HYBRID LAND USE REGRESSION MODELING OF FINE PARTICULATE MATTER AND METAL COMPONENTS FOR APPLICATION IN TWO PITTSBURGH COHORTS

by

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ABSTRACT

While numerous studies have linked exposure to ambient fine particulate matter ($PM_{2.5}$) to adverse health outcomes (e.g., asthma, cardiovascular disease), less is known about which specific components of $PM_{2.5}$ drive these associations. Because $PM_{2.5}$ composition varies spatially with sources, characterizing fine-scale variation in constituents is critical to improving epidemiological studies on health effects of source-specific $PM_{2.5}$. One approach for improving this characterization may be hybrid models wherein source-specific dispersion covariates are integrated into land use regression models (LURs).

The objective of this dissertation was to develop hybrid dispersion-LUR models for $PM_{2.5}$, black carbon (BC), and steel-related $PM_{2.5}$ constituents [lead (Pb), manganese (Mn), iron (Fe), and zinc (Zn)], by combining concentrations data from spatial saturation monitoring with daily Environmental Protection Agency (EPA) regulatory data. These models were used to assign residence-based exposure estimates for time windows of interest for two Pittsburgh-area epidemiological cohorts.

The first epidemiologic study examined associations between one-year pollutant exposures and levels of both circulating and lipopolysaccharide (LPS)-stimulated inflammatory mediators in the Adult Health and Behavior II (AHAB II) cohort. We found that exposures to $PM_{2.5}$ and BC were associated with higher LPS-stimulated IL-1β, IL-6, and TNF-α. Pb was associated with increased stimulated TNF-α ($p = 0.02$) and IL-1β ($p = 0.02$), but were insignificant after adjusting for multiple comparisons (Bonferroni correction). No pollutant exposures were associated with circulating IL-6 or CRP. The second epidemiological study explored associations between pollutant exposures and brain morphology indicators (i.e., total and cortical gray matter volumes, cortical white matter volume, total white matter surface area, mean cortical thickness) from magnetic resonance images of participants in the AHAB II and Pittsburgh Imaging Project Cohorts, finding no significant associations.

These results suggest that, although pollutants were not associated with circulating inflammatory mediators or brain morphology in these samples of healthy midlife adults, some chronic air pollution exposures may influence immune responsiveness, influencing risk for future inflammatory conditions. Taken together, these results indicate the public health importance of better understanding relationships between long-term source-specific $PM_{2.5}$ and component exposures with functional indicators of immune responsiveness and other processes shaping risk for future health effects.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to thank my advisor Dr. Jane E. Clougherty for helping me become a stronger researcher and writer. I am grateful to have had the opportunity to work on a wide range of exposure science and environmental epidemiology related projects. The work presented in this dissertation would not have been possible without contributions by Dr. Brett Tunno, Dr. Drew Michanowicz, and Ellen Kinnee. I am also grateful for the opportunity to have worked with and learned from Sara Gillooly and Dr. Jessie LC. Shmool.

The research presented in chapters 3 and 4 of this dissertation was the result of a collaboration with the University of Pittsburgh Department of Psychology and would not have been possible without contributions from Dr. Peter Gianaros, Dr. Anna Marsland, and Dr. Stephen Manuck.

Many thanks to the faculty and staff at the University of Pittsburgh Department of Environmental and Occupational Health as well as members of my dissertation committee Dr. Aaron Barchowsky and Dr. Fernando Holguin.

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Finally, I would like to thank my family and friends for their support throughout this process.

I dedicate this dissertation in memory of my dad, Dr. Sukant K. Tripathy, who came to the United States from India to pursue his Ph.D.

ABBREVIATIONS

ACHD	Allegheny County Health Department
AERMOD	American Meteorological Society/Environmental Protection Agency Regulatory Model
AHAB II	Adult Health and Behavior II
AQS	Air Quality System
BC	Black carbon
CAA	Clean Air Act
CRP	C-reactive protein
EPA	Environmental Protection Agency
Fe	Iron
GIS	Geographic information systems
IDW	Inverse distance weighted
IL -1β	Interleukin - 1 beta
IL-6	Interleukin-6
LPS	Lipopolysaccharide
LUR	Land use regression
Mn	Manganese

MRI	Magnetic resonance image
NAAQS	National Ambient Air Quality Standards
NEI	National Emissions Inventory
NLCD	National Land Cover Database
Pb	Lead
PIP	Pittsburgh Imaging Project
PM	Particulate matter
SD	Standard deviation
TNF-α	Tumor necrosis factor alpha
Zn	Zinc

1.0 INTRODUCTION

1.1 DISSERTATION OBJECTIVES

The overall objective of this dissertation was to develop hybrid dispersion-LUR models for $PM_{2.5}$, black carbon (BC), and steel-related $PM_{2.5}$ constituents [lead (Pb), manganese (Mn), iron (Fe), and zinc (Zn)], by combining concentrations data from spatial saturation monitoring with daily Environmental Protection Agency (EPA) regulatory data. These models were used to assign residence-based exposure estimates for time windows of interest for two Pittsburgh-area epidemiological cohorts. The first epidemiologic study examined associations between one-year pollutant exposures and levels of both circulating and lipopolysaccharide (LPS)-stimulated inflammatory mediators in the Adult Health and Behavior II (AHAB II) cohort. The second epidemiological study explored associations between pollutant exposures and brain morphology indicators from magnetic resonance images of participants in the AHAB II and Pittsburgh Imaging Project Cohorts. Specific goals for each dissertation chapter are as follows:

Chapter 2: Develop hybrid dispersion LUR models for $PM_{2.5}$, BC, and steel-related Pb, Mn, Fe, and Zn metal constituents for use in epidemiological studies.

Chapter 3: Examine associations between one-year residence-based pollutant exposures with circulating and LPS-stimulated inflammatory mediators in the AHAB II cohort.

Hypothesis: Elevated exposures to $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe will be associated with higher levels of circulating inflammatory mediators (Il-6 and CRP), and LPS-stimulated production of cytokines (IL-6, IL-1β, and TNF-α).

Chapter 4: Explore the relationship between one-year pollutant exposures with total and cortical gray matter volumes, cortical white matter volume, total white matter surface area, and mean cortical thickness measures of brain morphology in AHAB II and PIP cohorts.

Hypothesis: Higher residence-based exposures to $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe will be associated with reduced structural integrity of the brain in two Pittsburgh cohorts of health middle-aged adults.

The remainder of Chapter 1 includes background information pertaining to Chapters 2-4.

1.2 BACKGROUND

1.2.1 Particulate Matter

Particulate matter is composed of solid and/or liquid particles and composition varies depending on factors including location, temperature and emission sources. Depending on these and other factors, PM can be composed of acids, metals, and organic compounds including dust and allergens. Two commonly measured size fractions of PM are PM_{10} - coarse particles that have a diameter of 10 micrometers or less and $PM_{2.5}$ - fine particles with a diameter of 2.5 μm or less. Examples of PM_{10} include particles such as pollen and spores while $PM_{2.5}$ may include combustion-related particles such as smoke. (Anderson et al. 2012; Dockery 2009; EPA 2016c). $PM_{2.5}$ enters the lungs through normal breathing and smaller size fractions of PM such as $PM_{2.5}$

can penetrate deep into the lung and certain components may enter the blood stream, leading to a wide range of adverse health effects. For the purposes of this dissertation we focus here on cardiovascular and central nervous system related effects of $PM_{2.5}$ (EPA 2016c).

While $PM_{2.5}$ composition varies depending on the source, these components may also differ in toxicity potentially leading to differences in associated health effects (Bell et al. 2014; Franklin et al. 2008). The work in this dissertation focuses on health impacts associated with black carbon (BC), lead (Pb), manganese (Mn), iron (Fe), and zinc (Zn), components of $PM_{2.5}$.

Black carbon (BC) is a component of PM formed from incomplete combustion of fuels. The majority of BC emissions in the United States are from traffic-related sources including diesel sources. Other sources include biomass burning, residential heating, and industrial emissions (EPA 2016a). BC, similar to total $PM_{2.5}$, has been associated with numerous health effects including hospital admissions, cardiovascular mortality and morbidity (Bell et al. 2014; Grahame et al. 2014; Organization 2012). A review by the World Health Organization in 2012 suggests that BC may be a better indicator of combustion sources compared to $PM_{2.5}$ mass (Organization 2012).

Lead is a toxic metal that has both natural and anthropogenic sources. Some of these sources include industrial and traffic-related emissions, residual lead from gasoline, and lead paint in older homes. Lead paint was banned in the United States in 1978 and lead in gasoline was phased out and banned in 1995 but both persist in the environment. Urban soil may be contaminated due to paint from older buildings and industrial emissions deposited in soil. Lead targets the nervous system which can lead to adverse health effects including learning disabilities and behavioral issues. Lead disproportionately affects children because of their developing brains (Agency 1996; CDC 2016). In contrast to lead, Mn, Zn, and Fe metal constituents of

$PM_{2.5}$ examined in this dissertation are essential nutrients and toxicity depends on the dose of each metal. Pb, Mn, Zn, and Fe all have sources related to steel production and traffic-related emissions. In terms of traffic-related sources, Pb, Zn, and Fe had motor vehicle sources, all had soil, and dust suspension sources and Mn, Zn, and Fe had brake and tire wear related sources (Tunno et al. 2015a). All four of these metals have also been found to target the central nervous system (Gorell et al. 1998; Kim et al. 2011; Rouault 2013; Sensi et al. 2009).

1.2.2 $PM_{2.5}$ regulation in the United States

The Environmental Protection Agency (EPA) has set limits for exposure to both $PM_{2.5}$ and PM_{10} as part of the National Ambient Air Quality Standards as part of the Clean Air Act (CAA) established in 1970 (Anderson et al. 2012). The EPA sets exposure thresholds for six primary air pollutants: carbon monoxide, lead, nitrogen dioxide, ozone, sulfur dioxide, and PM_{10} and $PM_{2.5}$. Primary and secondary standards were developed for each of these pollutants. Primary standards are in place to protect public health, especially sensitive people like children, people with asthma, and the elderly. Secondary standards are limits set for protection of public welfare by managing reduced visibility, monitoring damage to animals, crops, and vegetation, and regulating buildings. Relevant to the research presented in this dissertation, the EPA sets exposure thresholds for $PM_{2.5}$ and airborne Pb. Current standards limit exposure to $PM_{2.5}$ (three year rolling average) at 12 $\mu g/m^3$ as a primary standard and 15 $\mu g/m^3$ as a secondary standard. Exposure to airborne Pb is not to exceed 0.15 $\mu g/m^3$ over a three month average as both a primary and secondary standard (EPA 2016b).

1.2.3 $PM_{2.5}$, cardiovascular disease, and systemic inflammation

Inflammation is an immune response to a biological, physical or chemical stimuli (e.g., $PM_{2.5}$) (Germolec et al. 2010). Examples of inflammatory mediators include cytokines and acute-phase reactants (Pearson et al. 2003). Chronic inflammation may occur due to persistent exposure to a stimulus and may contribute to diseases including asthma and cardiovascular disease (Germolec et al. 2010).

Substantial evidence links air pollution, particularly $PM_{2.5}$ to clinical and preclinical endpoints associated with chronic inflammatory conditions associated with aging, including cardiovascular morbidity and mortality (Robert D Brook et al. 2010; Dominici et al. 2006; Eftim et al. 2008; Gill et al. 2011; Halonen et al. 2009; Miller et al. 2007; Peel et al. 2005; Peters et al. 2000; Symons et al. 2006). Systemic inflammation is one possible mediating pathway (Robert D Brook et al. 2010; Cosselman et al. 2015; Pope et al. 2004; Thurston et al. 2015). Previous studies have found associations between long term exposure to $PM_{2.5}$ and circulating inflammatory markers in cohort studies with both healthy participants and potentially vulnerable subpopulations including older, obese, diabetic, and hypertensive people (Dubowsky et al. 2006; Zeka et al. 2006). For example, Ostro et al (2014)., found that a 10-$\mu g/m^3$ increase in annual $PM_{2.5}$ more than doubled the risk of CRP greater than 3 mg/l in women who were older diabetics, or smokers (Ostro et al. 2014). While long-term exposure to ambient $PM_{2.5}$ has been positively associated with circulating inflammatory mediators, some studies have also found inconsistent or null associations, potentially due to population differences in susceptibility or differences in $PM_{2.5}$ composition (Robert D Brook et al. 2010; Roux et al. 2006; Zeka et al. 2006).

Compared to circulating inflammatory mediators, stimulated inflammatory mediators may capture individual differences in the magnitude of immune response following exposure to

endotoxin [e.g., lipopolysaccharide (LPS), phytohaemagglutinin (PHA)], possibly indicating immune reactivity (Marsland et al. 2002; Marsland et al. 2017b). In contrast, circulating cytokines may reflect an individual's current condition, such as acute infection. In this sense, stimulated cytokine measures may identify under- or over-responsiveness of the immune system (Ai et al. 2013).

1.2.4 $PM_{2.5}$ and the central nervous system

The effects of air pollution on the central nervous system have become an emerging area of concern and growing evidence suggests a relationship between particulate air pollution exposures and adverse neurological outcomes (e.g., cognitive decline, ischemic stroke) (Lisabeth et al. 2008; Maheswaran et al. 2014; Ranft et al. 2009; Stafoggia et al. 2014).

Air pollution may increase risk for early cognitive decline (Calderon-Garciduenas et al. 2011; Chen and Schwartz 2009; Gatto et al. 2013; Loop et al. 2013; Power et al. 2011; Ranft et al. 2009; Weuve et al. 2012), possibly through inflammatory mechanisms that may adversely affect brain circuits for executive control, memory, and processing speed. In particular, fine particles can be inhaled and deposited into the airways and alveolar surfaces, entering pulmonary and systemic circulations (R. D. Brook et al. 2010). Second, ensuing effects may involve 1) the up-regulation of oxidative and inflammatory mediators, 2) direct suppression of cardiac vagal (parasympathetic) nerve traffic, impacting autonomic control over the heart, and 3) the down-regulation of nitric oxide synthase, affecting vascular resistance, compliance, and endothelial circulatory control (R. D. Brook et al. 2010; Gill et al. 2011). $PM_{2.5}$ may also impact CNS through olfaction by translocation across olfactory mucosa and penetration into olfactory bulb neural projection pathways to medial temporal

lobe regions, leading to cognitive decline and dementia (Calderon-Garciduenas et al. 2010; Donaldson et al. 2005; Elder et al. 2006; Tin Tin Win et al. 2006). $PM_{2.5}$ may also disrupt the blood-brain barrier leading to neurotoxicity and neuroinflammation (Calderon-Garciduenas et al. 2002; Calderon-Garciduenas et al. 2004; Calderon-Garciduenas et al. 2008a; Calderon-Garciduenas et al. 2008b; Calderon-Garciduenas et al. 2010; Calderon-Garciduenas et al. 2011; Campbell et al. 2009; Gerlofs-Nijland et al. 2010; Levesque et al. 2011; van Berlo et al. 2010).

1.2.5 Air pollution and brain morphology

Fine particle exposure may also impact the central nervous system possibly by mechanisms involving the effects of $PM_{2.5}$-related inflammation on brain tissue integrity (Ranft et al. 2009). Measures of brain morphology (e.g., cortical thickness, gray matter volume) have been associated with cognitive decline as well as neurological diseases such as Alzheimer's or Parkinson's disease (Block et al. 2012; Dickerson and Wolk 2012; Ferreira et al. 2014; Marsland et al. 2015; Whitwell et al. 2008).

Most of the epidemiologic literature linking air pollution and brain morphology has been performed in children or older adults. For example, some recent studies have examined associations between children's exposure to air pollution at schools with structural and functional brain changes from MRI scans. In one study of 263 children in Barcelona, a composite air pollution indicator combining indoor and outdoor elemental carbon and NO_2 at schools was developed indicative of traffic-related pollution; no significant associations were found with brain structure, however, children with higher pollution exposures had lower functional integration and segregation in certain brain networks (Pujol et al. 2016b).

Another study in the same cohort examined associations between copper (Cu) in PM2.5 measured in school courtyards with structural and functional brain measures obtained from anatomical MRI, diffusion tensor imaging, and functional MRI. Associations were found between higher exposures to Cu and poorer motor performance and alterations in basal ganglia structure and function (Pujol et al. 2016a).

A few epidemiological studies have explored relationships between ambient outdoor $PM_{2.5}$ and measures of brain structure in older adults. Wilker et al. (2015), found that a 2-$\mu g/m^3$ increase in one-year annual average $PM_{2.5}$ was associated with a 0.32% decrease in cerebral brain volume and 46% higher odds of covert brain infarcts but did not see any associations with hippocampal volume or white matter hyperintensity volume (Wilker et al. 2015). Participants in this study were in the Framingham Offspring Cohort (n = 943). A long running cohort study composed of community dwelling adults in the New England area with no history of dementia or stroke.

Chen et al. (2015) found significant associations between exposure to ambient $PM_{2.5}$ and decreased white matter volume in frontal and temporal lobes and in the corpus callosum of older women (Chen et al. 2015). They examined associations between long term exposure to $PM_{2.5}$ and brain volume, using volumetric measures of gray matter and normal-appearing white matter in MRI results from participants in the Women's Health Initiative Memory Cohort (n = 1403). All participants were free of dementia. They found that for each inter-quartile range (3.49 $\mu g/m^3$) increase in $PM_{2.5}$, mean white matter volume decreased by 6.23 ($\pm$ 1.28) cm^3 for total brain volume. Significant associations were also found between increased $PM_{2.5}$ with decreases in frontal, parietal, and temporal and corpus callosum white matter volume. No associations were found with gray matter or hippocampal volume (Chen et al. 2015).

1.2.6 Exposure Modeling

In large cohort studies where personal exposure monitoring is often not possible due to financial and time constraints, other methods have been used to predict and assign pollutant exposures. Examples include proximity based measurements (e.g., distance to road) (Baccarelli et al. 2009; Gauderman et al. 2005) and interpolation (e.g., inverse distance weighting, kriging) (Jerrett et al. 2001; Künzli et al. 2005; Stacy et al. 2015). Land use regression (LUR) modeling has become a common method for predicting pollutant concentrations. LUR models use observed associations between monitored pollution concentrations and GIS-based pollution source indicators, such as industrial emissions and land use zoning, to predict pollutant concentrations at unmonitored locations. LUR models have been widely used to identify key pollution sources, to predict pollutant concentrations, and to assign exposure estimates for epidemiological cohorts (Hoek et al. 2008; Jerrett et al. 2005). For cohort exposure estimates that are accurate in space and time, the spatial surfaces produced by LUR modeling are often combined with temporally-dense concentration measures, such as those provided by the EPA air quality system (AQS) monitors (Johnson et al. 2013; Ross et al. 2013). These approaches represent a great improvement over exposure assignments that rely solely on the nearest EPA air AQS monitor(s).

Although many studies have developed land use regression (LUR) models for $PM_{2.5}$ (Jerrett et al. 2005), relatively few have developed LURs to examine specific constituents. The European Study of Cohorts for Air Pollution Effects (ESCAPE) modeled eight components of $PM_{2.5,}$ the New York City Community Air Survey (NYCCAS) modeled 15 components of $PM_{2.5}$, and Brokamp et al., developed LUR models for 11 metals in Cincinnati, Ohio (Brokamp et al.

2016; de Hoogh et al. 2013; Ito et al. 2016). Several LUR models have also been made for constituents of different PM fractions (Zhang et al. 2015; Zhang et al. 2014).

Adding pollutant dispersion covariates into LUR models may improve models by incorporating source-specific emissions data particularly relevant for metals components of PM, increasing accuracy of exposure estimates near sources. Two spatial models for elemental components have incorporated dispersion parameters in a hybrid LUR approach. The Multi-Ethnic Study of Atherosclerosis modeled four constituents of $PM_{10-2.5}$ and included the CALINE3 line dispersion model as a traffic-related covariate (Zhang et al. 2014). The NYCCAS developed a commercial charbroiling variable using the AERMOD dispersion model in elemental component models (Ito et al. 2016). To our knowledge, LUR elemental components models developed in the ESCAPE study are the only constituent LUR models that have been applied to epidemiological health studies to examine potential associations of long term elemental components with adverse health effects (e.g., pneumonia and cardiovascular mortality) (Fuertes et al. 2014; Hampel et al. 2015; Pedersen et al. 2016; Wang et al. 2014).

The work presented in this dissertation builds on this literature by developing hybrid dispersion LUR models for $PM_{2.5}$ and BC, Pb, Mn, Fe, and Zn metal constituents in the Pittsburgh region. These models were then applied to estimate pollutant exposures at geocoded addresses of participants in two retrospective cohorts. Epidemiological studies were done to examine associations between one-year pollutant exposures with circulating and LPS-stimulated inflammatory mediators and measures of brain morphology.

2.0 HYBRID LAND USE REGRESSION MODELS FOR ESTIMATING EXPOSURES TO AIRBORNE METALS ACROSS PITTSBURGH

2.1 ABSTRACT

Land use regression (LUR) modeling has become a common method for predicting pollutant concentrations and assigning exposure estimates in epidemiological studies. However, few LUR models have been developed for metal constituents of fine particulate matter ($PM_{2.5}$) or have incorporated source-specific dispersion covariates. We developed hybrid AERMOD LUR models for $PM_{2.5}$, black carbon (BC), and steel-related $PM_{2.5}$ constituents lead (Pb), manganese (Mn), iron (Fe), and zinc (Zn), using fine-scale air pollution data from 36 sites across the Pittsburgh area. Models were designed for application to future epidemiological studies, by combining spatially saturated monitoring data with daily pollutant concentrations from an Environmental Protection Agency (EPA) regulatory monitor. We found that the hybrid LURs explained greater variability in $PM_{2.5}$ (R^2 = 0.79) compared to BC (R^2 = 0.59) and metal constituents (R^2 = 0.34 - 0.56). Approximately 70% of variation in $PM_{2.5}$ was attributable to temporal variance, compared to 36% for BC, and 17 - 26% for metals. An AERMOD dispersion covariate developed with industrial emissions data for 207 sources was significant in $PM_{2.5}$ and BC models; all metals models contained a steel mill-specific AERMOD term. Other significant covariates included industrial land use, commercial and industrial land use, percent impervious

surface, and summed railroad length. These models will be used to develop exposure estimates for relevant time points of interest in epidemiology studies.

2.2 INTRODUCTION

While numerous studies have linked exposure to ambient fine particulate matter ($PM_{2.5}$) to adverse health outcomes (e.g., asthma, cardiovascular disease) (Robert D Brook et al. 2010; Guarnieri and Balmes 2014), less is known about which specific components of $PM_{2.5}$ drive these associations. Because $PM_{2.5}$ composition varies across space, characterizing fine-scale intra-urban variation in constituents is critical to improving epidemiological studies aimed towards better understanding health effects of key emissions sources (Bell et al. 2007). Although many studies have developed land use regression (LUR) models for $PM_{2.5}$, relatively few have developed LURs to examine specific constituents. Because a greater proportion of spatial variation in metal constituents may be attributable to a few specific sources than is the case for total PM, emissions from these sources may need to be characterized with greater precision. One promising approach for improving this characterization may be hybrid models, where source-specific dispersion covariates are integrated into LURs.

LUR models have been widely used to identify key sources, to predict pollutant concentrations at unmonitored locations, and to assign exposure estimates for epidemiological cohorts (Hoek et al. 2008; Jerrett et al. 2005). For cohort exposure estimates that are accurate in space and time, the spatial surfaces produced by LUR modeling are often combined with temporally-dense concentration measures, such as those provided by the EPA air quality system

(AQS) monitors (Johnson et al. 2013; Ross et al. 2013). These approaches represent a great improvement over exposure assignments that rely solely on the nearest EPA AQS monitor(s).

Few previous studies have developed elemental LUR models for $PM_{2.5}$ components. The European Study of Cohorts for Air Pollution Effects (ESCAPE) modeled eight components of $PM_{2.5,}$ the New York City Community Air Survey (NYCCAS) modeled 15 components of $PM_{2.5}$, and Brokamp et al., developed LUR models for 11 metals in Cincinnati, Ohio (Brokamp et al. 2016; de Hoogh et al. 2013; Ito et al. 2016). Several LUR models have also been made for constituents of different PM fractions (Zhang et al. 2015; Zhang et al. 2014).

