case, M., Schmitt, M., Stone, S., Jones, P., Thornburg, J. and Devlin, R.B. (2012) "Multi-pollutant exposures in an asthmatic cohort", *Atmospheric Environment*, **61**, 244-252.

Williams, R.O.N., Rea, A., Vette, A., Croghan, C., Whitaker, D., Stevens, C., Mcdow, S., Fortmann, R.O.Y., Sheldon, L., Wilson, H., Thornburg, J., Phillips, M., Lawless, P., Rodes, C. and Daughtrey, H. (2009) "The design and field implementation of the Detroit Exposure and Aerosol Research Study", *Journal of Exposure Science & Environmental Epidemiology*, **19**, 643-659.