Despite the greater influence that one or a few key sources will have on spatial patterns when modeling constituents rather than total PM, only two spatial models for elemental components have incorporated dispersion parameters in a hybrid LUR approach. The Multi-Ethnic Study of Atherosclerosis modeled four constituents of $PM_{10-2.5}$ and included the CALINE3 line dispersion model as a traffic-related covariate (Zhang et al. 2014). The NYCCAS developed a commercial charbroiling variable using the AERMOD dispersion model in elemental component models (Ito et al. 2016). Adding pollutant dispersion covariates into LUR models may further improve models by incorporating source-specific emissions data particularly relevant for metals components of PM, increasing accuracy of exposure estimates near sources.

To our knowledge, LUR elemental components models developed in the ESCAPE study are the only constituent LUR models that have been applied to epidemiological health studies to examine potential associations of long term elemental components with adverse health effects (e.g., pneumonia and cardiovascular mortality) (Fuertes et al. 2014; Hampel et al. 2015; Pedersen et al. 2016; Wang et al. 2014).

We previously found that legacy industrial sources (e.g., Edgar Thomson Steel Works and Clairton Coke Works) substantially contribute to spatial variability in both $PM_{2.5}$ and metal constituent concentrations across the Pittsburgh area (ACHD 2011; EPA 2009; Kelly 2007; Michanowicz et al. 2016; Shmool et al. 2014; Tunno et al. 2015c). In this study, we developed AERMOD hybrid LUR models for $PM_{2.5,}$ black carbon (BC), and lead (Pb), manganese (Mn), zinc (Zn), and iron (Fe), metal constituents to develop spatial and temporal exposure estimates for retroactive and prospective cohort studies in the greater Pittsburgh area. We created two AERMOD Industrial $PM_{2.5}$ dispersion covariates to develop hybrid models. One was built using emissions profiles for 207 sources and the second was created using sources associated with Edgar Thomson Steel Works. We then used concentrations from a centrally-located EPA AQS monitor to temporally adjust pollutant concentrations to develop exposure estimates that can be modified for relevant time points of interest in epidemiology studies. These models will be used in future epidemiological studies examining chronic pollutant exposures and health effects.

2.3 METHODS

2.3.1 Air Pollution Data

$PM_{2.5}$ samples were collected during a spatial-saturation monitoring campaign with 36 sites monitored in both summer (June 5 to July 26, 2012) and winter (January 8 to March 10, 2013) as detailed previously (Shmool et al. 2014; Tunno et al. 2015c). Briefly, a sampling domain of approximately 388 km^2 was identified to include urban and rural areas in the greater Pittsburgh region, and included major industrial sources in Allegheny County (e.g., steel mill, two coke

(coal) works). A stratified random sampling approach was used to systematically choose monitoring sites based on cross-stratifications of elevation gradient, traffic density and industrial emissions using geographic information systems (GIS) (ArcMap 10.0-10.3, Redlands). A background reference site was chosen in Settler's Cabin Park, west of the city, due to its location in the lowest strata classes (high elevation, far from industry, and low traffic density) and location upwind in the predominant wind direction (Fig. 1).

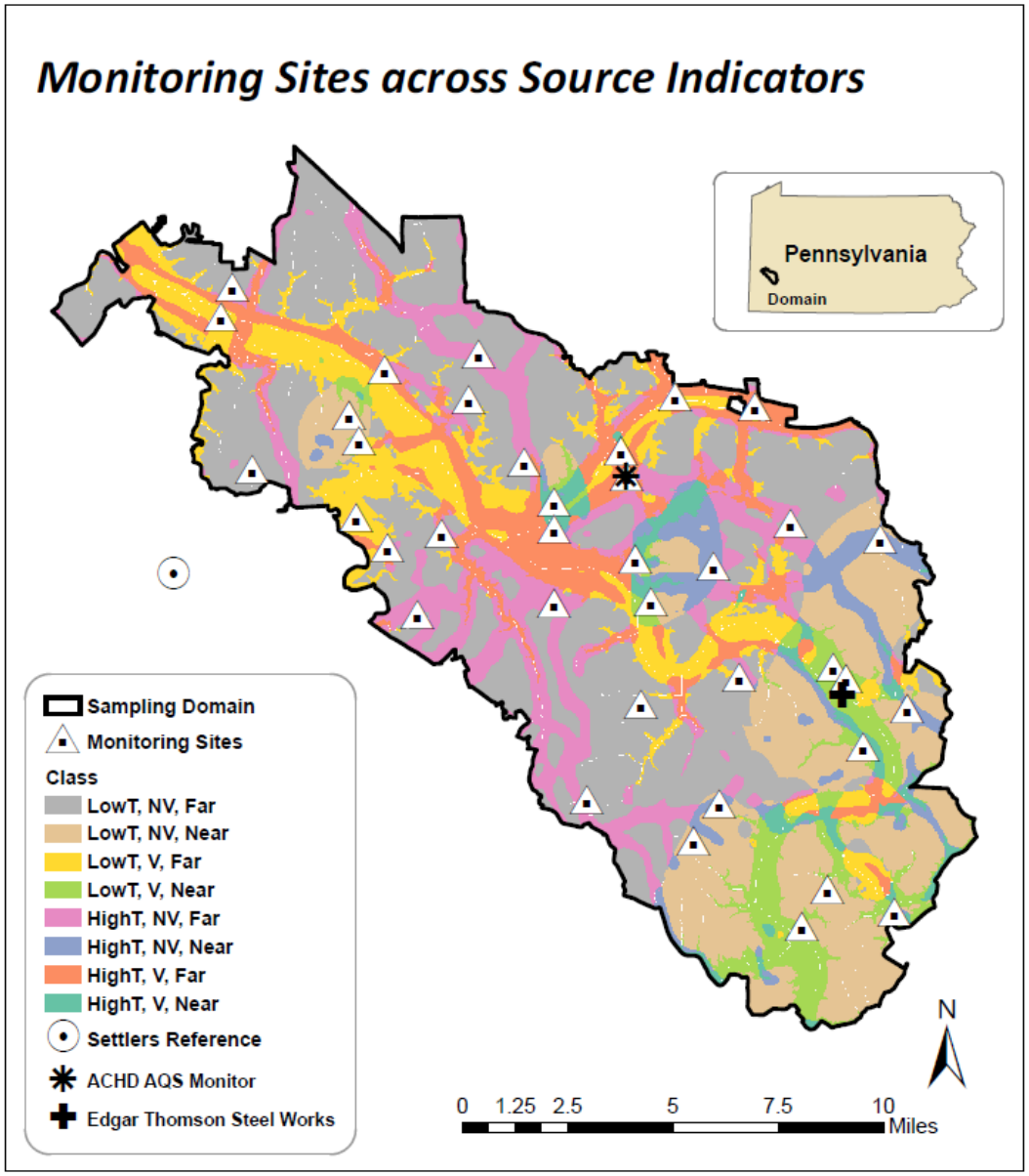

Figure 1. Monitoring locations and site selection strata

One-week integrated $PM_{2.5}$ samples were collected using Harvard Impactors (Air Diagnostics and Engineering Inc.) programmed to sample at a rate of 4.0 liters per minute. Integrated $PM_{2.5}$ concentrations were obtained at each site for the first 15 minutes of every hour for seven days, and eight sites were sampled per session. The reference site was monitored every session, to enable temporal adjustment across sessions. Pollutant concentrations were temporally adjusted to account for sampling sites across multiple weeks by dividing the raw concentration by the session-specific reference site concentration and then multiplying the result by the average concentration for the entire season (Shmool et al. 2014; Tunno et al. 2015c).

$PM_{2.5}$ concentrations were calculated from pre- and post-sampling Teflon filter weights and black carbon was measured using an EEL43M Smokestain Reflectometer (Diffusion Systems). Filters were then analyzed using inductively-coupled plasma mass spectrometry (ICP-MS) to determine elemental concentrations for 25 elements (Wisconsin State Laboratory of Hygiene).

2.3.2 Factor Analysis and Source Apportionment

We used factor analysis to identify spatially correlated suites of constituents associated with key urban sources, such as traffic, industry, and long-range transport. Unconstrained factor analysis with varimax rotation was performed on 25 $PM_{2.5}$ elemental constituents plus BC, and a previously-developed literature review on source tracers was used to interpret resulting factor sources (Tunno et al. 2015a). Temporally-adjusted metal concentrations for summer and winter were combined, and analysis was performed using PROC FACTOR in SAS 9.3 (Cary, NC, USA) per methods used by Tunno et al., and Clougherty et al (Clougherty et al. 2009; Tunno et al. 2015a). Factors explaining at least 5% of the total variance, and constituents with loadings greater than or equal to 0.60 on those factors were retained. Factor 3, which included barium

(Ba), cesium (Cs), Fe, lanthanum (La), Mn, and Zn, was of particular interest because it contained three metals (Fe, Mn, and Zn) previously associated with steel mill emissions (Almeida et al. 2015; Pancras et al. 2013; Tunno et al. 2015a). While Pb, also previously associated with steel emissions (Almeida et al. 2015; Pancras et al. 2013), did not meet the 0.60 threshold for any factor, its highest loading was 0.57, also on factor 3.

2.3.3 GIS-based Covariates

A wide range of covariates were developed using GIS to capture multiple source categories. Methods for covariate creation are described elsewhere (Tunno et al. 2015c). Table 1 includes all covariates created and examined in LUR models. Source categories included traffic density indicators, transportation indicators, road-specific measures, land use/ built environment, industrial emissions, population, and truck, bus, and diesel indicators. Several new covariates were also created in addition to covariates developed by Tunno et al (Tunno et al. 2015c). Under the industrial emissions source category, mean density of total Pb and Mn emitted per meter were developed from EPA National Emissions Inventory (NEI) data (EPA 2011). Three covariates were created using data from the 2011 National Land Cover Database (NLCD) including percent developed imperviousness, percent medium development, and percent high development ((USGS) 2011). Variables were created for varying buffer sizes around monitoring sites, ranging from 50-1000 meters.

Table 1. Covariates for LUR modeling

Source category for LUR modeling	Covariates examined (50m to 1000m buffers)	Data source
Traffic density indicators	Mean density traffic (primary roads) Mean density traffic (primary and secondary roads) Number of signaled intersections	Pennsylvania Department of Transportation (PADOT) Southwestern Pennsylvania Commission (SPC, 2011)
Road-specific measures	Average daily traffic on nearest primary road Distance to nearest major road Summed length of primary roadways Summed length of primary and secondary roads	PADOT
Truck, Bus, and Diesel	Mean bus traffic density Distance to nearest bus route Outbound and inbound trip frequency per week summed by route Mean density of heavy truck traffic on nearest primary roadway	Google Transit (11/11 -3/12) PADOT
Population	Census population density (blockgroup)	US Census Bureau (2010)
Land Use / Built Environment	Total area of industrial parcels Total area of commercial parcels Total area of industrial and commercial parcels Percent developed imperviousness Percent medium development Percent high development	Allegheny County Assessment Data, by parcel (2011) National Land Cover Dataset (NLCD, 2011)
Industrial emissions	Mean density of total $PM_{2.5}$ emitted per meter Mean density of total SO_2 emitted per meter Mean density of total Pb emitted per meter Mean density of total Mn emitted per meter AERMOD-predicted steel mill $PM_{2.5}$ emissions AERMOD-predicted industrial emissions	National Emissions Inventory (NEI, 2011)
Transportation Facilities	Distance to nearest active railroad Summed line length of active railroads Distance to nearest bus depot	Southwestern Pennsylvania Commission (SPC, 2011)

Adapted from Tunno et al 2015.

2.3.4 AERMOD Dispersion Covariates

Two industrial $PM_{2.5}$ dispersion covariates were developed using AERMOD, a Gaussian plume atmospheric dispersion model. These variables were developed with the goal of providing more accurate, source-specific emission profiles to explain greater variability in monitored concentrations. AERMOD is currently used for regulatory purposes by the EPA to assess NAAQS pollutants under the Clean Air Act (EPA 2016b). Both variables were developed using emissions data from the Allegheny County Health Department (ACHD) Air Quality/ Pollution

Control Program Division emissions inventory, meteorological data (e.g., wind speed, temperature) and elevation. Meteorological data used in AERMOD was averaged for 2012 to develop an annual average dispersion covariate. Emissions data was used for 207 individual point, volume, and area sources. Model processing was done using Lakes Environmental (Lakes Environmental Software, Waterloo, ON) version 7.3.0, corresponding to AERMOD version 11103. More information about this process is detailed by Michanowicz et al (Michanowicz et al. 2016). Two covariates were developed as follows: 1) AERMOD predicted industrial $PM_{2.5}$ emissions using all 207 sources, 2) AERMOD predicted steel mill $PM_{2.5}$ emissions using 14 sources from the Edgar Thomson Steel Works. Using AERMOD, $PM_{2.5}$ concentrations were predicted directly at monitoring locations as well as at each centroid of a 100 m^2 Cartesian receptor grid covering Allegheny County. Average concentrations were determined at monitoring point locations and for buffers 50 m-1000 m around sites.

2.3.5 Reference Site and Temporal Adjustment

While the Settler's Park background reference site was used as a temporal component in previously developed seasonal LUR models, an alternative method was needed that used data available for the entire sampling year (summer 2012 to spring 2013) to develop annual models. To accomplish this, an EPA AQS monitor maintained by the ACHD was used to adjust for temporal variation across sampling weeks. This particular AQS site was chosen for three main reasons: 1) its central location within the sampling domain (Fig. 1), 2) quality of data and comparable model agreement with the Settler's reference site, and 3) availability of data matching the time period cohort data was collected. Daily $PM_{2.5}$ data from all $PM_{2.5}$ reference monitors in Allegheny County were downloaded from the EPA air data website (Agency 2017).

We chose Allegheny monitor site 420030008 located in Lawrenceville, PA for 2003-2013 based on the criteria mentioned above. Preference was given to data from National Ambient Air Quality Standard (NAAQS) compliant monitors. One daily monitor in particular was used with only 176 missing days over the 10-year period. Concentrations for 141 additional missing days were filled with data from other monitors (e.g., speciation monitors) leaving a remaining 35 days with missing data. These 35 concentrations were imputed using PROC GLM with daily $PM_{2.5}$ concentrations as the dependent variable and year, month, and day of the week as categorical variables in SAS v. 9.3 (Cary, NC).

2.3.6 Land Use Regression Models

Prior to modeling, pollutant distributions were examined through scatter plots and histograms then tested for normality. Pb, Mn, Fe, and Zn concentrations were transformed using the natural logarithm due to right-skewed distributions.

LUR models use observed associations between monitored pollution concentrations and GIS-based pollution source indicators, such as industrial emissions and land use zoning, to predict pollutant concentrations at unmonitored locations. Pollutant concentrations collected from summer 2012 and winter 2013 were combined to create merged mixed models accounting for season as a random factor. Modeling was done using SAS v. 9.3 and Snijders/Bosker R^2 values were computed in STATA v. 13. We used GIS-based source indicators and LUR models built using a manual forward step-wise process to predict fine-scale $PM_{2.5}$, BC, Pb, Mn, Fe, and Zn concentration estimates with methods adapted from Tunno et al. and Clougherty et al (Clougherty et al. 2013; Tunno et al. 2015c).

Correlations were first tested for non-temporally adjusted metal concentrations versus covariates in each source group. The two highest covariates in each source group were retained. Scatter plots of these covariates versus pollutant concentrations were examined to make sure that predictors captured variability across the entire concentration range. Based on these scatter plots several covariates were natural log transformed to improve linearity in metal models, including the Lawrenceville temporal term and AERMOD-predicted steel-mill $PM_{2.5}$ emissions. Covariates were then grouped together and run using a random forest automation to determine a covariate ranking order using R version 3.1.0 (The R Foundation). Next, LUR models were built starting with the temporal covariate built using session specific concentrations from the AQS monitor described above. Covariates were then sequentially tested starting with the highest ranked covariate from the random forest analysis. The coefficient of determination (R^2) was used to retain covariates. Covariates with a p-value <.1 were removed at each stage. Next, modification of covariates by elevation was tested by examining interaction terms using a binary indicator for elevation (low, high, 50%) multiplied by source covariates. Models were then examined for collinearity by removing covariates with variance inflation factors greater than 2.0. $PM_{2.5}$ and BC models were built following the methods from Tunno et al. (Tunno et al. 2015c) but tested all new covariates built for metal models as well. Spatial R^2 values were determined by taking out the temporal term and predicting temporally adjusted concentrations using only spatial covariates. Using a 100 m grid spread across the sampling domain, pollutant concentrations were predicted at the centroid of each grid cell by applying LUR models to the spatial covariate values in each grid cell. Concentrations were then smoothed with inverse distance weighting with 100 nearest neighboring grid cells. Centroids with metal concentrations greater than the highest

temporally adjusted concentration measured during the monitoring campaign were capped at this concentration.

2.3.7 Sensitivity Analyses

Model residuals were assessed using scatter plots and examined for normality and heteroskedasticity. Residuals were also mapped using GIS to examine spatial patterns in model performance. In order to produce semivariograms and determine any additional spatial trends, residuals were mapped against the latitude and longitude of monitoring sites. Twenty percent of sites (n=14) were selected randomly in SAS and removed. The LUR model was then tested using the remaining sites. All four metal models were also built without natural log transformations to compare covariates with ln transformed models. Sensitivity analyses were done with temporal data. ACHD data for Pb, Mn, Fe, and Zn (concentrations collected every three days) was tested by replacing the $PM_{2.5}$ temporal term.

2.3.8 Exposure Assignment for Cohort Participants

Pollutant exposures will be assigned within a 300 m buffer of each participant's address by computing the mean centroid concentration within each buffer. These exposures will then be temporally extrapolated to relevant time points (e.g., 1 year, or 5 years before the date of the health outcome of interest). The same reference monitor that was used to temporally adjust the LUR models will also be used to develop residence-specific cohort participant exposure estimates as follows in Equation 2.1:

$$\textbf{pollution}_i = \textbf{pollution (300 m buffer)} - \beta_1 * \textbf{(mean EPA concentration during sampling year)} + \beta_1 * \textbf{(mean EPA concentration during time point of interest).}$$

(2.1)

Where $pollution_i$ is the LUR-derived pollutant-specific exposure estimate corresponding to participant i's address and temporally adjusted to the time point of interest.

β_1 corresponds to the temporal term from the corresponding LUR model. Mean pollutant concentrations will be assigned to each participant within a 300 m radial buffer surrounding each participant's geocoded residential location.

2.4 RESULTS

2.4.1 Summary Statistics

Descriptive statistics for temporally adjusted concentrations of monitored $PM_{2.5}$, BC, Pb, Mn, Zn and Fe are shown in Table 2.

Table 2. Descriptive statistics for temporally adjusted citywide air sampling concentrations

	Summer 2012			**Winter 2013**		
Pollutant	**Mean (SD)**	**Min**	**Max**	**Mean (SD)**	**Min**	**Max**
$PM_{2.5}$ ($\mu g/m^3$)`	13.9 (2.01)	11.26	22.6	11.3 (2.01)	8.01	18.9
BC (abs)	1.06 (0.36)	0.61	2.47	0.93 (0.35)	0.50	2.15
Pb (ng/m^3)	3.87 (2.20)	0.11	10.4	4.21 (5.43)	0.56	26.4
Mn (ng/m^3)	5.00 (5.42)	0.17	29.4	9.08 (22.0)	0.40	96.3
Zn (ng/m^3)	23.8 (15.0)	5.22	75.4	39.0 (84.1)	0.71	391.9
Fe (ng/m^3)	110.8 (86.3)	3.41	515.6	260.0 (675.8)	6.03	3661.3

Adapted from Tunno et al 2015.

2.4.2 Factor Analysis

Five distinct factors resulted from this analysis (Fig. 2). Factor 1 includes metals related to traffic. Metals aluminum (Al), potassium (K), molybdenum (Mo), antimony (Sb), and strontium (Sr) loaded onto this factor. Copper (Cu) almost loaded onto this factor (0.59). Each of these metals have been linked to traffic sources. Mo, Sb, Sr, and Cu have been linked more specifically to brake and tire wear as well. Factor 2 also consists of components mainly linked to traffic sources. Calcium (Ca), cadmium (Cd), cerium (Ce), chromium (Cr), vanadium (V), and magnesium (Mg, 0.59) loaded onto factor 2. Ca, Cd, and Mg have been linked to traffic in general, Cr to brake/tire wear, and V to fuel and oil sources. Barium (Ba), cesium (Cs), Fe, lanthanum (La), Mn, Zn, and Pb (0.57) loaded on factor 3. Fe, Zn, Mn, and Pb have all been

linked to steel mill emissions. Factor 4 included arsenic (As) and thallium (Tl) and factor 5 had selenium (Se). All of these metals have been associated with coal sources (Tunno et al. 2015a).

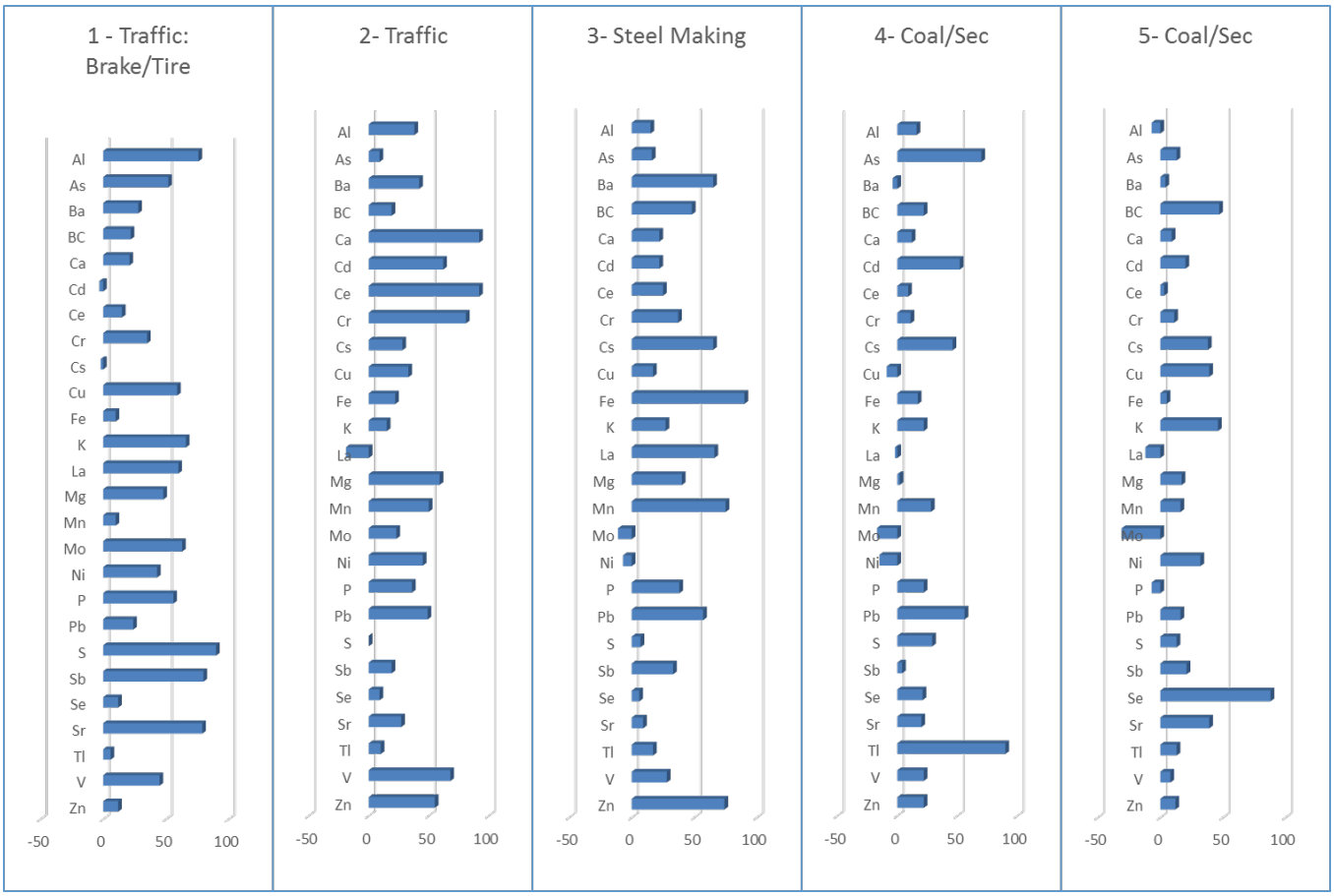

Figure 2. Factor loadings of $PM_{2.5}$ elemental constituents and BC

2.4.3 LUR Models

Most of the variability in the metal models was explained by spatial covariates. The temporal reference contributed to 17-26% of the total variance in metal models compared to 70% in $PM_{2.5}$ and 36% in BC as shown in Table 3. All models contained AERMOD covariates. $PM_{2.5}$ and BC models incorporated the AERMOD predicted industrial $PM_{2.5}$ emissions covariate while the metal models included the steel mill specific AERMOD term in every model. Zn, Fe, and Mn models all included percent impervious surface within a 500 m buffer. Summed railroad length within a 300 m buffer was in Pb, Mn, and Fe models. In addition to the AERMOD covariate, the $PM_{2.5}$ model also included industrial land use within a 500 m buffer and percent impervious surface within a 200 m buffer. Commercial and industrial land use within a 200 m buffer was found in the BC model. Spatial R^2 values were 0.33 for $PM_{2.5}$, 0.32 for BC, 0.25 for Pb, 0.47 for Mn, 0.36 for Fe, and 0.32 for Zn. Spatial surfaces developed from these models are shown in Figure 3.

Table 3. LUR model results

LUR Models			
Pollutant	Covariates	β	Seq. R^2
$PM_{2.5}$ (μg/m^3)	Intercept	-2.53 (1.14)	--
	Reference $PM_{2.5}$	1.08 (0.08)**	0.70
	Industrial land use 500 m	$7.40x10^{-6}$ ($2.45x10^{-6}$)*	0.75
	Percent Impervious surface 200 m	0.03 (0.01)*	0.77
	AERMOD predicted industrial $PM_{2.5}$ emissions	0.50 (0.20)*	**0.79**
Pb (ng/m^3)	Intercept	-2.84 (1.11)	--
	Reference $PM_{2.5}$	1.65 (0.43)*	0.18
	AERMOD steel mill $PM_{2.5}$ emissions 1000 m	0.26 (0.09)*	0.26
	Sum rail length 300 m	0.00011 (0.000036)*	**0.35**
Mn (ng/m^3)	Intercept	-4.42 (1.22)	--
	Reference $PM_{2.5}$	2.16 (0.47)**	0.20
	AERMOD steel mill $PM_{2.5}$ emissions 1000 m	0.38 (0.10)**	0.34
	Sum rail length 300m	0.00016 (0.00005)*	0.52
	Percent Impervious surface 500 m	0.01 (0.005)*	**0.55**
Fe (ng/m^3)	Intercept	-2.21 (1.21)	--
	Reference $PM_{2.5}$	2.50 (0.47)**	0.26
	AERMOD steel mill $PM_{2.5}$ emissions 1000 m	0.32 (0.10)*	0.36
	Sum rail length 300 m	0.00015 (0.000044)*	0.52
	Percent impervious surface 500 m	0.01 (0.005)*	**0.55**
Zn (ng/m^3)	Intercept	-1.81 (1.34)	--
	Reference $PM_{2.5}$	1.71 (0.52)*	0.17
	AERMOD steel mill $PM_{2.5}$ emissions 1000 m	0.24 (0.11)**	0.26
	Percent impervious surface 500 m	0.02 (0.005)*	**0.37**
BC (abs)	Intercept	-0.55 (0.17)	--
	Reference $PM_{2.5}$	0.10 (0.01)**	0.36
	Commercial and industrial land use 200 m	$7.31x10^{-6}$ ($1.41\ x10^{-6}$)*	0.54
	AERMOD predicted industrial $PM_{2.5}$ emissions	0.11 (0.04)**	**0.59**

(*p-value <.05, **p-value<.0001).

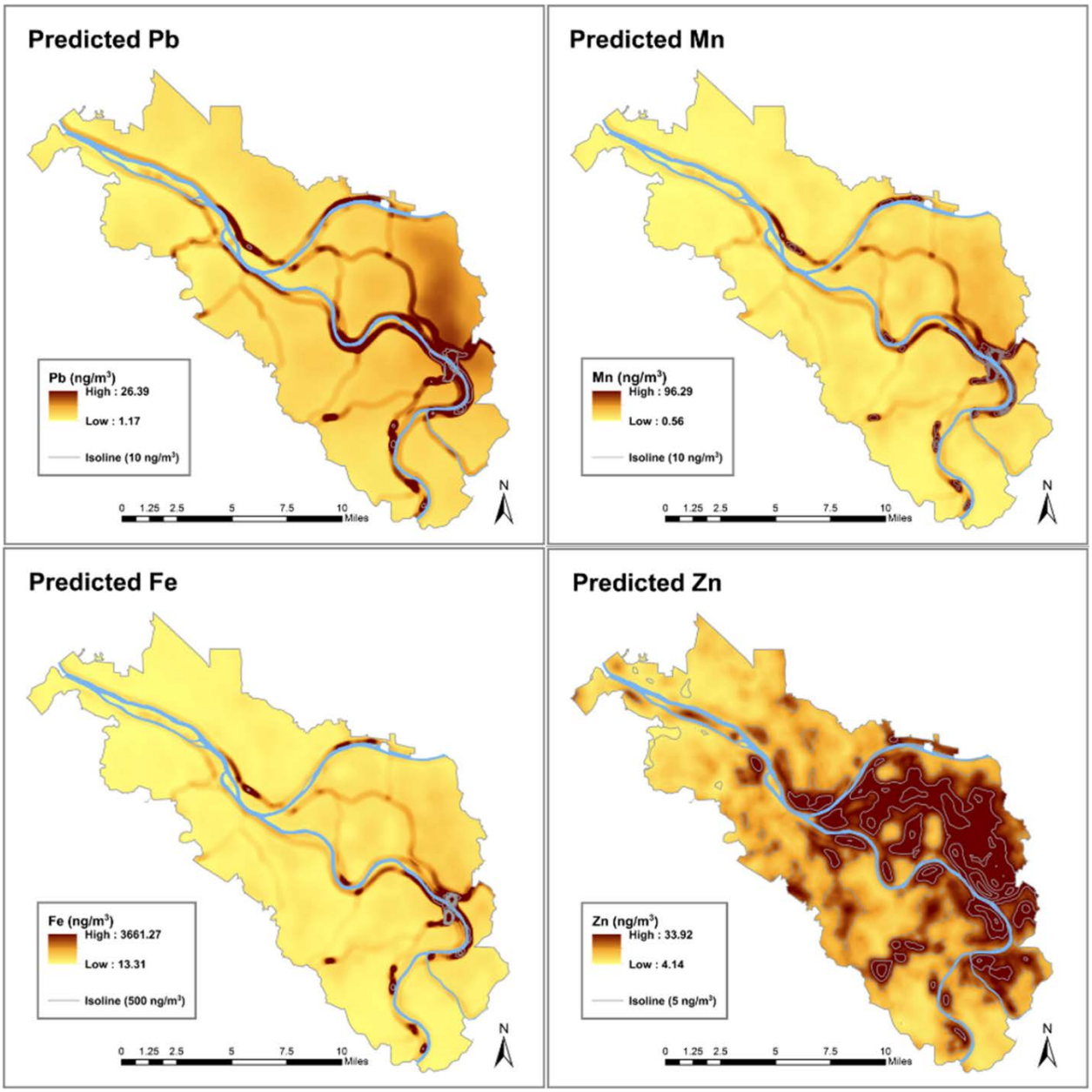

Figure 3 continued below

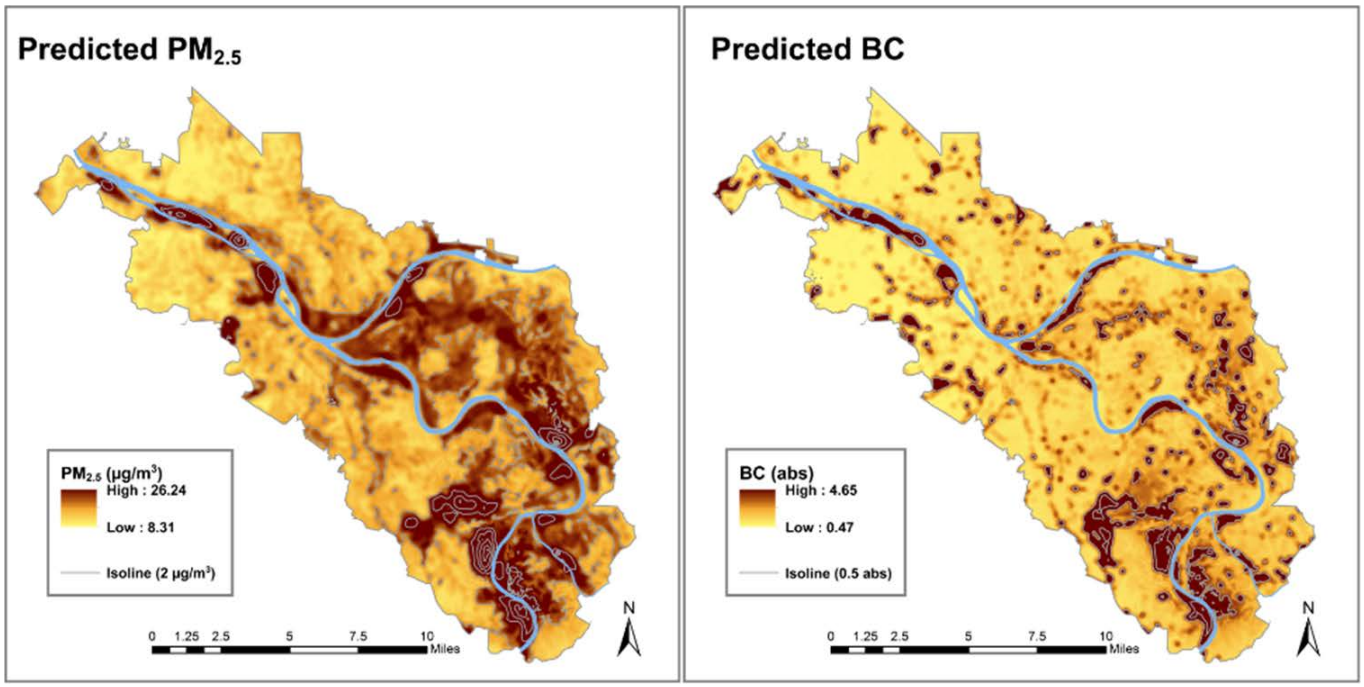

Figure 3. Hybrid LUR annual pollutant surfaces

2.4.4 Sensitivity Analyses

After reviewing semivariograms of model residuals, no additional spatial patterns were found for any of the pollutants. When models were re-fit after deleting 20% of sites, all covariates were retained with a p-value less than 0.1.

Compared to the models developed using ln transformed concentrations, models built with non-transformed concentrations had considerably higher R^2 values. However, the distribution of residuals for all non-transformed models were heteroskedastic compared to transformed models. All non-transformed models contained the AERMOD-predicted steel mill $PM_{2.5}$ emissions covariate. The Mn and Fe models also included percent impervious surface and

industrial land use within a 500 m buffer. Pb and Zn both had industrial land use at 500 m and percent of medium developed land within a 500 m buffer.

Using daily $PM_{2.5}$ from the AQS monitor performed better in models compared to using speciation data collected once every 3 days from the same site (higher R^2, lower p-value).

2.5 DISCUSSION

Hybrid AERMOD LUR models were developed for $PM_{2.5,}$ BC, Pb, Mn, Zn, and Fe metal constituents by combining spatial source-based covariates developed in GIS with industry specific $PM_{2.5}$ dispersion covariates developed using AERMOD. These models were specifically designed to assign exposure estimates to participants in cohort studies in Allegheny County using an EPA AQS monitor to temporally extrapolate LUR spatial surfaces to cohort specific time points.

Significant covariates found in the metal models were mostly consistent with known Pb, Mn, Zn, and Fe sources. All metal models included AERMOD-predicted steel mill $PM_{2.5}$ emissions compared to $PM_{2.5}$ and BC models which incorporated the AERMOD-predicted industrial emissions variable containing 207 sources. This corroborates our factor analysis and source apportionment results which grouped these metals together and pointed to a "steel making" source in the literature. The NYCCAS also included industry (industrial land use) as a covariate in their Mn, Fe, and Pb LUR models (Ito et al. 2016).

Our seasonal models for $PM_{2.5}$ and BC previously contained a covariate developed from inverse distance weighted NEI $PM_{2.5}$ emissions instead of AERMOD-predicted $PM_{2.5}$ emissions. We found in our seasonal $PM_{2.5}$ models that AERMOD-predicted industrial $PM_{2.5}$ emissions

increased the accuracy of exposure estimates compared to IDW emissions by incorporating wind speed/direction and detailed emission profiles of local industries. This was found near specific industrial sources where wind direction and elevation may play a role in transport of pollutants from a point source, which is particularly important for the metal models that were heavily influenced by specific industrial sources (Michanowicz et al. 2016). AERMOD covariates contributed more variability to the metal models compared to $PM_{2.5}$ and BC. However, $PM_{2.5}$ and BC models also contained industrial land use. Inclusion of the steel-mill related AERMOD covariate in all metal models demonstrates the importance of developing accurate source-specific covariates for modeling metal constituents.

Summed railroad length was a significant covariate for Pb, Fe and Mn models. Buikowieki et al., found Mn and Fe were emitted from railways in Zurich, Switzerland (Bukowiecki et al. 2007). Brokamp et al. found that summed railroad length within a 1000 m buffer was significant in their Mn LUR model. Percent impervious surface within a 500 m buffer developed from the NLCD (2011) were also significant for Mn, Zn, and Fe models. Brokamp et al., included Developed High Intensity area which is an NLCD variable including 80-100% impervious surface in Mn and Fe models (Brokamp et al. 2016).

We found lower R^2 values for metal constituent models compared to total $PM_{2.5}$. One reason for this could be because less variability was explained by the temporal term in the metal models. Another possibility could be due to our monitoring design which included sites “near” or “far” from industry based on IDW NEI pollutant emissions but did not include a range of distances from industrial locations.

2.5.1 Limitations

While we developed annual average models, sampling was not completed in fall or spring seasons. However, many of the spatial covariates were developed from source data averaged over one or several years. Spatial surfaces were also temporally adjusted using data for the entire monitoring year from the EPA AQS monitor. Models will be temporally extrapolated for use in cohort studies using this same AQS site. A limitation of LUR models is that the analysis is based on associations and LUR model results cannot establish causation between source covariates and pollutants.

2.5.2 Strengths

The $PM_{2.5}$ concentrations used for this analysis were obtained from two seasons of data from 36 sites and modeled to generate concentrations for every 100 m grid cell within the sampling domain. This provided a much higher spatial resolution compared to the established EPA AQS monitoring network locations within the county. In addition, our hybrid AERMOD LUR models may be more accurate by incorporating meteorology and topography into AERMOD covariates. The AQS monitor used to adjust the models also contributed to high temporal resolution providing daily concentrations.

3.0 LONG-TERM AMBIENT AIR POLLUTION EXPOSURES AND CIRCULATING AND STIMULATED INFLAMMATORY MEDIATORS IN A COHORT OF MIDLIFE ADULTS

3.1 ABSTRACT

While long term exposure to ambient air pollution has been found to impact the immune system through systemic inflammation, it is unclear whether chronic pollutant exposures are associated with endotoxin stimulated inflammatory mediators. We examined associations between chronic exposures to outdoor air pollution and levels of both circulating and lipopolysaccharide (LPS) stimulated inflammatory mediators in a cohort of healthy adults. Circulating levels of Interleukin-6 (IL-6), C-reactive protein (CRP) (n=392), and LPS-stimulated production of Interleukin-1β (IL-1β), IL-6, and Tumor Necrosis Factor-α (TNF-α) were measured in blood samples collected from 379 participants in the Adult Health and Behavior II cohort. Spatial air pollution exposure models developed for fine particulate matter ($PM_{2.5}$), black carbon (BC), and lead (Pb), manganese (Mn), zinc (Zn), and iron (Fe) metal constituents of $PM_{2.5}$ were used to assign pollutant exposures at participant's geocoded addresses. Associations between pollutant exposures with circulating and stimulated inflammatory mediators were examined using linear regression models adjusting for age, sex, race, smoking status, body mass index (BMI), and years of education. Exposure to $PM_{2.5}$ and BC were associated with higher LPS-stimulated IL-

1β, IL-6, and TNF-α. Pb was associated with increased stimulated TNF-α (p=0.02) and IL-1β (p=0.02), but were insignificant after applying a Bonferroni correction for multiple comparisons. No pollutant exposures were associated with circulating levels of IL-6 or CRP. Exposure to $PM_{2.5}$ and BC was associated with increased LPS-stimulated pro-inflammatory cytokine production in a cohort of middle-aged adults. These results suggest that some chronic air pollution exposures may influence the responsiveness of the immune system, possibly increasing risk for future inflammatory conditions.

3.2 INTRODUCTION

Exposure to fine particulate matter ($PM_{2.5}$) has been consistently associated with increased cardiovascular morbidity and mortality and systemic inflammation is one possible mediating pathway (Robert D Brook et al. 2010; Cosselman et al. 2015; Pope et al. 2004; Thurston et al. 2015). While studies have found associations between long term exposure to $PM_{2.5}$ and circulating inflammatory markers [e.g., Interleukin-6 (IL-6), C-reactive protein (CRP)] (Dubowsky et al. 2006; Hampel et al. 2015; Hoffmann et al. 2009; Ostro et al. 2014), little is known about how chronic $PM_{2.5}$ exposures may impact immune competence. While long-term exposure to ambient $PM_{2.5}$ has been positively associated with circulating inflammatory mediators, some studies have also found inconsistent or null associations, potentially due to population differences in susceptibility or differences in $PM_{2.5}$ composition (Robert D Brook et al. 2010; Roux et al. 2006; Zeka et al. 2006). These results indicate the need for more research examining associations between long-term $PM_{2.5}$ exposures with cohorts of different ages and health status.

Compared to circulating inflammatory mediators, stimulated inflammatory mediators may provide an indicator of immune response, as they capture individual differences in the magnitude of immune response following exposure to endotoxin [e.g., lipopolysaccharide (LPS), phytohaemagglutinin (PHA)], possibly indicating immune *reactivity* (Marsland et al. 2002; Marsland et al. 2017b). Circulating cytokines may reflect an individual's current condition, such as acute infection. In this sense, stimulated cytokine measures may identify under- or over-responsiveness of the immune system (Ai et al. 2013). Better understanding how chronic $PM_{2.5}$ exposures relate to stimulated cytokine levels may indicate whether and how air pollution exposures may be associated immune response.

Only a few studies have explored the association of environmental pollutants with stimulated cytokine production. To date, results have been mixed. For example, Grosse et al. found that induced iron oxide nanoparticles suppressed the ability of LPS to induce a stimulated inflammatory response in monocytes, while Kronborg et al., found that exposing isolated human cells to polybrominated diphenyl ether (DE-71) flame retardants in vitro, followed by LPS stimulation, exhibited increased production of cytokines including IL-6, IL-1β and TNF-α (Grosse et al. 2016; Kronborg et al. 2016).

$PM_{2.5}$ constituents may differ in toxicity (Bell et al. 2014; Franklin et al. 2008), and some prior studies have identified a heightened effect of steel-related metals components on inflammation (Ghio and Devlin 2001). In Pittsburgh, we previously identified elevated concentrations of Pb, Mn, Fe, and Zn related to steel mill emissions, and developed hybrid land use regression models predicting concentrations of each across the urban area (Tripathy et al. 2017). Here, we associate annual-average residence-based exposures to ambient $PM_{2.5}$ and metals components with circulating and stimulated levels of proinflammatory mediators among

middle-aged adults in the AHAB-II cohort. We hypothesized that elevated exposures to $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe would be associated with higher levels of circulating inflammatory mediators (Il-6 and CRP), and LPS-stimulated production of cytokines (IL-6, IL-1β, and TNF-α).

3.3 METHODS

3.3.1 AHAB-II Cohort

AHAB-II is a cohort study of healthy middle-aged adults in Western Pennsylvania. It was developed to identify neural and bio-behavioral correlates of physical and mental health in midlife. Cohort participants were recruited between March 2008 and October 2011 through mass mailings of invitation letters to individuals randomly selected from voter registration and other public domain lists. Individuals eligible for AHAB-II were aged 30–54 years, were working at least 25 h per week outside of the home, and spoke English as their first language. Individuals were further excluded if they: (a) had a history of cardiovascular disease, schizophrenia or bipolar disorder, chronic hepatitis, renal failure, major neurological disorder, chronic lung disease, or stage 2 hypertension (SBP/DBP ≥ 160/100); (b) consumed ⩾ 5 alcoholic drinks 3–4 times (> approximately 201 g of alcohol) per week; (c) took fish-oil supplements, took prescribed insulin or glucocorticoid, anti-arrhythmic, antihypertensive, lipid-lowering, psychotropic, or prescription weight-loss medications; (e) were pregnant; (f) had less than 8th grade reading skills; or (g) were shift workers. Finally, all participants were screened for prior and current DSM-IV Axis-I disorders using the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al. 1998). The University of Pittsburgh Institutional Board approved the

study; all participants provided informed consent in accordance with its regulations and were remunerated for their participation (Marsland et al. 2017a).

3.3.2 Circulating Inflammatory Mediators

Blood samples were taken from participants to determine levels of circulating IL-6 and C-reactive protein from 2008-2011. Plasma levels of IL-6 and CRP were assessed from blood samples drawn between 7:30AM and 12:35PM (M = 9:16 ± 0:54 min). Prior to the blood draw, participants were asked to fast for 8 h, avoid vigorous exercise for 12 h and alcohol for 24 h, and refrain from using tobacco products that morning. The blood draw was rescheduled if the participant reported symptoms of acute infection or use of antibiotics or antivirals in the previous 2 weeks. At the blood draw visit, a registered nurse completed a medical history and medication use interview and obtained measurements of height and weight to determine body mass index (BMI in kg/m^2). The nurse also drew a 40 cc blood sample. Plasma samples were collected from citrated tubes, frozen at −80 °C until analysis in batches. IL-6 levels were determined in duplicate by high sensitivity quantitative sandwich enzyme immunoassay kit (R & D Systems, Minneapolis, MN, standard range = 0.156–10 pg/mL) run per manufacturer's directions. CRP was measured at the University of Vermont's Laboratory of Clinical Biochemistry Research with the BNII nephelometer from Dade Behring utilizing a particle enhanced immunonephelometric assay. Average inter- and intra-assay coefficients of variation were <10% for both IL-6 and CRP (Marsland et al. 2017a).

3.3.3 Stimulated Cytokines

Whole blood was collected in citrate-treated vacutainer tubes and stimulated with LPS (serotype 026:B6, Sigma) at a final concentration of 2.5 ug/ml under sterile conditions and incubated at 37°C with 5.0% CO_2 for 24 hours. The tubes were then centrifuged at 1000g for 10 minutes and the plasma was frozen at -80°C until the completion of the study.

Samples were assayed in one batch using a multiplex analysis system. Multiplex bead kits (Biosource, Camarillo, CA), based on the principle of solid phase sandwich immunoassays, were employed and stimulated levels of IL-6, IL-1β, and TNF-α were determined using Bio-Plex Manager Software (Bio-rad Corporation, Hercules, CA), interpolating from the standard curve (Logisitc-5PL curve fit). Pooled plasma controls were included on all plates to determine assay reliability. Inter- and intra- assay coefficients of variability were less than 10%. Stimulated cytokine production was quantified by subtracting cytokine levels in unstimulated samples from the stimulated levels (Prather et al. 2007).

3.3.4 Air Pollution Data

Pollutant concentrations were measured during a multi-pollutant monitoring campaign in Allegheny County previously described (Shmool et al. 2014; Tunno et al. 2015c). Our sampling domain, including both urban and suburban areas in the greater Pittsburgh region, was determined using geographic information systems (GIS) (ArcMap 10.0-10.3, Redlands), to capture major industrial sources in Allegheny county (e.g., steel mill, coke works). A stratified random sampling design was used to select 36 monitoring sites based on cross-stratified classes of elevation, traffic density, and industrial emissions. Monitoring was completed during summer

(June-July) 2012, and the same sites were repeated in winter (January-March) 2013. $PM_{2.5}$ samples were collected using Harvard Impactors (Air Diagnostics and Engineering Inc.) at 4.0 liters per minute. Integrated $PM_{2.5}$ concentrations were obtained at each site for the first 15 minutes of every hour for 7 days. Eight sites were sampled per session. $PM_{2.5}$ concentrations were calculated based on gravimetric analysis of Teflon filters before and after sampling, and black carbon was measured using an EEL43M Smokestain Reflectometer (Diffusion Systems). Elemental concentrations were determined using inductively-coupled plasma mass spectrometry (ICP-MS) (Wisconsin State Laboratory of Hygiene) (Shmool et al. 2014; Tunno et al. 2015c).

3.3.5 Hybrid LUR Models

To estimate average one-year air pollution exposures at the homes of each AHAB II participant, we used previously-developed hybrid LUR models for $PM_{2.5}$, BC, Pb, Mn, Fe, and Zn. Model development is detailed elsewhere (Tripathy et al. 2017; Tunno et al. 2015c). Briefly, covariates were created using GIS to capture a variety of potential pollutant sources - including traffic density indicators, transportation indicators, road-specific measures, land use/built environment, industrial emissions, population, and truck, bus, and diesel indicators (Tunno et al. 2015c) - across locations. Following our hybrid AERMOD-LUR modeling approach, detailed in Michanowicz et al. 2016 (Michanowicz et al. 2016), two additional covariates were developed using the AERMOD atmospheric dispersion model. One dispersion variable was built using emissions profiles for 207 sources (AERMOD-predicted *industrial* $PM_{2.5}$ emissions), A second was developed using only the 14 point source profiles associated with the Edgar Thomson Steel Works (AERMOD-predicted *steel mill* $PM_{2.5}$ emissions).

Hybrid LUR models were built using a manual forward step-wise approach combined with random forest analyses using SAS version 9.3 (Cary, NC) and R version 3.1.0 to select covariates that contributed the most to variability in pollutant concentrations. In addition to spatial covariates, a temporal term was incorporated into models using daily concentrations from an Environmental Protection Agency (EPA) Air Quality System (AQS) maintained by the Allegheny County Health Department (ACHD) centrally located within the sampling domain. These models were used to predict pollutant concentrations across the monitoring domain using source layers in GIS. Model predictions were then spatially extrapolated outside of the original sampling domain to include all of Allegheny County where most AHAB-II participants lived (Tripathy et al. 2017).

3.3.6 Geocoding

AHAB-II participant addresses were geocoded using a three-tiered system in GIS, following methods we have used successfully in other cities as shown in Figure 1 (Shmool et al. 2016). Briefly, addresses were first run through a U.S. Postal Service reference dataset using ZP4™ address standardization software (Semaphore Corporation, Monterey, CA). Incomplete addresses, P.O. Box numbers, and addresses outside of Allegheny County were excluded. We first attempted to match addresses using an address point based locator, unmatched addresses were then matched via a parcel centroid locator. Finally, any remaining addresses were matched using a street network locator. Buffers were created 300 m around geocoded addresses in preparation for exposure assignment. Participants with unmatched addresses or 300 m buffers that were not completely contained within the Allegheny County boundary were excluded.

3.3.7 Exposure Assignment

$PM_{2.5}$, BC, Pb, Mn, Fe, and Zn exposure estimates were assigned using pollutant LUR surfaces. Mean concentration estimates were assigned within a 300 m buffer of geocoded addresses. These exposures were then temporally extrapolated to a 1 year average predicted concentration prior to the date of participant blood draw. This was done using the same AQS monitoring data used during the LUR modeling process using the procedure by Tripathy et al. 2017 (Tripathy et al. 2017).

3.3.8 Statistical Analysis

IL-6, CRP, and stimulated IL-6, IL-1β, and TNF-α were tested for normality with PROC UNIVARIATE and examination of histogram distributions. Each exposure-outcome relationship was tested for linearity by reviewing scatter plots of exposures versus outcomes. Bivariate linear regression models were run for each pollutant by each inflammatory marker. Next, pollutants were tested in a second model adjusting for age, sex, race, smoking status (current, former, never), body mass index (BMI), and education (years) as potential confounders. Potential interaction of pollutants by sex was also tested. Statistical analyses were generated using SAS versions 9.3-9.4 (Cary, NC) and scatter plots were displayed using STATA version 13.0 (StataCorp, TX).

3.3.9 Sensitivity Analyses

Bivariate linear regression models and linear regression models adjusting for confounders were run again excluding participants that did not live within the original monitoring domain to ensure results were not due to misclassification by sources that may not have been represented in the original sampling domain.

3.4 RESULTS

3.4.1 AHAB II Sample Size

The original AHAB II dataset contained 490 participants. Twenty-nine participants were excluded due to incomplete addresses, P.O. Box numbers, and addresses outside of Allegheny County as shown in Figure 4. Remaining addresses were geocoded using the composite locator resulting in 4 unmatched addresses (n=463) and 2 additional addresses were excluded with 300 m buffers extending outside of Allegheny County. Exposures were assigned at 461 geocoded locations using our hybrid LUR pollutant exposure surfaces.

A separate sample size was determined for circulating cytokines and stimulated cytokines due to missing inflammatory outcome data resulting in 393 participants with circulating cytokine data and 379 with stimulated cytokine data. One additional participant was excluded from the circulating cytokine sample due to missing data on smoking status. Participant geocoded addresses are shown in Figure 4.

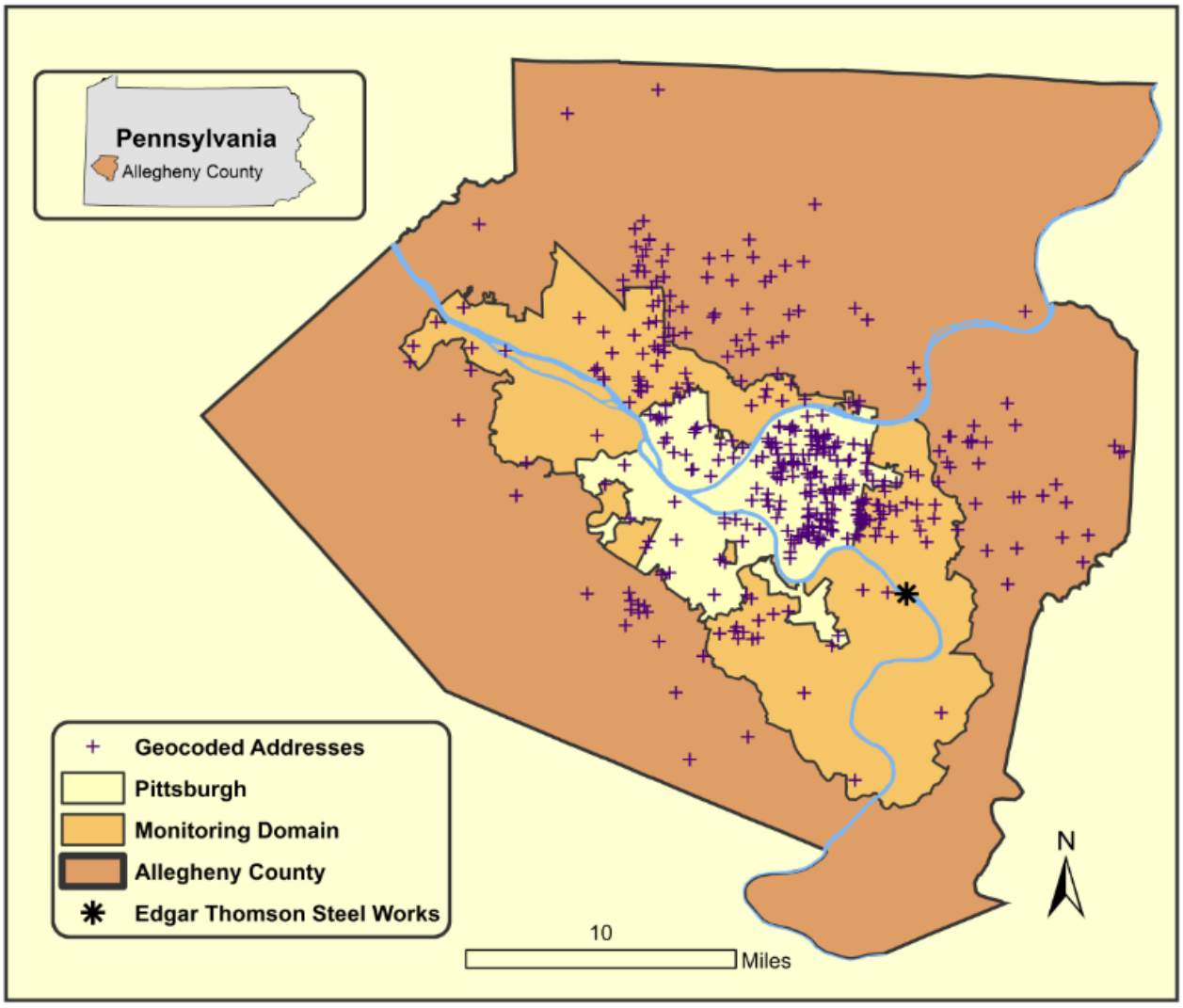

Figure 4. Geocoded addresses for AHAB II participants with valid circulating IL-6 and CRP

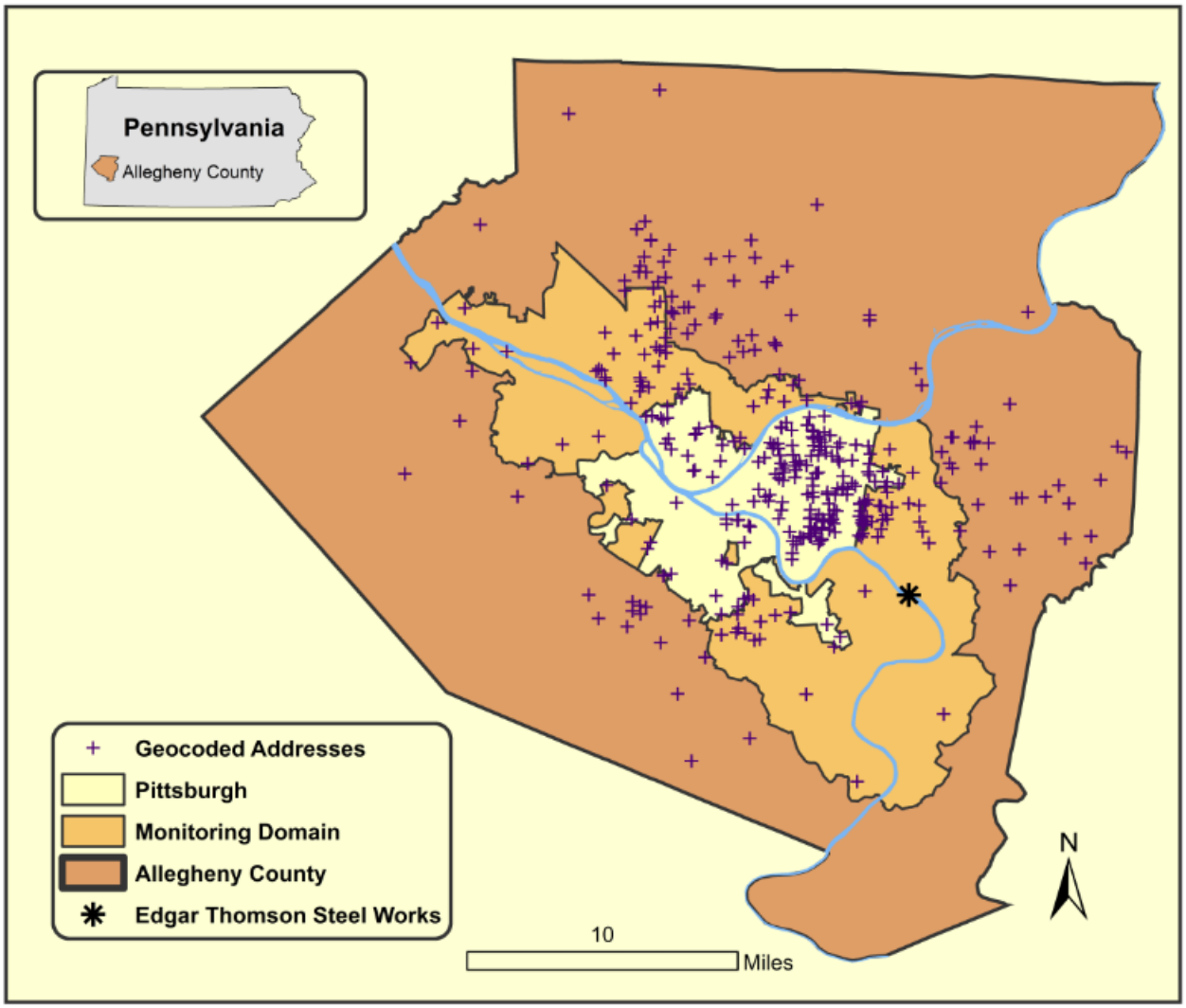

Figure 5. Geocoded addresses for AHAB II participants with valid stimulated cytokines

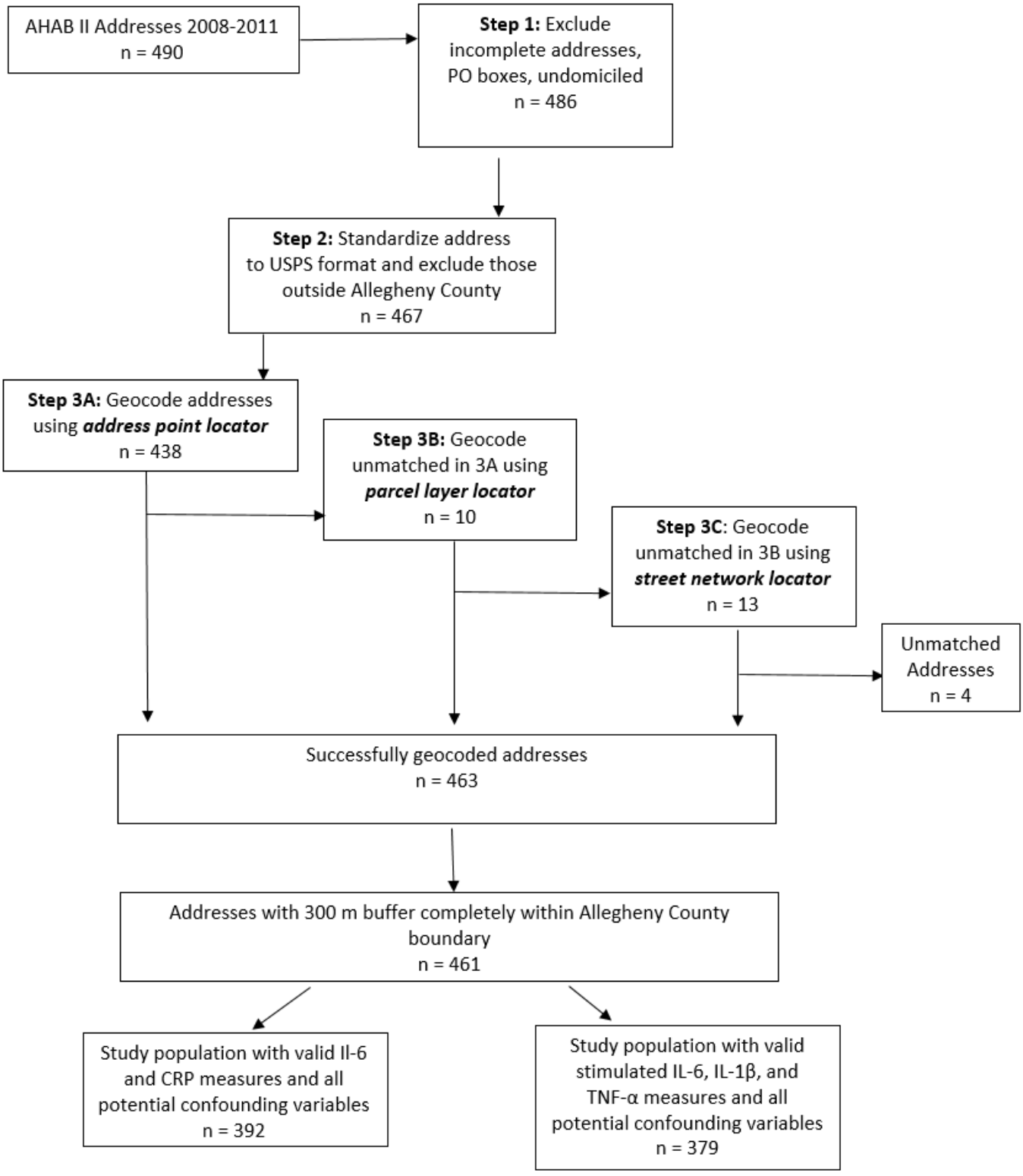

Figure 6. AHAB II participant exclusions and geocoding methodology

3.4.2 Sample Characteristics

As shown in Table 4 and Table 5, sample characteristics were similar for participants with valid circulating cytokines (n=392) compared to participants with valid stimulated cytokine data (n=379). AHAB II had slightly more women than men and the average age was approximately 43 years. Participants were predominately white and had completed college on average. Most participants had never smoked.

Table 4. AHAB II Participant Characteristics: Circulating Cytokines

Sample characteristics	mean (SD) or %	5%	95%
Sex (%)	48% male, 52% female		
Age (years)	43.1 (7.2)	31	53
Race (%)	81.7% white, 16.3% black, 2.0% other		
Education (years)	16.9 (2.9)	12	23
BMI (kg/m2)	27.2 (5.1)	19.9	36.2
Smoking status	20.4% former, 16.6% current, 63% never		

Table 5. AHAB II Participant Characteristics: Stimulated Cytokines

Sample characteristics	mean (SD) or %	5%	95%
Sex (%)	46.4% male, 53.6% female		
Age (years)	42.8 (7.4)	31	53
Race (%)	82.3% white, 15% black, 2.7% other		
Education (years)	17.0 (2.9)	12	23
BMI (kg/m2)	26.9 (5.3)	19.9	36.7
Smoking status	21.1% former, 14.5% current, 64.4% never		

3.4.3 Statistical Analysis

Natural-log transformation was applied to all outcome variables to correct skewed distributions. Scatter plots were made for each pollutant versus outcome to assess linearity (Fig. 5-9). Pb, Mn, Zn, and Fe exposures were transformed using the natural log to improve linear fit.

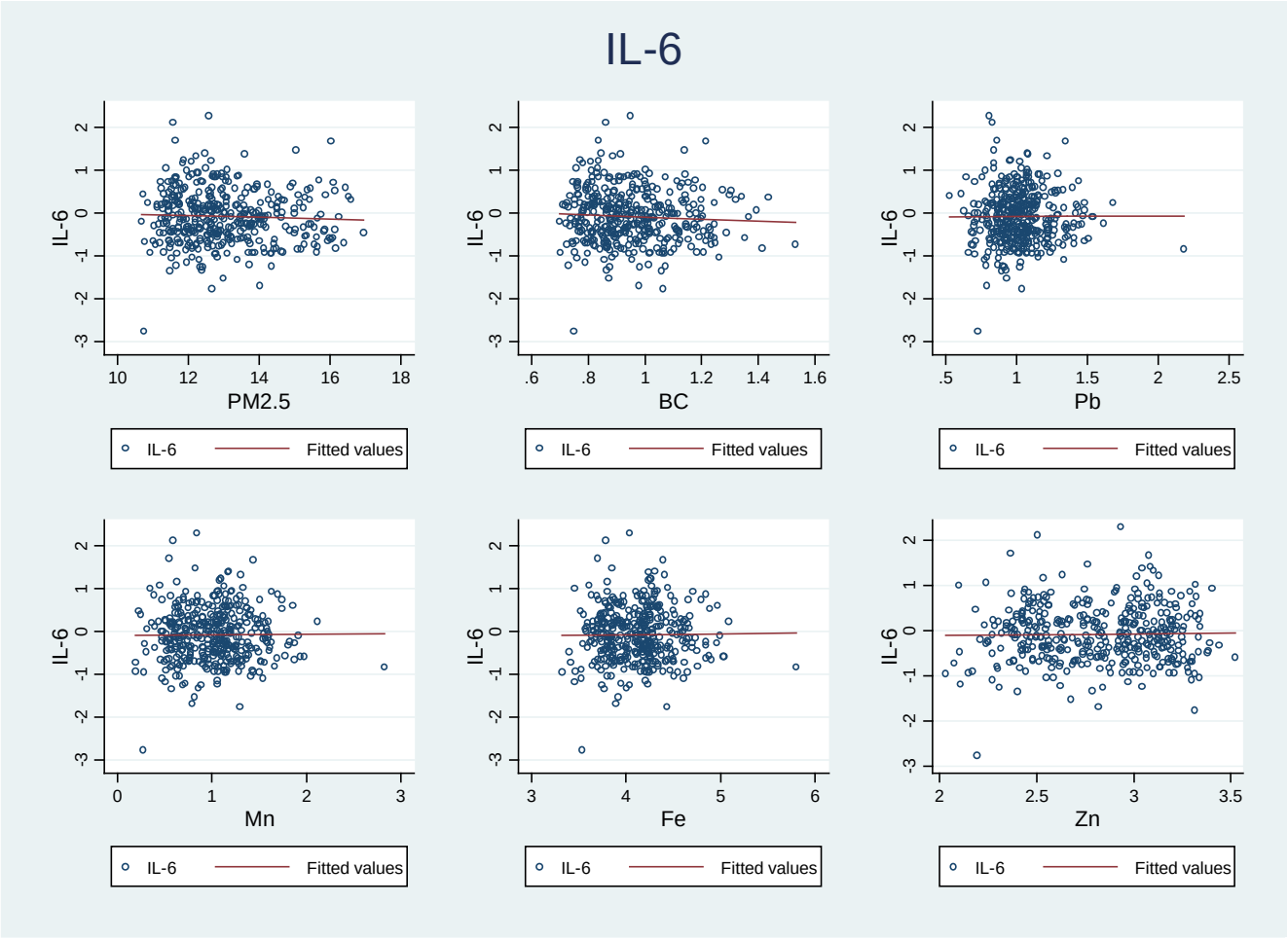

Figure 7. Scatter plots for pollutants versus IL-6

Figure 8. Scatter plots for pollutants versus CRP

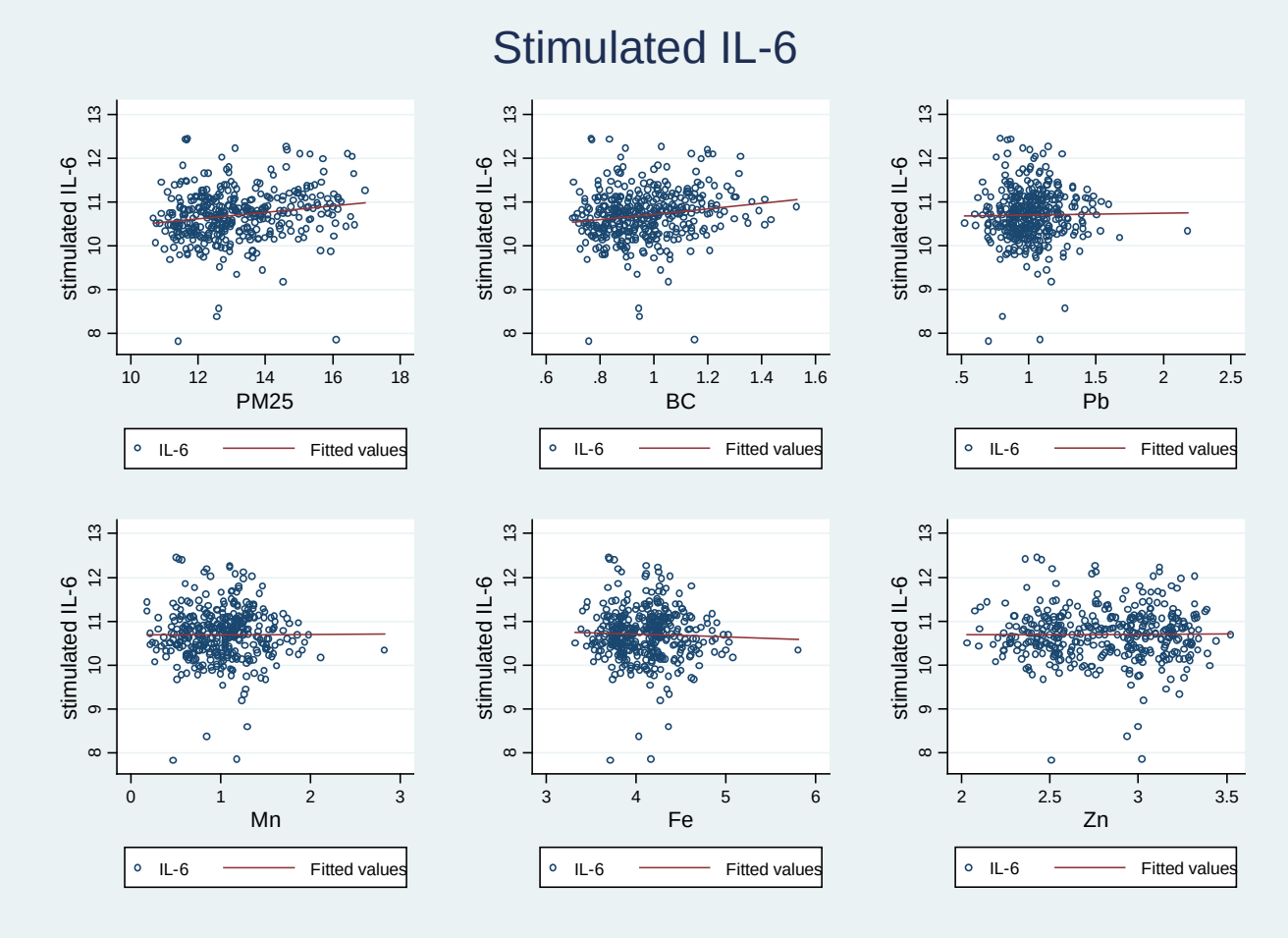

Figure 9. Scatter plots for pollutants versus stimulated IL-6

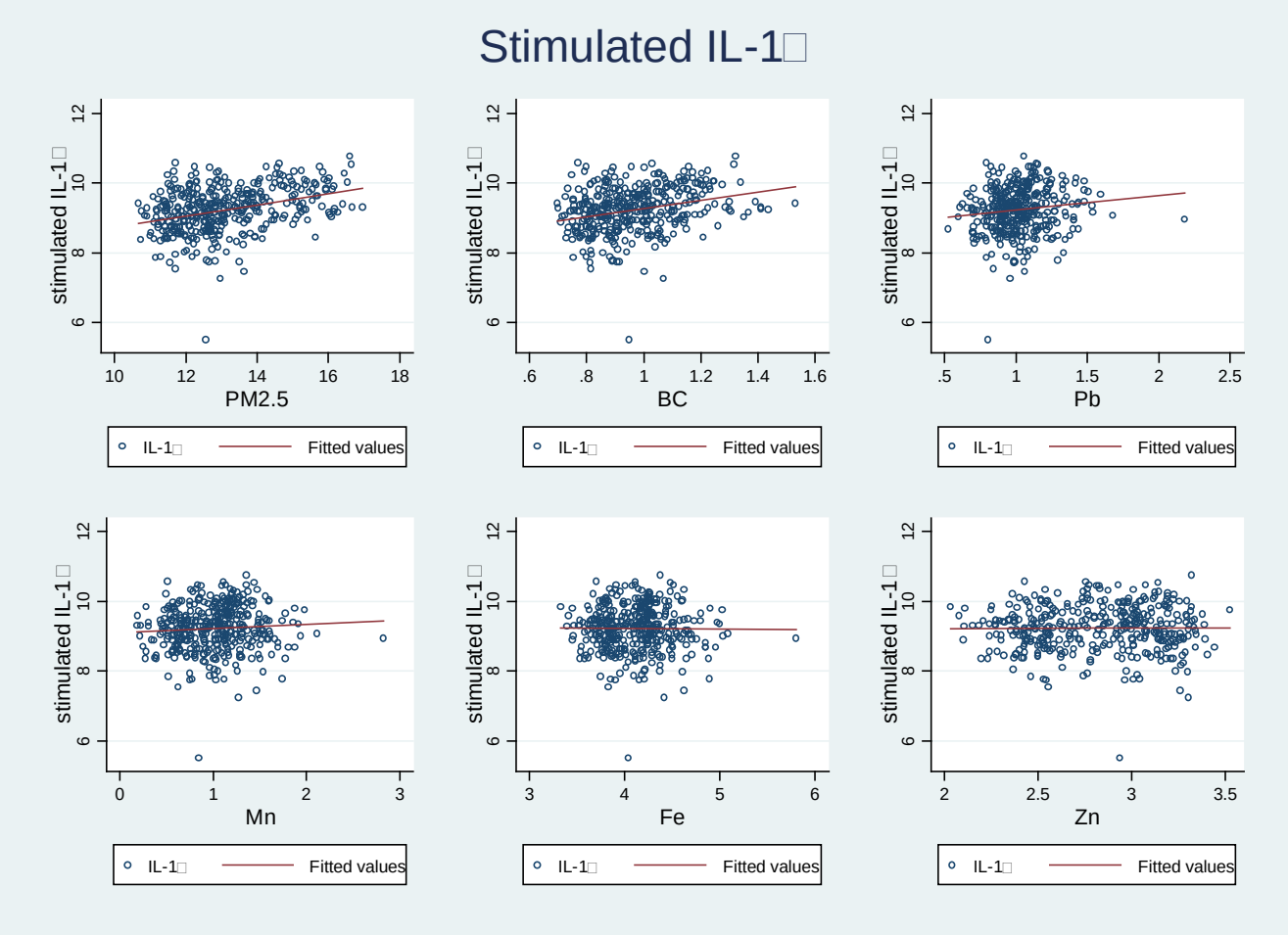

Figure 10. Scatter plots for pollutants versus stimulated IL-1β

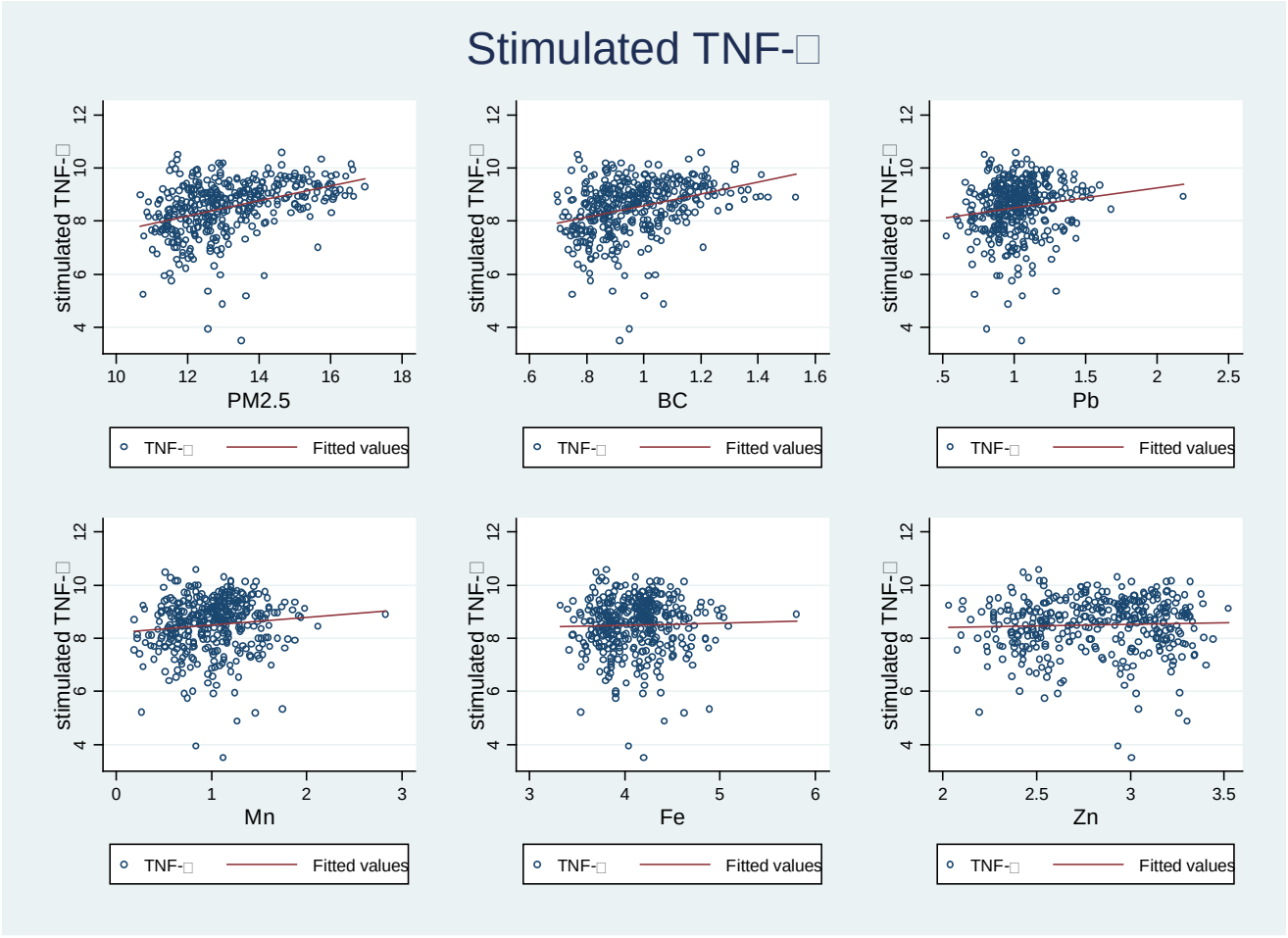

Figure 11. Scatter plots for pollutants versus stimulated TNF-α

Pollutant exposures, confounders, and outcomes are summarized in Tables 6-8. Average pollutant exposures were similar between participants with valid circulating cytokines and participants with valid stimulated cytokines, as shown in Table 6 and Table 7.

Table 6. AHAB II 1-year residential pollutant exposure estimates: circulating cytokines (n=392)

Exposure estimates	**mean (SD)**	**5%**	**95%**
$PM_{2.5}$ ($\mu g/m^3$)	13.0 (1.35)	11.3	15.7
BC (abs)	0.95 (0.15)	0.75	1.23
Pb (ng/m^3)	2.84 (0.63)	2.06	4.06
Mn (ng/m^3)	2.95 (1.32)	1.60	5.00
Fe (ng/m^3)	65.3 (26.9)	37.9	110.1
Zn (ng/m^3)	17.6 (5.57)	9.81	27.1

Table 7. AHAB II 1-year residential pollutant exposure estimates: stimulated cytokines (n=379)

Exposure estimates	**mean (SD)**	**5%**	**95%**
$PM_{2.5}$ ($\mu g/m^3$)	13.1 (1.41)	11.3	15.9
BC (abs)	0.97 (0.15)	0.76	1.24
Pb (ng/m^3)	2.83 (0.63)	2.05	4.03
Mn (ng/m^3)	2.95 (1.33)	1.60	4.99
Fe (ng/m^3)	65.1 (27.0)	37.0	110.1
Zn (ng/m^3)	17.6 (5.65)	9.71	27.1

Table 8. AHAB II outcomes

Inflammatory marker	**n**	**mean (SD)**	**5%**	**95%**
Il-6 (pg/ml)	392	1.14 (0.94)	0.39	2.51
CRP (ng/ml)	392	1.67 (1.93)	0.21	5.87
stimulated Il-6 (pg/ml)	379	52415.6 (35648.9)	19371.4	116938.1
stimulated Il-1β (pg/ml)	379	12322.4 (7632.6)	3528.5	27943.1
stimulated tnf-α (pg/ml)	379	7319.1 (6071.8)	808.3	18570.1

Results from bivariate linear regression models are shown in Tables 9-10. We found significant positive associations ($p < 0.05$) of exposure to $PM_{2.5}$ and BC with all stimulated cytokines. Pb was significantly associated with higher stimulated production of IL-1β and TNF-α, and Mn with TNF-α production. Associations of Pb with IL-1β and Mn with TNF-α did not withstand Bonferroni correction for multiple testing ($p < 0.008$). No significant associations were found among pollutants with circulating IL-6 or CRP.

Table 9. Bivariate linear regression models for inflammatory markers by pollutant including intercept (β), standard error (SE) and p-value: circulating cytokines

	IL-6		**CRP**	
pollutant	**β (SE)**	**p-value**	**β (SE)**	**p-value**
$PM_{2.5}$	-0.02 (0.02)	0.36	-0.04 (0.04)	0.27
BC	-0.25 (0.21)	0.24	-0.19 (0.34)	0.57
Mn	0.02 (0.09)	0.85	0.11 (0.14)	0.41
Pb	0.01 (0.16)	0.93	0.31 (0.26)	0.25
Fe	0.02 (0.09)	0.79	0.15 (0.15)	0.31
Zn	0.03 (0.10)	0.74	0.08 (0.16)	0.63

Table 10. Bivariate linear regression models for inflammatory markers by pollutant including intercept (β), standard error (SE) and p-value: stimulated cytokines

	IL-1β		**IL-6**		**Tnf-α**	
pollutant	**β (SE)**	**p-value**	**β (SE)**	**p-value**	**β (SE)**	**p-value**
$PM_{2.5}$	0.16 (0.02)	<.0001**	0.07 (0.02)	0.0007*	0.28 (0.03)	<.0001**
BC	1.18 (0.21)	<.0001**	0.62 (0.20)	0.002*	2.21 (0.33)	<.0001**
Mn	0.12 (0.09)	0.18	0.003 (0.08)	0.97	0.30 (0.14)	0.04*
Pb	0.42 (0.17)	0.01*	0.04 (0.16)	0.81	0.75 (0.27)	0.006*
Fe	-0.01 (0.10)	0.88	-0.06 (0.09)	0.47	0.08 (0.15)	0.59
Zn	0.02 (0.10)	0.87	0.008 (0.09)	0.93	0.13 (0.16)	0.41

*p<.05 **p<.0001

Fully-adjusted model results are shown in Tables 11-13. After adjusting for age, sex, race, smoking status, and BMI, significant positive associations were found for $PM_{2.5}$ and BC with all stimulated cytokine concentrations. Significant associations were also found for Pb with IL-1β ($p=0.02$) and TNF-α ($p=0.02$) in fully-adjusted models, before accounting for multiple comparison. Applying a Bonferroni correction to account for multiple comparisons produced…. ($p<0.008$). There were no significant interactions by sex.

Table 11. Linear regression model results for stimulated cytokines by pollutants adjusting for BMI, education, sex, age, race, and smoking status: IL-1β

	$PM_{2.5}$	BC	Pb	Mn	Fe	Zn
Parameter	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	7.53 (0.44)**	8.46 (0.39)**	9.12 (0.39)**	9.41 (0.40)**	9.54 (0.53)**	9.38 (0.44)**
Pollutant	0.16 (0.02)**	1.11 (0.21)**	0.4 (0.17)*	0.12 (0.09)	-0.004 (0.10)	0.05 (0.10)
BMI	0.001 (0.006)	0.001 (0.006)	-0.0003 (0.007)	-0.0009 (0.007)	-0.001 (0.01)	-0.001 (0.01)
Education	0.008 (0.01)	0.01 (0.010	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)
Age	-0.01 (0.004)*	-0.01 (0.005)*	-0.01 (0.005)*	-0.01 (0.005)*	-0.01 (0.005)*	-0.01 (0.005)*
Sex	-0.02 (0.06)	-0.04 (0.07)	-0.04 (0.07)	-0.04 (0.07)	-0.04 (0.07)	-0.04 (0.07)
Race (black)	0.03 (0.09)	0.01 (0.10)	0.03 (0.10)	0.04 (0.10)	0.05 (0.10)	0.05 (0.10)
Race (other)	-0.1 (0.20)	-0.05 (0.20)	-0.06 (0.21)	-0.01 (0.21)	0.02 (0.21)	0.02 (0.21)
Race (white)	0	0	0	0	0	0
Smoking (former)	0.01 (0.08)	0.02 (0.08)	0.01 (0.08)	0.02 (0.08)	0.03 (0.09)	0.03 (0.09)
Smoking (current)	0.06 (0.09)	0.05 (0.10)	0.04 (0.10)	0.05 (0.10)	0.05 (0.10)	0.05 (0.10)
Smoking (never)	0	0	0	0	0	0

*p<.05 **p<.0001

Table 12. Linear regression model results for stimulated cytokines by pollutants adjusting for BMI, education, sex, age, race, and smoking status: IL-6

	$PM_{2.5}$	BC	Pb	Mn	Fe	Zn
Parameter	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	9.6 (0.41)**	9.97 (0.36)**	10.53 (0.35)**	10.54 (0.32)**	10.78 (0.47)**	10.45 (0.39)**
Pollutant	0.08 (0.02)*	0.61 (0.19)*	0.02 (0.15)	0.009 (0.08)	-0.06 (0.09)	0.04 (0.09)
BMI	0.009 (0.006)	0.009 (0.006)	0.007 (0.006)	0.007 (0.006)	0.007 (0.006)	0.007 (0.006)
Education	0.004 (0.01)	0.007 (0.01)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)	0.01 (0.01)
Age	-0.008 (0.004)	-0.008 (0.004)*	-0.009 (0.004)*	-0.009 (0.004)*	-0.009 (0.004)	-0.009 (0.004)*
Sex	0.29 (0.06)**	0.28 (0.06)**	0.28 (0.06)**	0.28 (0.06)**	0.28 (0.06)**	0.28 (0.06)**
Race (black)	-0.02 (0.09)	-0.03 (0.09)	-0.01 (0.09)	-0.01 (0.09)	-0.01 (0.09)	-0.01 (0.09)
Race (other)	-0.12 (0.18)	-0.10 (0.18)	-0.06 (0.19)	-0.06 (0.19)	-0.04 (0.19)	-0.06 (0.19)
Race (white)	0	0	0	0	0	0
Smoking (former)	0.005 (0.07)	0.009 (0.07)	0.01 (0.08)	0.01 (0.08)	0.01 (0.08)	0.01 (0.08)
Smoking (current)	0.16(0.09)	0.15 (0.09)	0.16 (0.09)	0.16 (0.09)	0.16 (0.09)	0.16 (0.09)
Smoking (never)	0	0	0	0	0	0

*p<.05 **p<.0001

Table 13. Linear regression model results for stimulated cytokines by pollutants adjusting for BMI, education, sex, age, race, and smoking status: TNF-α

	PM2.5	BC	Pb	Mn	Fe	Zn
Parameter	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	5.71 (0.68)**	7.25 (0.61)**	8.56 (0.61)**	8.95 (0.56)**	8.89 (0.83)**	8.71 (0.69)**
Pollutant	0.28 (0.035)**	2.06 (0.33)**	0.65 (0.27)*	0.26 (0.14)	0.08 (0.15)	0.19 (0.16)
BMI	-0.01 (0.01)	-0.01 (0.01)	-0.01 (0.01)	-0.02 (0.01)	-0.02 (0.01)	-0.02 (0.02)
ducation	0.003 (0.02)	0.01 (0.02)	0.02 (0.02)	0.03 (0.02)	0.03 (0.02)	0.03 (0.02)
Age	-0.02 (0.007)*	-0.02 (0.007)*	-0.02 (0.007)*	-0.02 (0.007)*	-0.02 (0.007)*	-0.02 (0.007)*
Sex	0.19 (0.10)	0.15 (0.10)	0.14 (0.11)	0.15 (0.11)	0.15 (0.11)	0.15 (0.11)
Race (black)	0.33 (0.14)*	0.30 (0.15)*	0.33 (0.15)*	0.35 (0.15)*	0.35 (0.15)*	0.35 (0.33)*
Race (other)	0.01 (0.31)	0.09 (0.31)	0.10 (0.33)	0.15 (0.33)	0.21 (0.33)	0.23 (0.33)
Race (white)	0	0	0	0	0	0
Smoking (former)	0.01 (0.12)	0.026 (0.13)	0.02 (0.13)	0.03 (0.13)	0.04 (0.13)	0.04 (0.13)
Smoking (current)	0.03 (0.14)	0.01 (0.15)	0.009 (0.15)	0.02 (0.15)	0.02 (0.16)	0.03 (0.15)
Smoking (never)	0	0	0	0	0	0

*p<.05 **p<.0001

3.4.4 Sensitivity Analyses

The bivariate regression association between Mn with TNF-α and the adjusted model association of Pb with IL-1β and TNF-α did not reach statistical significance after excluding participants that did not live within the original air pollution monitoring domain.

3.5 DISCUSSIONS

We found that chronic air pollution exposures were associated with higher production of pro-inflammatory cytokines in response to ex vivo stimulation with endotoxin. In contrast, there was no association of pollutant exposures with circulating levels of inflammatory mediators. $PM_{2.5}$, and BC exposure associated positively with LPS-stimulated IL-6, IL-1β, and TNF-α among a cohort of adults living in Allegheny County, PA. Pb was associated with stimulated TNF-α, and IL-1β, although neither association was significant after adjustment for multiple comparisons.

Our results suggest that chronic air pollution exposures may influence the magnitude of inflammatory response to endotoxin. While stimulated cytokine measures are in direct response to endotoxin, individual differences in the magnitude of response may also predict future cardiovascular risk (Marsland et al. 2017b). For example, Brydon et al., found that the magnitude of stimulated Il-6 predicted ambulatory blood pressure three years after measurement (Brydon and Steptoe 2005). Individuals with larger increases in LPS-stimulated inflammatory mediators may also be prone to increases in mediators of systemic inflammation (Lockwood et al. 2016).

We did not find significant associations between pollutant exposures with IL-6 or CRP. Several factors may have influenced these results. While we examined associations not only with $PM_{2.5}$ but also with BC, Pb, Mn, Zn, and Fe components, a different source of $PM_{2.5}$ could be associated with systemic inflammation. For example, Zeka et al., (2006) found significant positive associations between traffic-related particles with inflammatory markers but not with $PM_{2.5}$ or sulphates (Zeka et al. 2006). Duration of the pollutant exposures and population susceptibility may also influence associations (Robert D Brook et al. 2010). While positive associations have been found between pollutant exposures with markers of systemic inflammation in healthy cohorts, studies have also found associations in potentially vulnerable subpopulations including older, obese, diabetic, and hypertensive people (Dubowsky et al. 2006; Zeka et al. 2006). For example, Ostro et al (2014)., found that a 10-$\mu g/m^3$ increase in annual $PM_{2.5}$ more than doubled the risk of CRP greater than 3 mg/l in women who were older diabetics, or smokers (Ostro et al. 2014). One reason for the lack of association between pollutants with circulating inflammatory mediators in this study, could be because AHAB II is composed of relatively healthy, middle-aged participants, with no history of clinical cardiovascular disease, angina, or claudication, and taking no cardiovascular medications

3.5.1 Strengths and Limitations

A clear limitation of this study is its cross-sectional design. Having inflammatory mediator data at multiple time points would allow us to disentangle relevant exposure windows, and to examine changes in both pollutant exposures and cytokine levels over time, within and between participants.

Another limitation was that we needed to spatially extrapolate predicted pollutant concentrations outside of the original monitoring domain to include all of Allegheny County. Sensitivity analyses including only those participants within the original sampling domain, however, revealed comparable results, with the exception that associations for Pb with stimulated cytokines became non-significant after adjusting for multiple comparisons.

In addition, we also needed to temporally adjust predicted pollutant concentrations, because our air monitoring campaign and participant blood draws were performed at two different points in time. However, using regulatory data from the ACHD AQS monitor provided daily temporal resolution, improving the accuracy of spatio-temporal exposure estimates. While associations with inflammatory markers were tested using only one-year exposure estimates, correlations between 1- and 5-year participant-specific pollutant exposure estimates were highly correlated ($r > 0.90$ for all pollutants), indicating stable exposure contrast across the cohort over time.

Though $PM_{2.5}$ and BC exposures were significantly associated with all stimulated cytokine concentrations, metal constituent exposures were either insignificant or became insignificant after adjusting for multiple comparisons. One explanation could be that relatively few AHAB II participants lived in close proximity to the Edgar Thomson Steel Works (one participant lived within a mile of it), and thus more participants may have been exposed to other sources of $PM_{2.5,}$ such as traffic-related sources.

AHAB-II was a predominately white, relatively healthy, and well-educated cohort, and thus results may not be generalizable to other populations. There is a need for future studies examining the impact of pollutants on stimulated cytokines among more diverse populations

from a variety of different locations in order to assess generalizability, particularly among vulnerable subgroups including older populations.

3.6 CONCLUSIONS

The goal of this study was to examine whether chronic exposure to ambient outdoor air pollution altered systemic inflammation and/ or magnitude of inflammatory response to endotoxin. We found that one-year outdoor residential exposures to $PM_{2.5}$ and BC were associated with significant increases in concentrations of LPS-stimulated production of IL-6, IL-1β, and TNF-α, in a cohort of healthy middle-aged adults living in the Pittsburgh area. We found no significant associations between pollutant concentrations and circulating IL-6 or CRP. Results of this study suggest that chronic exposure to pollution may prime the innate immune system to be more reactive, increasing inflammatory responses to immune stimulation. It is possible that this provides a pathway connecting exposure to pollution to increased risk for inflammatory diseases, including allergies, asthma and CVD. Further research is needed, using longitudinal cohorts, and examining associations across more diverse populations, geographic locations, and source mixtures.

4.0 OUTDOOR AIR POLLUTION AND BRAIN MORPHOLOGY IN THE ADULT HEALTH BEHAVIOR II AND PITTSBURGH IMAGING PROJECT COHORTS

4.1 ABSTRACT

Exposure to ambient fine particulate matter ($PM_{2.5}$) has been associated with adverse neurological outcomes (e.g., cognitive decline), possibly mediated through systemic inflammation, disruption of the blood brain barrier, or translocation via olfactory mucosa. A few recent studies have also linked $PM_{2.5}$ to indicators of brain morphology, although little is known about which components of $PM_{2.5}$ may drive these associations. We examined relationships between ambient exposures to $PM_{2.5}$ and multiple components [i.e., black carbon (BC), lead (Pb), manganese (Mn), iron (Fe), zinc (Zn)] with measures of brain morphology [i.e., total and cortical gray matter volumes, cortical white matter volume, total white matter surface area, mean cortical thickness] from magnetic resonance images (MRIs) of participants in the Adult Health Behavior II and Pittsburgh Imaging Project Cohorts (n = 702). Annual average pollutant exposure estimates were assigned for the 300 m buffer around each participant's address using hybrid land use regression models. Linear regression models were developed to examine associations between pollutant exposures and brain morphology measures, adjusting for intracranial volume, age, sex, race, education, and smoking status. No significant associations were found between $PM_{2.5}$, BC, or metal constituent exposures with any of the brain morphology outcomes. Both

AHAB II and PIP cohorts include relatively healthy middle aged participants. While we did not find associations between pollutant exposures and measures of brain morphology, examining associations in these same adults later in life, in older cohorts, or using more refined measures of brain morphology (e.g., voxel analysis) may provide greater insights into potential associations.

4.2 INTRODUCTION

Growing evidence suggests a relationship between particulate air pollution exposures and adverse neurological outcomes (e.g., cognitive decline, ischemic stroke) (Lisabeth et al. 2008; Maheswaran et al. 2014; Ranft et al. 2009; Stafoggia et al. 2014), potentially mediated through systemic inflammation, disruption of the blood-brain barrier (Calderon-Garciduenas et al. 2008a; Calderon-Garciduenas et al. 2008b), translocation via olfactory mucosa (Ajmani et al. 2016; Maher et al. 2016), or other mechanisms (Robert D Brook et al. 2010; Calderón-Garcidueñas et al. 2010; Costa et al. 2014; Genc et al. 2012; Peters et al. 2006). These effects likely vary by $PM_{2.5}$ composition, and the literature linking metals exposures to adverse neurological outcomes suggests that urban airborne metals [e.g., lead (Pb), manganese (Mn), iron (Fe), zinc (Zn)] may be critical components of $PM_{2.5}$ impacting this effect (Finkelstein and Jerrett 2007; Lucchini et al. 2012; White et al. 2007).

Fine particle exposure may engender early cognitive decline, possibly by mechanisms involving the effects of $PM_{2.5}$-related inflammation on brain tissue integrity (Ranft et al. 2009). Measures of brain morphology (e.g., cortical thickness, gray matter volume) have been associated with cognitive decline as well as neurological diseases such as Alzheimer's or Parkinson's disease (Block et al. 2012; Dickerson and Wolk 2012; Ferreira et al. 2014; Marsland

et al. 2015; Whitwell et al. 2008). Although the relationship between brain morphology and neurological outcomes is complex, better understanding potential effects of air pollution on brain morphology may help to elucidate pollutant impacts on neurological outcomes, and suggest opportunities for intervention towards preventing neurocognitive decline (Genc et al. 2012).

In occupational settings, high airborne metals concentrations have been associated with brain structure; for example, welders chronically exposed to Mn have shown significantly decreased globus pallidus and cerebellar brain regions, compared to age-matched controls (Chang et al. 2013). In animal models, exposures to individual metals (e.g., Fe, Pb, Mn, Zn) and metals mixtures (Wright and Baccarelli 2007) have been shown to induce neurotoxic effects, including impacts on specific brain regions (Lucchini et al. 2012). For example, Pb exposures have been linked to altered hippocampal morphology in mice (Verina et al. 2007).

To date, most of the epidemiologic literature linking air pollution and brain morphology has been performed in children or older adults. For example, some recent studies have examined associations between children's exposure to air pollution at schools with structural and functional brain changes from MRI scans. In one study of 263 children in Barcelona, a composite air pollution indicator combining indoor and outdoor elemental carbon and NO_2 at schools was developed indicative of traffic-related pollution; no significant associations were found with brain structure, however, children with higher pollution exposures had lower functional integration and segregation in certain brain networks (Pujol et al. 2016b). Another study in the same cohort examined associations between copper (Cu) in $PM_{2.5}$ measured in school courtyards with structural and functional brain measures obtained from anatomical MRI, diffusion tensor imaging, and functional MRI. Associations were found between higher exposures to Cu and

poorer motor performance and alterations in basal ganglia structure and function (Pujol et al. 2016a).

A few epidemiological studies have explored relationships between ambient outdoor $PM_{2.5}$ and measures of brain structure in older adults. Wilker et al. (2015), found that a 2-$\mu g/m^3$ increase in one-year annual average $PM_{2.5}$ was associated with a 0.32% decrease in cerebral brain volume and 46% higher odds of covert brain infarcts (Wilker et al. 2015). Chen et al. (2015) found significant associations between exposure to ambient $PM_{2.5}$ and decreased white matter volume in frontal and temporal lobes and in the corpus callosum of older women (Chen et al. 2015).

$PM_{2.5}$ composition varies by pollutant source, and therefore epidemiologic studies of metals components may require finer-scale source-specific exposure assessment than is needed for PM. Previous studies have developed land use regression (LUR) models for metal constituents of $PM_{2.5}$ from multi-pollutant monitoring campaigns (de Hoogh et al. 2013; Zhang et al. 2015), though few have yet applied these metals LURs in epidemiological studies (Fuertes et al. 2014; Wang et al. 2014).

We aimed to contribute to this literature by applying fine-scale spatial models for $PM_{2.5}$ and metals constituents to examine associations between spatially-varying airborne metals and measures of brain structure in healthy adults. Pittsburgh, PA is a city with legacy industry (e.g., steel mills, coke works) and, consequently, relatively high airborne metals concentrations with substantial intra-urban variation (Tunno et al. 2015a). We applied previously-developed hybrid LUR models (Tripathy et al. 2017), to distinguish associations between multiple source-specific airborne metals and a broad suite of brain morphology measures. We hypothesized that higher residence-based exposures to $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe would be associated with reduced

structural integrity of the brain in two Pittsburgh cohorts of health middle-aged adults. Observed associations may have implications for pollution effects on brain-based functional outcomes including early cognitive decline and neurological disorders.

4.3 METHODS

4.3.1 AHAB-II and PIP Cohorts

AHAB-II and PIP are prospective cohorts of healthy middle-aged adults in Western Pennsylvania, developed to identify neural and bio-behavioral predictors of physical and mental health in midlife. AHAB II Participants were recruited between March 2008 and October 2011 through mass mailings of invitation letters to individuals randomly selected from voter registration and other public domain lists. Individuals eligible for AHAB-II were aged 30–54 years, were working at least 25 h per week outside of the home, and spoke English as their first language. Individuals were further excluded if they: (a) had a history of cardiovascular disease, schizophrenia or bipolar disorder, chronic hepatitis, renal failure, major neurological disorder, chronic lung disease, or stage 2 hypertension (SBP/DBP $\geq$ 160/100); (b) consumed $\geqslant$ 5 alcoholic drinks 3–4 times (> approximately 201 g of alcohol) per week; (c) took fish-oil supplements, took prescribed insulin or glucocorticoid, anti-arrhythmic, antihypertensive, lipid-lowering, psychotropic, or prescription weight-loss medications; (e) were pregnant; (f) had less than 8th grade reading skills; or (g) were shift workers. Finally, all participants were screened for prior and current DSM-IV Axis-I disorders using the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al. 1998). The University of Pittsburgh Institutional Board

approved the study; all participants provided informed consent in accordance with its regulations and were remunerated for their participation (Marsland et al. 2017a). PIP had comparable requirements, except for the employment, fish oil, and shift work criteria (Jennings et al. 2015). Here, data was combined from both cohorts to increase sample size, and participant characteristics in Table 14 indicate similar characteristics.

Table 14. Cohort participant characteristics

	AHABII (n=394)			**PIP (n=308)**		
	mean (SD) or %	**5th Percentile**	**95th Percentile**	**mean (SD) or %**	**5th Percentile**	**95th Percentile**
Sex (%)	47.2% male 52.8% female			51.6% male 48.4% female		
Race (%)	82.5% white 15.0% black 2.5% other			68.5% white 24.5% black 6.8% other		
Age (years)	42.9 (7.4)	31	53	40.5 (6.3)	31	49
Education (years)	16.9 (2.8)	12	22	16.6 (3.4)	12	24
Smoking status	63.5% never 21.1% former 15.5% current			61.7% never 20.1% former 18.2% current		

MRIs were performed for AHAB-II participants from 2008-2011, and for PIP participants from 2011-2014, to assess cortical and subcortical brain morphology.

4.3.2 MR Image Acquisition and Processing

MRI scans were collected on a 3T Trio TIM whole-body scanner. FreeSurfer software version 5.3.0 (http://surfer.nmr.mgh.harvard.edu) was used to compute cortical and subcortical volumetric data, total cortical surface area, and mean cortical thickness (Fischl and Dale 2000).

4.3.3 Air Pollution Data

Pollutant concentrations were measured during a previously-described multi-pollutant monitoring campaign in Allegheny County (Shmool et al. 2014; Tunno et al. 2015c). Our sampling domain, including both urban and suburban areas in greater Pittsburgh, was identified using geographic information systems (GIS) ESRI ArcMap software version10.3 (Redlands, CA), to capture the urban area and major industrial sources in Allegheny county (e.g., steel mill, coke works). Cross-stratified random sampling was used to select 36 monitoring sites based on elevation, traffic density, and emissions-weighted inverse distance to industry. The same sites were monitored during summer (June-July) 2012 and winter (January-March) 2013. $PM_{2.5}$ samples were collected using Harvard Impactors (Air Diagnostics and Engineering Inc.) at 4.0 liters per minute. Integrated $PM_{2.5}$ samples were obtained at each site for the first 15 minutes of every hour for 7 days. Eight sites were sampled per session. $PM_{2.5}$ concentrations were calculated based on gravimetric analysis of Teflon filters before and after sampling, and black carbon estimated using an EEL43M Smokestain Reflectometer (Diffusion Systems). Elemental concentrations were determined using inductively-coupled plasma mass spectrometry (ICP-MS) (Wisconsin State Laboratory of Hygiene) (Shmool et al. 2014; Tunno et al. 2015c).

4.3.4 Hybrid LUR models

To estimate one-year average air pollution exposures at the homes of each cohort participant, we used previously-developed hybrid LUR models for $PM_{2.5}$, BC, Pb, Mn, Fe, and Zn. Model development is detailed elsewhere (Tripathy et al. 2017; Tunno et al. 2015c). Briefly, covariates were created using GIS to capture a variety of potential pollutant sources - including traffic

density indicators, transportation indicators, road-specific measures, land use/ built environment characteristics, industrial emissions, population, and truck, bus, and diesel indicators (Tunno et al. 2015c) - across locations. Following our hybrid AERMOD-LUR modeling approach, detailed in Michanowicz et al. (2016) (Michanowicz et al. 2016), two additional covariates were developed using the AERMOD atmospheric dispersion model; one dispersion variable was built using emissions profiles for 207 sources (AERMOD-predicted *industrial* $PM_{2.5}$ emissions). A second was developed using only the 14 point source profiles located within Edgar Thomson Steel Works (AERMOD-predicted *steel mill* $PM_{2.5}$ emissions). Both of these covariates included additional temporal components (e.g., meteorology, wind speed) averaged for 2012.

Hybrid LUR models were built using a manual forward step-wise approach combined with random forest analyses to determine covariate ranking order. This method was implemented to select covariates that most strongly correlated with variability in pollutant concentrations. A temporal term was incorporated into models using daily concentrations from an Environmental Protection Agency (EPA) Air Quality System (AQS) maintained by the Allegheny County Health Department (ACHD) centrally located within the sampling domain. These models were used to predict pollutant concentrations across the monitoring domain using source layers in GIS. Model predictions were then spatially extrapolated outside of the original sampling domain to include all of Allegheny County, and the majority of cohort participants lived (Tripathy et al. 2017). Analyses were implemented in SAS version 9.3 (Cary, NC) and R version 3.1.0.

4.3.5 Geocoding

Cohort participant addresses were geocoded using a three-tiered system in GIS, following methods we have previously developed and validated (Fig. 10-11) (Shmool et al. 2016). Briefly,

we standardized addresses using the U.S. Postal Service reference dataset in ZP4™ software (Semaphore Corporation, Monterey, CA). Incomplete addresses, P.O. Box numbers, and addresses outside of Allegheny County were excluded. We first attempted to match addresses using 2015 Allegheny County address points, unmatched addresses were then matched via Allegheny County 2014 tax parcel centroids. Finally, any remaining addresses were matched using Streetmap for ArcPad 10.2 (North America Tom Tom 2013). Participants with unmatched addresses, or for whom a 300 m buffer around residence was not completely contained within Allegheny County, were excluded.

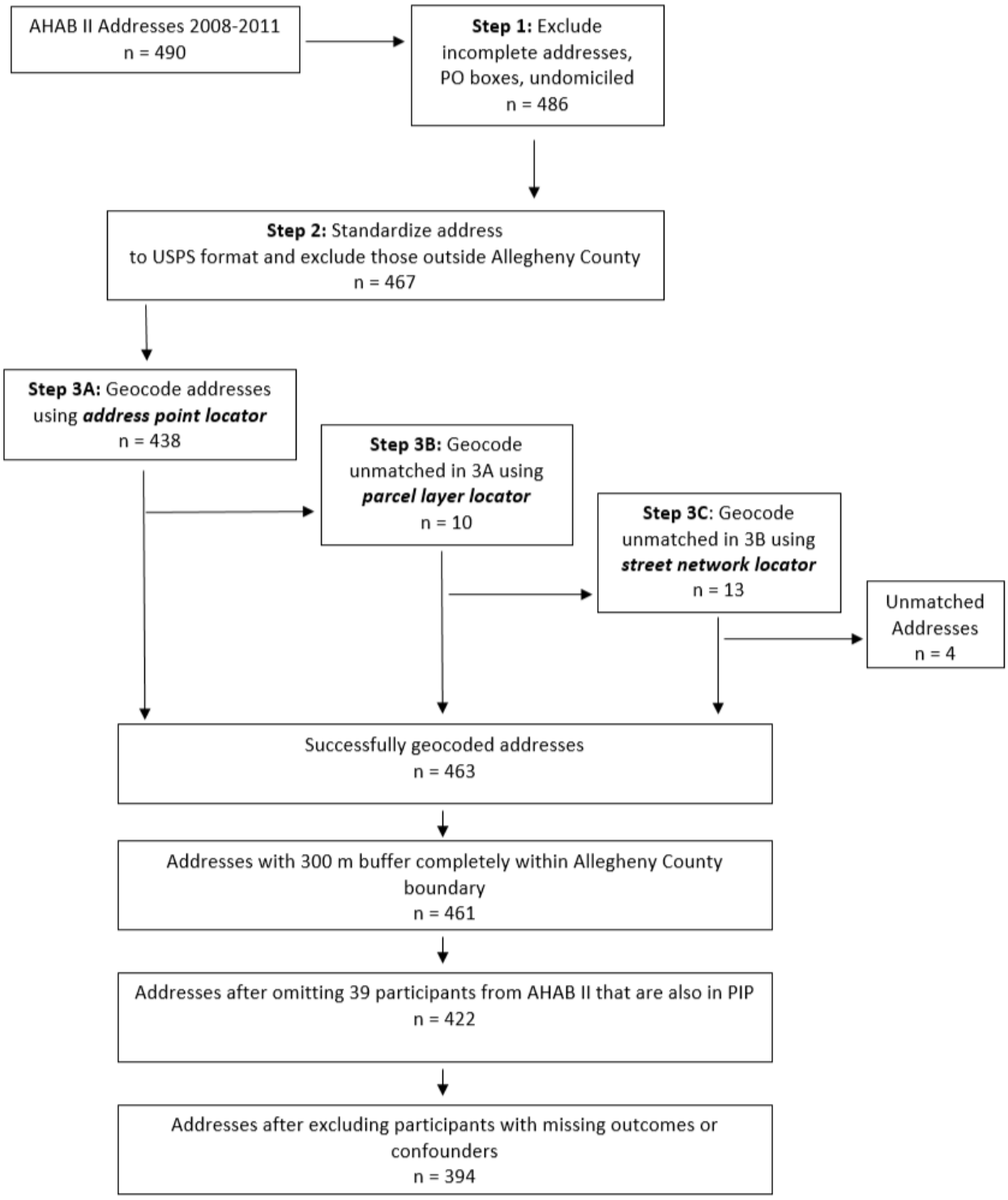

Figure 12. AHAB II cohort participant exclusions and geocoding methodology

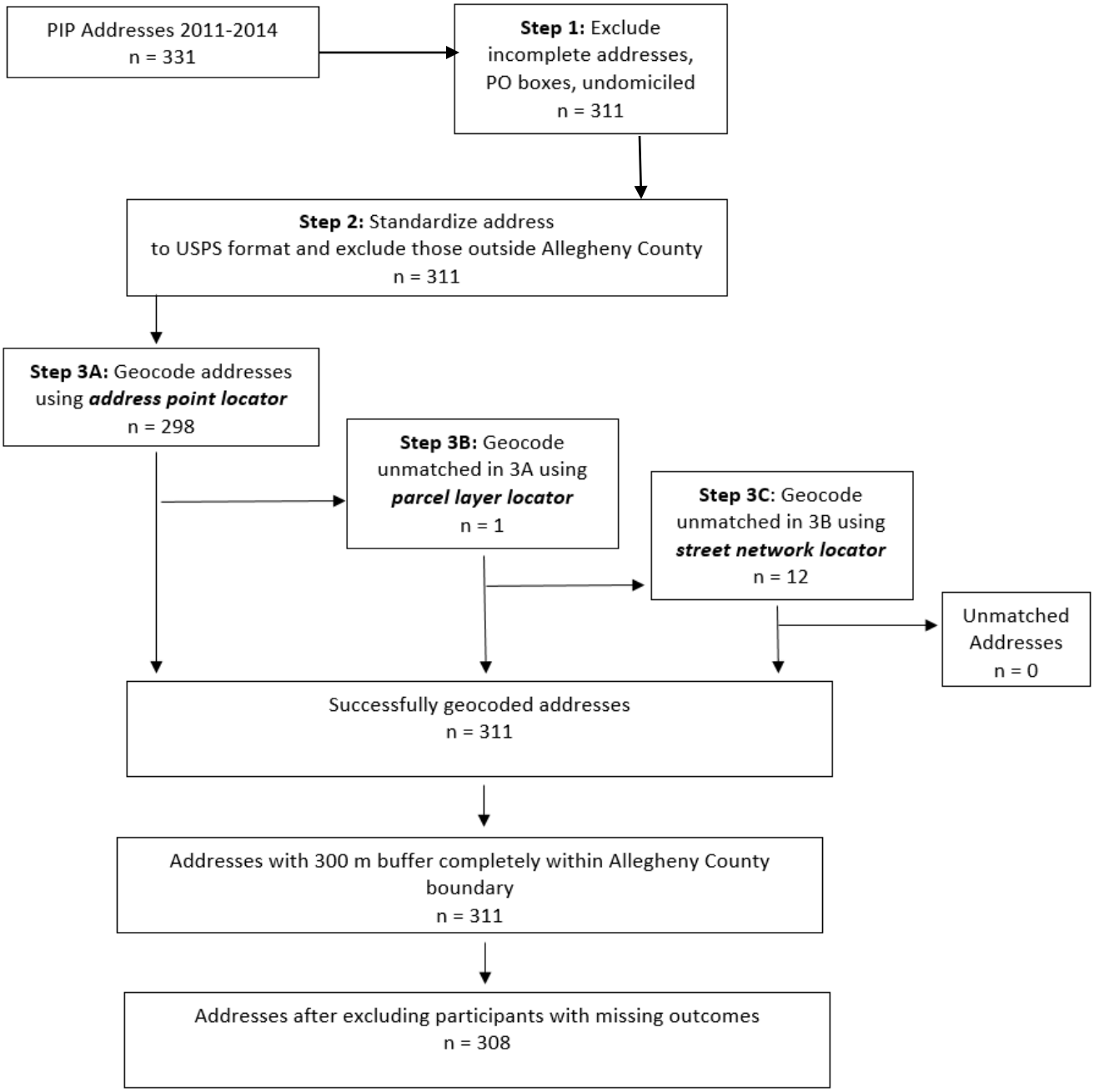

Figure 13. PIP cohort participant exclusions and geocoding methodology

4.3.6 Exposure Assignment

$PM_{2.5}$, BC, Pb, Mn, Fe, and Zn spatial exposure estimates were assigned by averaging concentrations from the pollutant LUR surfaces within the 300 m buffer around each geocoded address. We have found this buffer distance effective in our prior work and validated elsewhere (Ross et al. 2013). Hybrid LUR exposure estimates were then temporally extrapolated to produce exposure estimates for one year prior to MRI date for each participant. The AQS regulatory monitoring data, to create average exposure estimates for one year prior to the date of each participant MRI, using the procedure we have previously developed (Tripathy et al. 2017).

4.3.7 Statistical Analysis

Brain morphology measures were tested for normality using histograms, and scatter plots and raw correlations for each combination of exposures and outcomes were tested for significance and linearity. Linear regression models were developed for each outcome-exposure relationship adjusting only for intracranial volume (Whitwell et al. 2001). Multivariable regression models were then developed also adjusting for age, sex, race, smoking status (former, current, never) and education attainment (years). Statistics were generated using SAS 9.4 (Cary, NC). Two additional analyses were performed using fully adjust models: 1) stratifying by sex and 2) dichotomizing by median age (43 years).

4.4 RESULTS

4.4.1 Sample Size

The original AHAB II dataset contained 490 participants. Twenty-nine participants were excluded due to incomplete addresses, P.O. Box numbers, and addresses outside of Allegheny County as shown in Figure 10. Remaining addresses were geocoded using the composite locator, resulting in four unmatched addresses (n = 463). Two addresses were excluded with 300 m buffers extending outside of Allegheny County. Exposures were assigned for 461 geocoded locations using our hybrid LUR pollutant exposure surfaces. An additional 39 participants who had participated in both studies were excluded from the AHAB II cohort only. Twenty-seven participants were excluded for missing MRI data, and one participant was excluded lacking information on smoking status, resulting in a final AHAB II dataset of 394 participants. The PIP dataset originally included 331 participants; 311 were successfully geocoded, and three participants were excluded due to lacking MRI data (n = 308). Geocoded addresses for our final dataset of 702 cohort participants are shown in Figure 12.

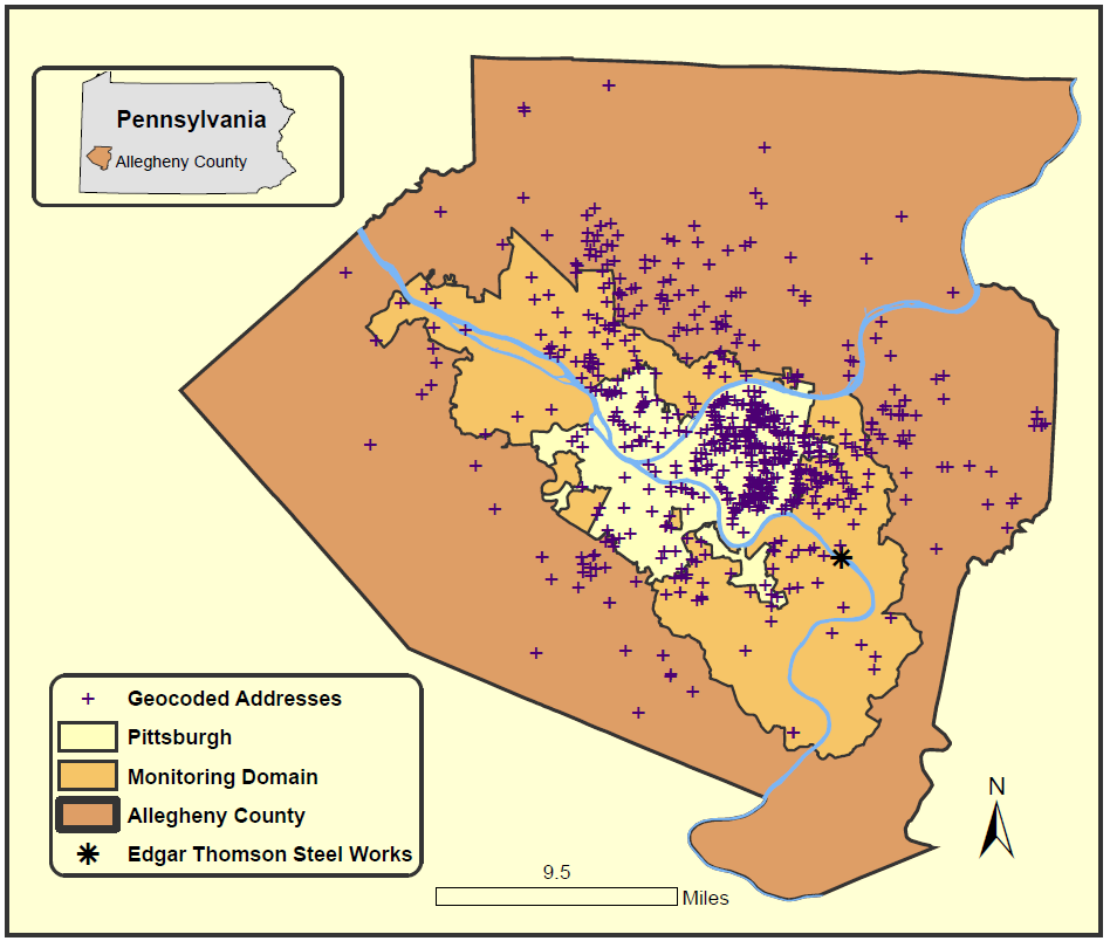

Figure 14. Cohort participant geocoded addresses

4.4.2 Statistical Analysis

Scatter plots were made for each pollutant versus outcome to assess linearity (Figures 13-17).

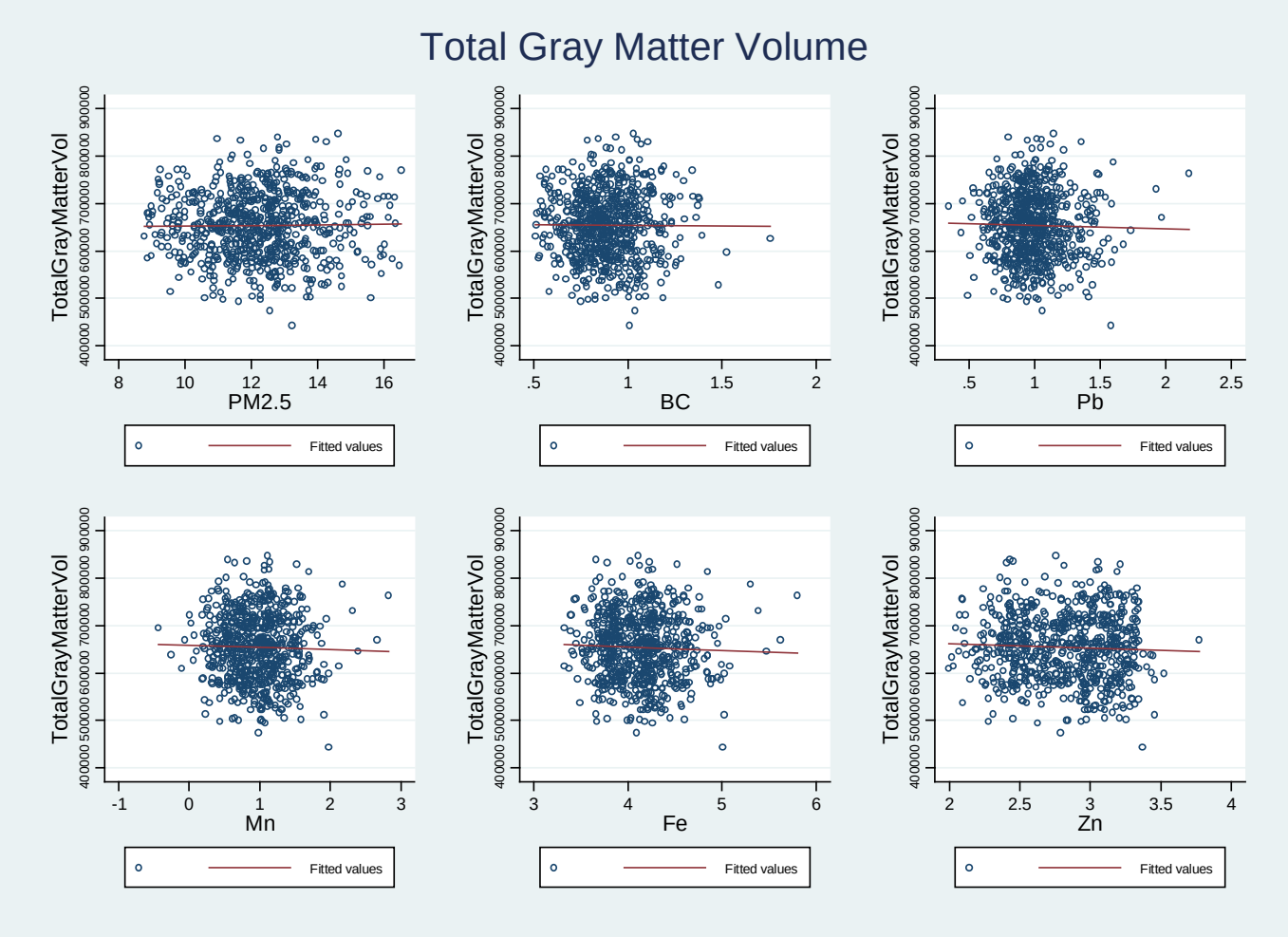

Figure 15. Scatter plots for pollutants versus total gray matter volume

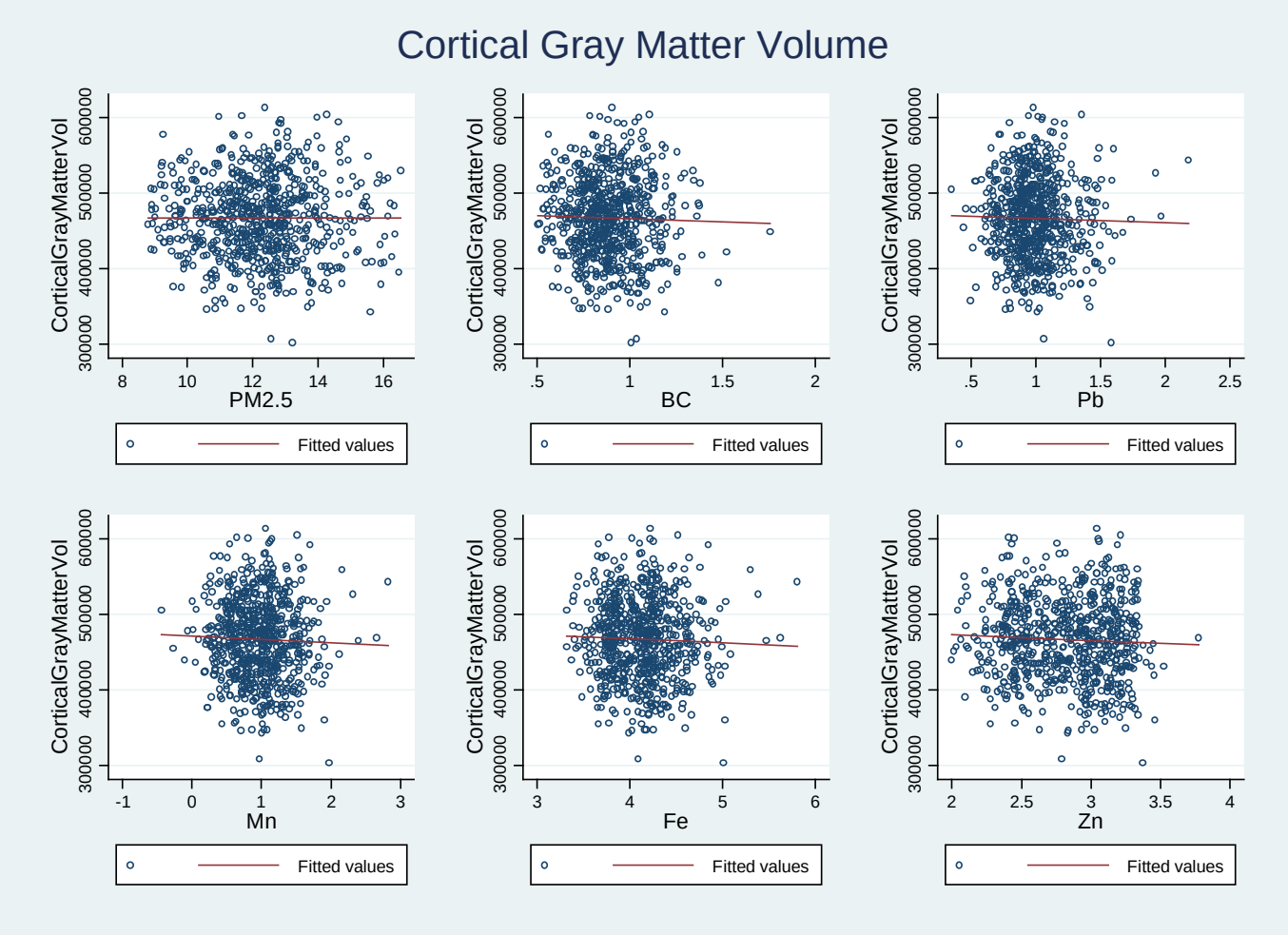

Figure 16. Scatter plots for pollutants versus cortical gray matter volume

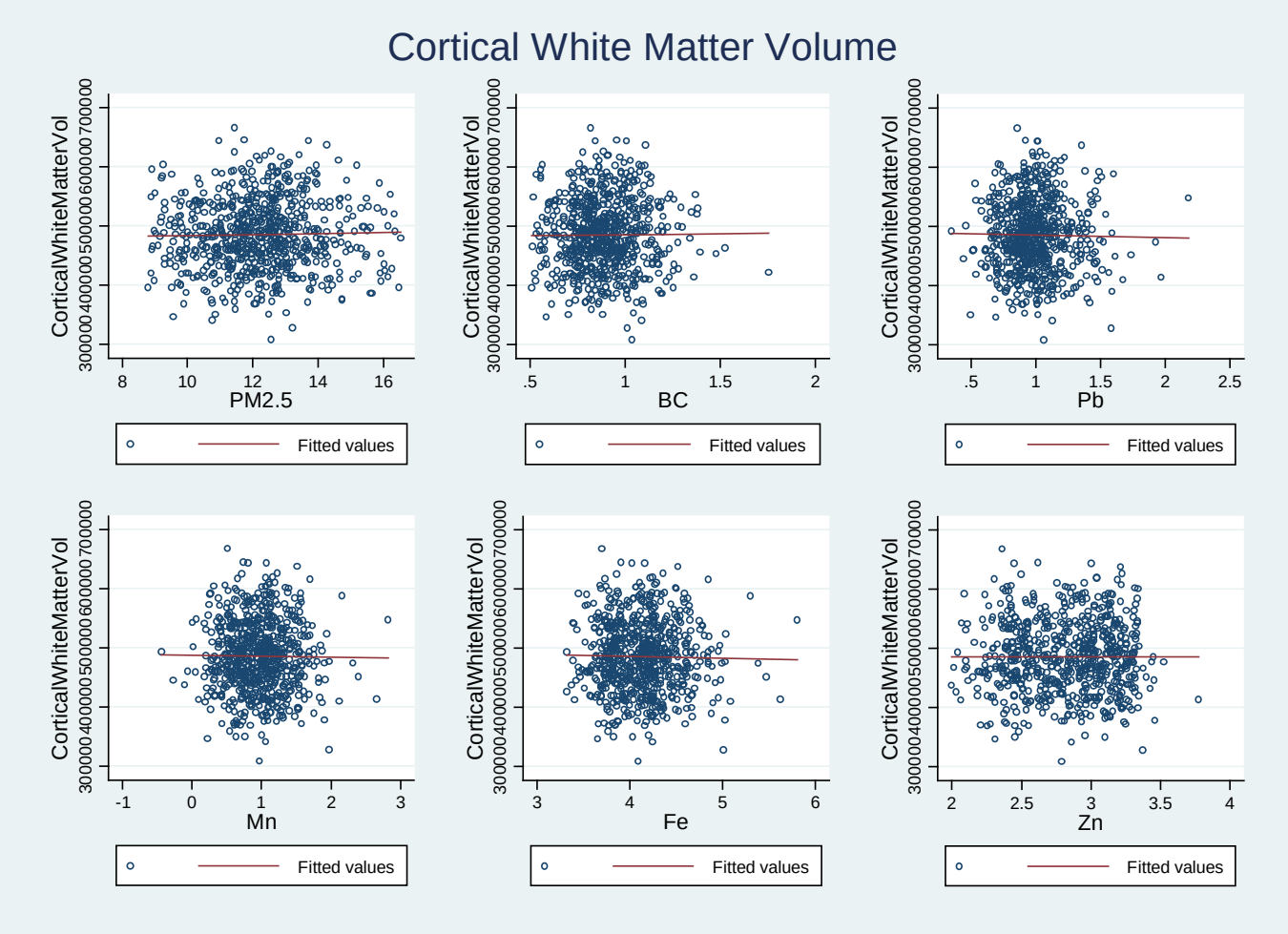

Figure 17. Scatter plots for pollutants versus cortical white matter volume

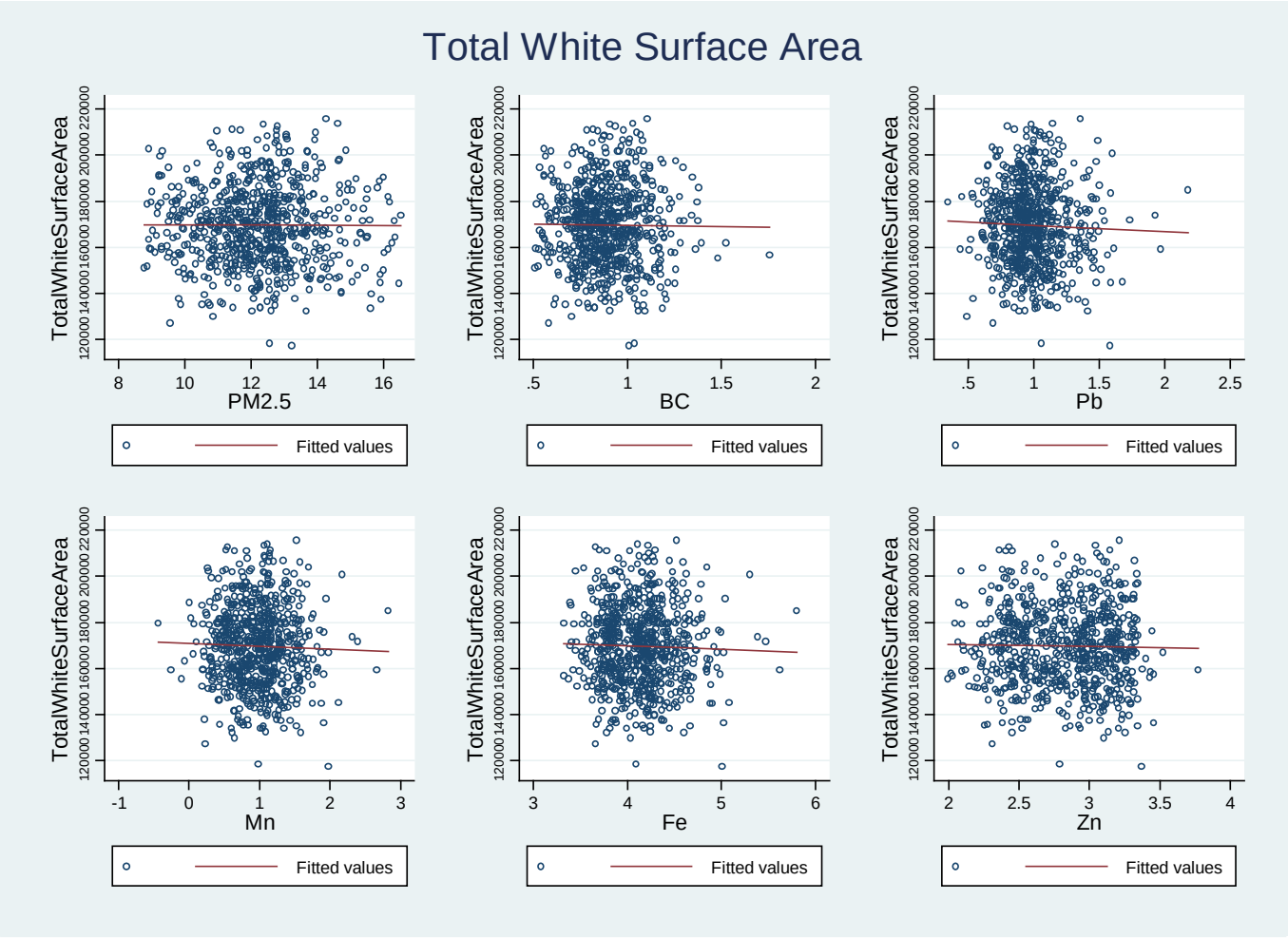

Figure 18. Scatter plots for pollutants versus total white surface area

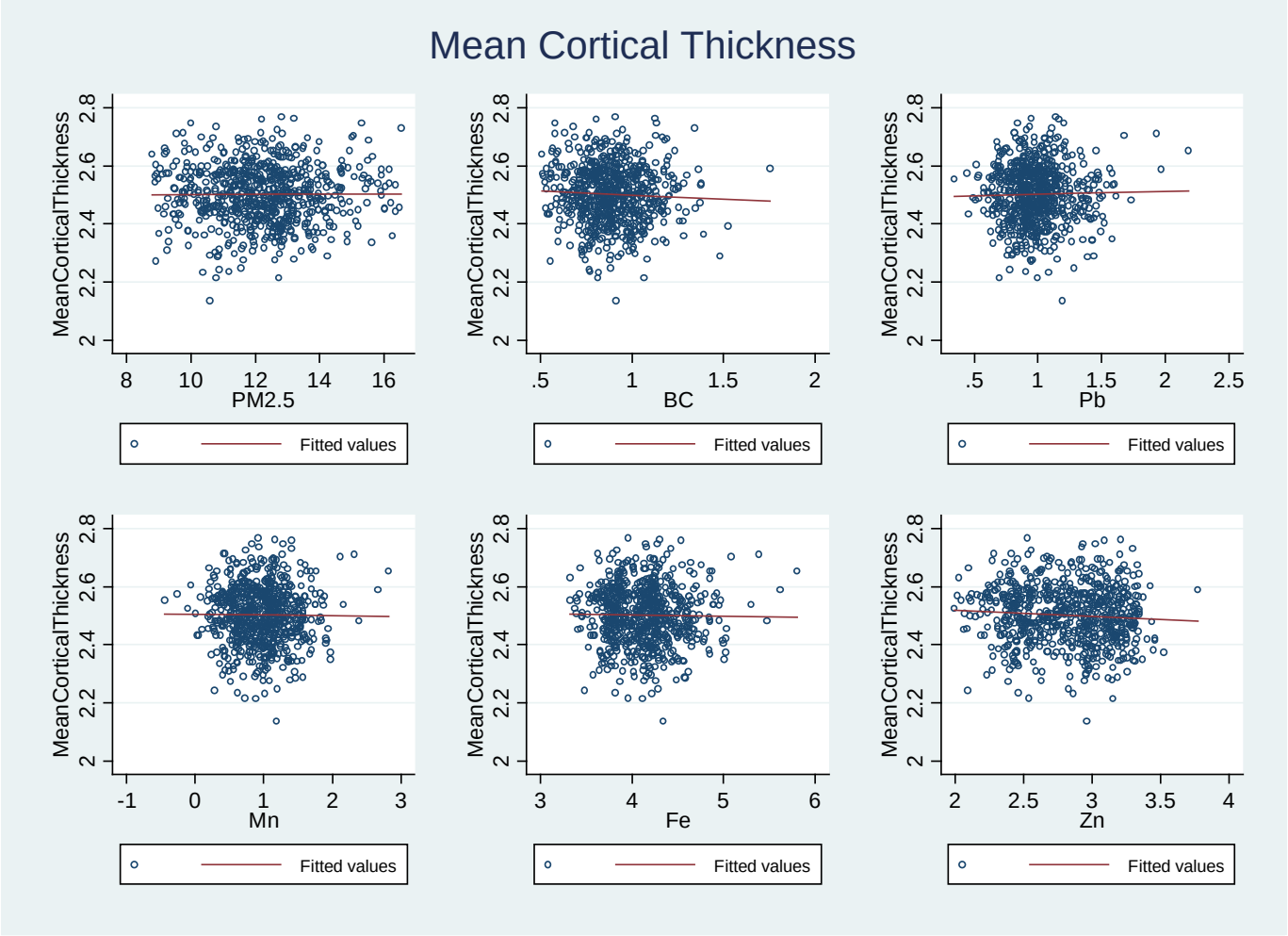

Figure 19. Scatter plots for pollutants versus mean cortical thickness

Pb, Mn, Zn, and Fe exposures were transformed using the natural logarithm to improve linear fit. Pollutant exposures, confounders, and outcomes are summarized in Tables 14-16.

Table 15. Cohort exposures

Pollutant	**mean (SD)**	**5%**	**95%**
$PM_{2.5}$ ($\mu g/m^3$)	12.2 (1.5)	9.0	15.0
Pb (ng/m^3)	2.8 (0.69)	2.0	4.1
Mn (ng/m^3)	2.9 (1.4)	1.4	5.0
Fe (ng/m^3)	66.5 (28.4)	37.7	112.9
Zn (ng/m^3)	17.7 (5.7)	9.7	27.1

Table 16. Cohort outcomes

	mean (SD)	**5%**	**95%**
Total Gray Matter Volume (mm^2)	654,051.3 (69,965.9)	540,234.0	764,941.0
Cortical Gray Matter Volume (mm^2)	467,109.2 (52,792.7)	380,168.0	554,544.0
Cortical White Matter Volume (mm^2)	485,852.8 (59,491.5)	390,608.0	589,199.0
Total White Matter Surface Area (mm^2)	169,628.9 (17,344.5)	142,241.0	199,873.0
Mean Cortical Thickness (mm)	2.5 (0.1)	2.3	2.7

The mean participant $PM_{2.5}$ exposure estimate (12.2 μg/m^3) was at the EPA National Ambient Air Quality Standard (NAAQS) threshold of 12 μg/m^3 for annual average exposure (based on a three year average) (EPA 2016b). Table 17 shows Pearson correlations between pollutants. $PM_{2.5}$ and BC were highly correlated (0.85) and correlations between $PM_{2.5}$ with the metals ranged from 0.30 – 0.45.

Table 17. Pearson correlations between pollutant exposures

	$PM_{2.5}$	BC	Pb	Mn	Zn	Fe
$PM_{2.5}$	1.00	0.85	0.40	0.43	0.45	0.30
BC	0.85	1.00	0.42	0.42	0.45	0.32
Pb	0.40	0.42	1.00	0.91	0.45	0.85
Mn	0.43	0.42	0.91	1.00	0.67	0.98
Zn	0.45	0.45	0.45	0.67	1.00	0.69
Fe	0.30	0.32	0.85	0.98	0.69	1.00

Results from linear regression models adjusting for intracranial volume are shown in Table 18. We found no significant associations between any of the pollutants and brain outcomes ($p < 0.05$).

Table 18. Linear regression models adjusting for ICV: exposures vs. outcomes

Brain Morphology Outcomes	**Pollutant**					
	$PM_{2.5}$	**BC**	**Mn**	**Pb**	**Fe**	**Zn**
Total Gray Matter Volume						
β (SE)	1018.4 (1055.9)	-14.7 (9494.7)	-3184.5 (3932.5)	-5618.1 (7427.2)	-5200.7 (4594.4)	-8160.5 (4853.6)
p-value	0.34	0.99	0.42	0.45	0.26	0.09
Cortical Gray Matter Volume						
β (SE)	234.3 (848.5)	-6589.0 (7620.7)	-2966.0 (3157.5)	-4960.7 (5963.9)	-4373.2 (3689.2)	-6938.5 (3896.8)
p-value	0.78	0.39	0.35	0.41	0.24	0.08
Cortical White Matter Volume						
β (SE)	1047.9 (986.6)	4341.9 (8870.9)	-243.7 (3676.4)	-3861.6 (6941.7)	-2064.8 (4296.5)	327.3 (4544.7)
p-value	0.29	0.62	0.95	0.58	0.63	0.94
Total White Surface Area						
β (SE)	11.6 (298.7)	-406.1 (2683.9)	-826.9 (1111.7)	-2611.4 (2098.1)	-1138.9 (1299.2)	-643.4 (1374.6)
p-value	0.97	0.88	0.46	0.21	0.38	0.64
Mean Cortical Thickness						
β (SE)	0.0005 (0.002)	-0.03 (0.02)	-0.002 (0.009)	0.01 (0.02)	-0.004 (0.01)	-0.02 (0.01)
p-value	0.84	0.20	0.81	0.55	0.71	0.06

Fully-adjusted model results are shown in Tables 19-23. After adjusting for intracranial volume, age, sex, race, and smoking status. No significant associations were found between any pollutants with brain morphology measures. No significant results were found after stratifying by sex or stratifying by median age.

Table 19. Fully adjusted linear regression model results for total gray matter volume

	$PM_{2.5}$	**BC**
Parameter	β (SE)	β (SE)
Intercept	478761.1 (17967.7)**	533279.5 (19892.2)**
Pollutant	651.9 (914.0)	-920.2 (8156.9)
Age	-2047.3 (200.4)**	-2047.6 (200.5)**
Sex	23681.9 (4008.3)**	-23439.0 (4001.7)**
Education	987.6 (505.9)	1044.3 (504.6)*
ICV	0.17 (0.008)**	0.17 (0.008)**
Race (black)	-26493.7 (3783.5)**	-26535.3 (3786.1)**
Race (other)	-21561.2 (6906.9)*	-21619.8 (6909.6)*
Race (white)	0	0
Smoking (former)	229.6 (3564.9)	330.6 (3565.1)
Smoking (current)	-13372.9 (4023.4)*	-13320.1 (4025.2)*
Smoking (never)	0	0

*p<.05 **p<.0001

	Pb	**Mn**	**Fe**	**Zn**
Parameter	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	486376.2 (16129.8)**	486095.90 (15375.10)**	492305.4 (22308.7)**	494112.8 (18939.9)**
Pollutant	-748.9 (6369.3)	-453.64 (3388.38)	-1594.9 (3970.9)	-3085.8 (4200.1)
Age	-2047.6 (200.5)**	-2048.41 (200.64)**	-2051.3 (200.7)**	-2051.8 (200.5)**
Sex	23444.7 (3999.2)**	23443.76 (3999.35)**	23438.9 (3997.5)**	23406.9 (3996.7)**
Education	1040.1 (501.5)*	1040.40 (501.54)*	1038.9 (501.1)*	1048.6 (501.2)*
ICV	0.17 (0.008)**	0.17 (0.008)**	0.17 (0.008)**	0.17 (0.008)**
Race (black)	-26523.5 (3790.3)**	-26504.12 (3798.95)**	-26390.7 (3804.2)**	-26181.3 (3815.6)**
Race (other)	-21576.6 (6914.9)*	-21547.60 (6924.83)*	-21378.6 (6932.4)*	-21287.0 (6920.4)*
Race (white)	0	0	0	0
Smoking (former)	346.7 (3571.0)	346.48 (3569.34)	382.2 (3566.9)	372.6 (3563.3)
Smoking (current)	-13305.8 (4029.2)*	-13292.40 (4033.64)*	-13219.9 (4033.1)*	-13153.5 (4029.9)*
Smoking (never)	0	0	0	0

Table 20. Fully adjusted linear regression model results for cortical gray matter volume

	$PM_{2.5}$	**BC**
Parameter	β (SE)	β (SE)
Intercept	346864.8 (14779.6)**	375006.3 (16341.2)**
Pollutant	-192.8 (751.8)	-7867.6 (6700.8)
Age	-1593.4 (164.9)**	-1595.2 (164.7)**
Sex	12253.1 (3297.1)*	-12143.8 (3287.4)*
Education	797.9 (416.1)	840.0 (414.5)*
ICV	0.13 (0.007)**	0.13 (0.006)**
Race (black)	-22128.1 (3112.2)**	-21993.9 (3110.2)**
Race (other)	-15081.2 (5681.4)*	-15148.8 (5676.1)*
Race (white)	0	0
Smoking (former)	87.4 (2932.4)	149.6 (2928.7)
Smoking (current)	-9579.7 (3309.5)*	-9517.2 (3306.6)*
Smoking (never)	0	0

*p<.05 **p<.0001

	Pb	**Mn**	**Fe**	**Zn**
Parameter	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	346057.8 (13262.9)**	345585.3 (12642.1)**	351070.5 (18343.7)**	352744.8 (15572.3)**
Pollutant	-1403.9 (5237.2)	-912.7 (2786.1)	-1514.1 (3265.2)	-2914.3 (3453.3)
Age	-1593.7 (164.9)**	-1595.4 (164.9)**	-1597.1 (165.0)**	-1597.5 (164.9)**
Sex	12291.1 (3288.7)*	12287.2 (3288.4)*	12299.3 (3287.03)*	12269.3 (3286.1)*
Education	787.5 (412.4)	788.5 (412.4)	784.3 (412.0)	793.3 (412.1)
ICV	0.13 (0.007)**	0.13 (0.006)**	0.13 (0.006)**	0.13 (0.006)**
Race (black)	-22063.8 (3116.6)**	-22021.3 (3123.7)**	-21961.4 (3128.04)**	-21764.3 (3137.2)**
Race (other)	-15003.8 (5685.9)*	-14940.7 (5693.9)*	-14846.8 (5700.2)*	-14761.5 (5689.9)*
Race (white)	0	0	0	0
Smoking (former)	110.2 (2936.3)	113.5 (2934.9)	119.4 (2932.9)	110.2 (2929.7)
Smoking (current)	-9549.5 (3313.1)*	-9519.2 (3316.6)*	-9489.3 (3316.3)*	-9427.1 (3313.4)*
Smoking (never)	0	0	0	0

Table 21. Fully adjusted linear regression model results for cortical white matter volume

	$PM_{2.5}$	**BC**
Parameter	β (SE)	β (SE)
Intercept	271078.6 (19409.8)**	286706.5 (21487.1)**
Pollutant	692.3 (987.4)	2776.9 (8810.9)
Age	-570.2 (216.5)*	-569.7 (216.6)*
Sex	5341.9 (4330.1)	-5167.6 (4322.6)
Education	824.9 (546.5)	857.9 (545.1)
ICV	0.16 (0.009)**	0.16 (0.009)**
Race (black)	-10129.4 (4087.2)*	-10229.8 (4089.6)*
Race (other)	-7425.7 (7461.3)	-7448.7 (7463.6)
Race (white)	0	0
Smoking (former)	2578.9 (3851.0)	2643.7 (3850.9)
Smoking (current)	-5240.8 (4346.4)	-5220.7 (4347.9)
Smoking (never)	0	0

*p<.05 **p<.0001

	Pb	**Mn**	**Fe**	**Zn**
Parameter	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	280749.6 (17422.6)**	277841.5 (16608.6)**	280501.8 (24101.5)**	271722.0 (20463.3)**
Pollutant	-2578.2 (6879.8)	724.2 (3660.2)	-494.5 (4290.1)	2473.5 (4537.9)
Age	-570.9 (216.6)*	-568.7 (216.7)*	-571.5 (216.8)*	-566.8 (216.6)*
Sex	5054.6 (4320.2)	5130.9 (4320.2)	5099.3 (4318.8)	5147.9 (4318.2)
Education	886.3 (541.7)	873.8 (541.8)	878.5 (541.4)	869.4 (541.5)
ICV	0.16 (0.009)**	0.16 (0.009)**	0.16 (0.009)**	0.16 (0.009)**
Race (black)	-10100.2 (4094.1)*	-10260.0 (4103.7)*	-10139.1 (4109.9)*	-10483.1 (4122.5)*
Race (other)	-7362.2 (7469.1)	-7577.7 (7480.4)	-7405.9 (7489.4)	-7736.8 (7477.1)
Race (white)	0	0	0	0
Smoking (former)	2766.3 (3857.3)	2633.1 (3855.7)	2694.4 (3853.5)	2633.1 (3849.9)
Smoking (current)	-5114.6 (4352.1)	-5252.4 (4357.3)	-5160.3 (4357.2)	-5334.7 (4354.1)
Smoking (never)	0	0	0	0

Table 22. Fully adjusted linear regression model results for total white surface area

	$PM_{2.5}$	**BC**
Parameter	β (SE)	β (SE)
Intercept	128049.0 (5623.4)**	136820.4 (6223.4)**
Pollutant	-52.7 (286.1)	-583.5 (2551.9)
Age	-405.3 (62.7)**	-405.4 (62.7)**
Sex	4442.5 (1254.5)*	-4447.4 (1251.9)*
Education	205.9 (158.3)	206.2 (157.9)
ICV	0.04 (0.002)**	0.04 (0.002)**
Race (black)	-4867.9 (1184.1)**	-4854.7 (1184.5)**
Race (other)	-3695.2 (2161.7)	-3697.3 (2161.7)
Race (white)	0	0
Smoking (former)	835.7 (1115.7)	834.9 (1115.4)
Smoking (current)	-837.5 (1259.2)	-835.5 (1259.3)
Smoking (never)	0	0

*p<.05 **p<.0001

	Pb	**Mn**	**Fe**	**Zn**
Parameter	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	129189.1 (5043.2)**	127889.5 (4809.7)**	130265.1 (6978.9)**	127077.5 (5927.9)**
Pollutant	-1913.9 (1991.4)	-473.1 (1059.9)	-671.8 (1242.3)	150.5 (1314.6)
Age	-405.7 (62.7)**	-406.3 (62.8)**	-406.9 (62.8)**	-405.1 (62.8)**
Sex	4422.5 (1250.5)*	4443.9 (1251.1)*	4451.7 (1250.6)*	4463.0 (1250.9)*
Education	208.1 (156.8)	204.8 (156.9)	202.5 (156.8)	201.4 (156.9)
ICV	0.04 (0.002)**	0.04 (0.002)**	0.04 (0.002)**	0.04 (0.002)**
Race (black)	-4798.1 (1185.1)**	-4816.6 (1188.4)**	-4796.8 (1190.1)**	-4881.4 (1194.2)**
Race (other)	-3605.4 (2162.0)	-3625.9 (2166.3)	-3593.6 (2168.7)	-3706.9 (2165.9)
Race (white)	0	0	0	0
Smoking (former)	896.0 (1116.5)	855.8 (1116.6)	854.5 (1115.9)	825.8 (1115.3)
Smoking (current)	-782.1 (1259.8)	-802.9 (1261.8)	-795.2 (1261.7)	-849.6 (1261.3)
Smoking (never)	0	0	0	0

Table 23. Fully adjusted linear regression model results for mean cortical thickness

	$PM_{2.5}$	**BC**
Parameter	β (SE)	β (SE)
Intercept	2.5 (0.05)**	2.5 (0.05)**
Pollutant	-0.0005 (0.002)	-0.03(0.02)
Age	-0.003 (0.0005)**	-0.003 (0.0005)**
Sex	0.001 (0.01)	-0.0007 (0.01)
Education	0.0009 (0.001)	0.001 (0.001)
ICV	8.3E-08 (2E-08)**	8.4E-08 (2E-08)**
Race (black)	-0.03 (0.01)*	-0.03 (0.01)*
Race (other)	-0.02 (0.02)	-0.02 (0.02)
Race (white)	0	0
Smoking (former)	-0.008 (0.009)	-0.008 (0.009)
Smoking (current)	-0.03 (0.01)*	-0.03(0.01)*
Smoking (never)	0	0

*p<.05 **p<.0001

	Pb	**Mn**	**Fe**	**Zn**
Parameter	β (SE)	β (SE)	β (SE)	β (SE)
Intercept	2.5 (0.04)**	2.5 (0.04)**	2.5 (0.06)**	2.5 (0.05)**
Pollutant	0.02 (0.02)	0.001 (0.009)	0.001 (0.01)	-0.01 (0.01)
Age	-0.003 (0.0005)**	-0.003 (0.0005)**	-0.003 (0.0005)**	-0.003 (0.0005)**
Sex	0.002 (0.01)	0.001 (0.01)	0.001 (0.01)	0.001 (0.01)
Education	0.0008 (0.001)	0.0009 (0.001)	0.0009 (0.001)	0.0009 (0.001)
ICV	8.3E-08 (2E-08)**	8.3E-08 (2E-08)**	8.3E-08 (2E-08)**	8.4E-08 (2E-08)**
Race (black)	-0.03 (0.01)*	-0.03 (0.01)*	-0.03 (0.01)*	-0.03 (0.01)*
Race (other)	-0.02 (0.02)	-0.02 (0.02)	-0.02 (0.02)	-0.01 (0.02)
Race (white)	0	0	0	0
Smoking (former)	-0.009 (0.009)	-0.008 (0.009)	-0.008 (0.009)	-0.008 (0.009)
Smoking (current)	-0.03 (0.01)*	-0.03 (0.01)*	-0.03 (0.01)*	-0.03 (0.01)*
Smoking (never)	0	0	0	0

4.5 DISCUSSION

We found no significant associations between annual average $PM_{2.5}$, BC, Pb, Mn, Fe, or Zn exposures with participant brain morphology measures of total gray matter volume, cortical gray matter volume, total white matter surface area, and mean cortical thickness.

Some of these results are consistent with prior studies. The two previous studies that found significant associations between predicted $PM_{2.5}$ exposures with indicators of brain morphology. Wilker et al., studied participants in the Framingham Offspring Cohort (n = 943) – composed of community dwelling adults in the New England area with no history of dementia or stroke. The age of participants at the time of MRIs was higher in their study than ours [median = 68 years vs. 43 years in our study]. (They considered total cerebral brain volume, hippocampal volume, white matter hyperintensity volume, and covert brain infarcts, and hypothesized that higher long-term exposure to ambient air pollution would be associated with subclinical damage as indicated by smaller total cerebral brain volume and hippocampal volume , larger white matter hyperintensity volume, and higher odds of covert brain infarcts While they found that a 2-$\mu g/m^3$ increase in $PM_{2.5}$ was associated with 0.32% smaller total cerebral brain volume and 46% higher odds of having covert brain infarcts, they did not see any associations for hippocampal volume or white matter hyperintensity volume (Wilker et al. 2015).

Chen et al. (2015) examined associations between long term exposure to $PM_{2.5}$ and brain volume, using volumetric measures of gray matter and normal-appearing white matter in MRI results from participants in the Women's Health Initiative Memory Cohort (n = 1403). All participants we free of dementia, but this was also a much older cohort than ours (range = 71 to

89 years). They found that for each inter-quartile range (3.49 μg/m^3) increase in $PM_{2.5}$, mean white matter volume decreased by 6.23 (± 1.28) cm^3 for total brain volume. Significant associations were also found between increased $PM_{2.5}$ with decreases in frontal, parietal, and temporal and corpus callosum white matter volume. No associations were found with gray matter or hippocampal volume (Chen et al. 2015).

In comparison to these two studies, our cohort participants were much younger (mean = 43 years (range = 30 to 54). As a result, it is highly possible that our participants have not yet developed the premature aging or damage to brain structures that may be associated with longer-term exposures to airborne metals. Following up with participants later in life may be beneficial to examine potential changes in brain morphology measures across the lifespan.

4.5.1 Strengths and Limitations

While this was a cross-sectional study, we were able to assign retrospective pollutant exposure estimates for one year prior to participant MRIs. Having MRI data at multiple time points would allow us to further examine relationships between long term pollutant exposure estimates with changes in brain morphology measures. In addition, we only had addresses for participants at the time of each study, so we were not able to account for participants that moved during that year. Along with temporally adjusting models using regulatory monitoring data, they were also spatially extrapolated outside of the sampling domain to encompass all of Allegheny County. This assumes stationary spatial surfaces/covariates. There may be different source-concentration relationships outside of this domain that we were unable to capture in our monitoring campaign resulting in exposure misclassification. One of the key covariates in our hybrid metal LUR models was AERMOD-predicted $PM_{2.5}$ emissions from the Edgar Thomson Steel Works within

our sampling domain. While cohort addresses appear to be well distributed spatially (Figure 12), only two participants lived within a one-mile radius of the steel mill.

4.6 CONCLUSIONS

We explored associations between annual-average ambient $PM_{2.5}$, BC, and Pb, Mn, Fe, and Zn metal constituent exposures with brain morphology measures of total and cortical gray matter volume, cortical white matter volume, total white matter surface area, and mean cortical thickness from MRIs of 702 participants in two Pittsburgh-based cohorts of mid-life adults. We found no significant associations between pollutant exposures and any of the brain morphology indicators. Further study is needed to examine effects of chronic air pollution and airborne metals exposures in older or more vulnerable populations.

5.0 OVERALL SUMMARY AND PUBLIC HEALTH SIGNIFICANCE

We developed hybrid dispersion-LUR models for $PM_{2.5}$, black carbon (BC), and steel-related $PM_{2.5}$ constituents [lead (Pb), manganese (Mn), iron (Fe), and zinc (Zn)] and applied them to assign residence-based exposure estimates for time windows of interest for two Pittsburgh-area epidemiological cohorts. Specific objectives, hypotheses, and conclusions are listed below followed by public health significance, strengths and limitations.

Chapter 2: Develop hybrid dispersion LUR models for $PM_{2.5}$, BC, and steel-related Pb, Mn, Fe, and Zn metal constituents for use in epidemiological studies.

Conclusions: We found that the hybrid LURs explained greater variability in $PM_{2.5}$ (R^2 = 0.79) compared to BC (R^2 = 0.59) and metal constituents (R^2 = 0.34 - 0.56). Approximately 70% of variation in $PM_{2.5}$ was attributable to temporal variance, compared to 36% for BC, and 17 - 26% for metals. Dispersion covariates were included in all models. A dispersion covariate developed with $PM_{2.5}$ industrial emissions data for 207 sources was significant in $PM_{2.5}$ and BC models while all metals models contained a steel mill-specific $PM_{2.5}$ emissions term.

Chapter 3: Examine associations between one-year residence-based pollutant exposures with circulating and LPS-stimulated inflammatory mediators in the AHAB II cohort.

Hypothesis: Elevated exposures to $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe will be associated with higher levels of circulating inflammatory mediators (Il-6 and CRP), and LPS-stimulated production of cytokines (IL-6, IL-1β, and TNF-α).

Conclusions: Exposure to $PM_{2.5}$ and BC was associated with increased LPS-stimulated pro-inflammatory cytokine production in a cohort of middle-aged adults. These results suggest that some chronic air pollution exposures may influence the responsiveness of the immune system, possibly increasing risk for future inflammatory conditions.

Chapter 4: Explore the relationship between one-year pollutant exposures with total and cortical gray matter volumes, cortical white matter volume, total white matter surface area, and mean cortical thickness measures of brain morphology in AHAB II and PIP cohorts.

Hypothesis: Higher residence-based exposures to $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe will be associated with reduced structural integrity of the brain in two Pittsburgh cohorts of health middle-aged adults.

Conclusions: No significant associations were found between $PM_{2.5}$, BC, or metal constituent exposures with any of the brain morphology outcomes.

Overall, the results of this dissertation indicate the public health importance of better understanding relationships between long-term source-specific $PM_{2.5}$ and component exposures with health outcomes including associations with circulating and stimulated inflammatory mediators and measures of brain morphology.

5.1.1 Strengths and Limitations

Using hybrid LUR models to assign pollutant exposures in cohort studies offers an improvement over exposure assignments that rely solely on the nearest EPA air quality system (AQS) monitor(s) and are more cost effective compared to personal monitoring. One limitation of LUR models is that the analysis is based on associations and LUR model results cannot

establish causation between source covariates and pollutants. The $PM_{2.5}$ concentrations used for this analysis were obtained from two seasons of data from 36 sites in the greater Pittsburgh region. This provided a much higher spatial resolution compared to the established EPA AQS monitoring network locations within the county. In addition, our hybrid LUR models may have improved accuracy by incorporating meteorology and topography into AERMOD covariates. The AQS monitor used to temporally adjust the models also contributed to high temporal resolution providing daily concentrations.

While both epidemiological studies presented in Chapter 3 and 4 used a cross-sectional study design, we were able to assign retrospective pollutant exposure estimates for one year prior to participant blood draws or MRIs. Having outcome data at multiple time points would allow us to further examine relationships between long term pollutant exposure estimates with circulating and stimulated inflammatory mediators and brain morphology measures. In addition, we only had addresses for participants at the time of each study, so we were not able to account for participants that moved during that year. Along with temporally adjusting models using regulatory monitoring data, they were also spatially extrapolated outside of the sampling domain to encompass all of Allegheny County. This assumes stationary spatial surfaces/covariates. There may be different source-concentration relationships outside of this domain that we were unable to capture in our monitoring campaign resulting in exposure misclassification. Using data collected in AHAB II and PIP cohorts provided the unique opportunity to study relatively healthy middle-aged adults in Allegheny County and allowed us to study emerging health outcomes of interest with predicted air pollutant exposures.

The hybrid LUR models developed for to $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe described in this dissertation will continue to be used in prospective and retrospective cohort studies in Allegheny County.

APPENDIX: MISSING $PM_{2.5}$ CONCENTRATIONS ACHD AQS LAWRENCEVILLE MONITOR

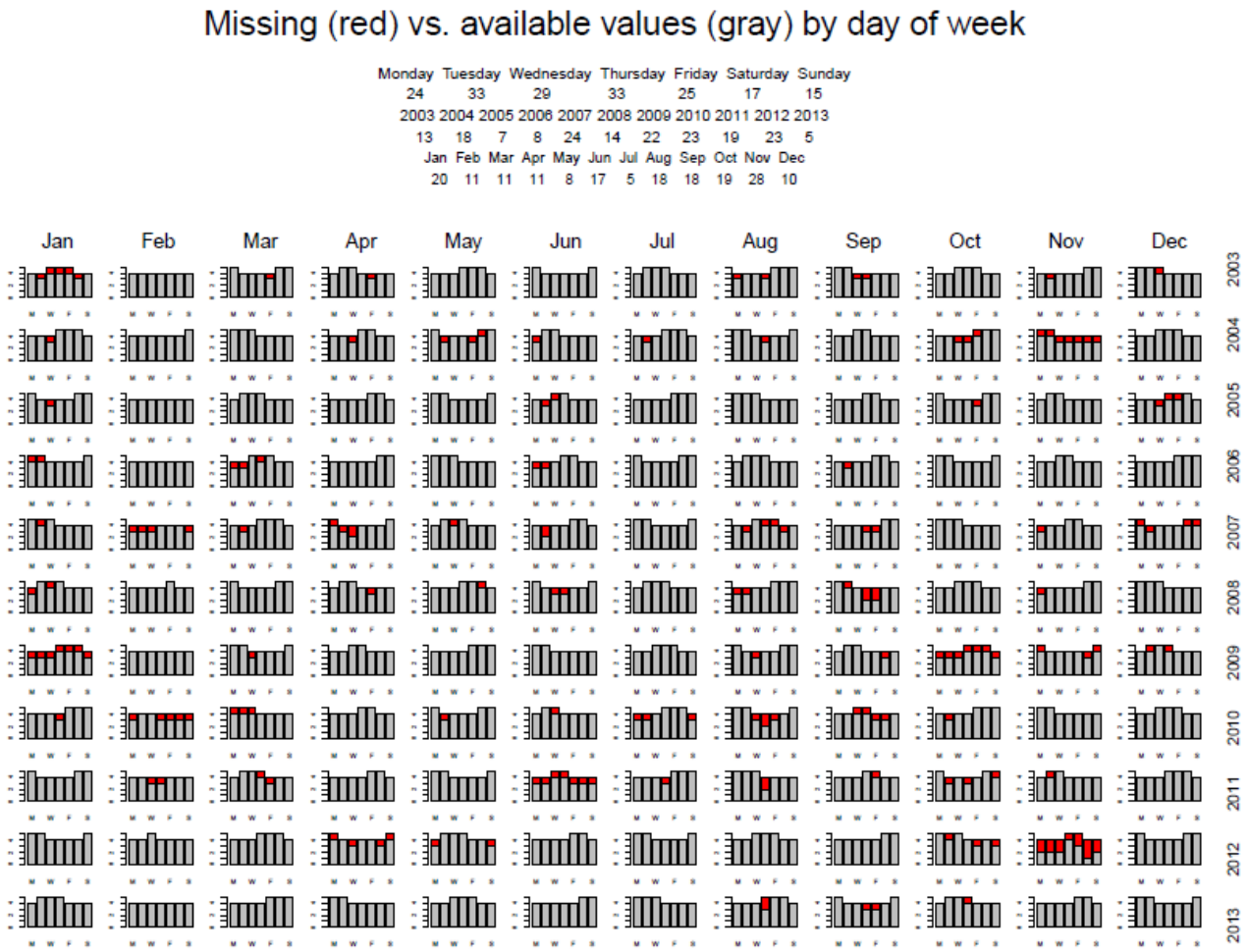

Figure 20. Missing daily $PM_{2.5}$ concentrations from ACHD Lawrenceville AQS station from 2003-2013

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