

**ASSOCIATION BETWEEN PARTICULATE COMPOSITIONAL
CHANGES DURING FILTER EXTRACTION AND THE
INTERPRETATION OF FILTER-BASED PM_{2.5} TOXICOLOGY**

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ABSTRACT

Ambient fine particulate matter (PM_{2.5}) is a global public health concern as it has well-established adverse respiratory and cardiovascular outcomes. Additionally, health effects have been shown to vary based on PM_{2.5} composition highlighting the importance of the contribution of metallic and organic species. To better understand the biological plausibility of epidemiology associations to PM_{2.5}, ambient filter-based toxicology studies are routinely performed. These studies require extraction of ambient PM_{2.5} from a filter and the methods for this extraction vary between research groups and differences in extraction methods utilized have been shown to result in differential toxicology outcomes.

This study compared characterization data for both ambient filter-based and corresponding extraction solutions prepared for toxicology research to identify compositional changes that occur due to the extraction methods. PM_{2.5} was characterized for concentration, metals, and organic compounds present in both ambient and extracted samples. While total PM_{2.5} mass recovery was high following extraction, there were significant and near complete losses of health relevant compounds. Following these findings, a study to assess the impact these compositional changes have on the interpretation of associations to inflammatory responses was designed.

The release of a pro-inflammatory cytokine, interleukin (IL)-6, was measured in an alveolar macrophage cell line and associations were made between IL-6 release and PM_{2.5} constituents in both ambient and extracted samples. When using ambient composition data, significant positive associations were made between IL-6 and a number of organic constituents, however these constituents were not detected in the extraction solution. Additionally, use of ambient composition values displayed significant negative associations to several health relevant metals, these associations were found to be positive when using the extracted values. This research established compositional changes from ambient PM_{2.5} due to extraction procedures and these changes led to a misinterpretation of associations between constituents and the release of a pro-inflammatory mediator

The public health significance of PM_{2.5} exposure is evident as it is a ubiquitous exposure with established adverse health outcomes. Further developing toxicology studies that accurately assess ambient exposures of PM_{2.5} are essential to ultimately create constituent specific regulations to protect human health.

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To infinity and beyond, reach for the sky...

1.0 INTRODUCTION

Outdoor air pollution is a ubiquitous exposure of significant public health importance throughout the world. Air pollution is comprised of solids, liquids, and gases; of particular interest is the solid particulate matter (PM) portion of air pollution.

1.1 FINE PARTICULATE MATTER (PM_{2.5})

1.1.1 Overview

Fine particulate matter (PM_{2.5}) is defined as particles that are less than 2.5 μm in aerodynamic diameter, this is approximately 1/25th the diameter of a human hair (EPA 2015). Because particles present in the air are irregularly shaped and difficult to measure, the aerodynamic diameter, the diameter of a spherical particle that has the same settling velocity as an irregularly shaped particle, is used as the unit of measure (Park 2015).

Exposure to air pollution is the world's single largest environmental health risk factor, accounting for nearly 60% of environmentally-related human health impacts in urban areas (Chen and Goldberg 2009). PM_{2.5} is estimated to cause 3.7 million deaths annually (WHO 2014) including approximately 11% of chronic obstructive pulmonary disease (COPD) and 16% of lung cancer deaths (WHO 2015). Beyond mortality, over 20% of ischemic heart disease and

stroke is caused by PM_{2.5} exposure (WHO 2015). Due to the obvious public health burden from ambient PM_{2.5}: the measurement, sources, and specific health effects of PM_{2.5} are described in detail below.

1.1.2 Measurement

In order to monitor ambient PM_{2.5}, measurements can be made to assess the concentration in the environment as well as the composition of PM_{2.5}.

1.1.2.1 Concentration

The concentration of PM_{2.5} is determined through measurement of the mass per unit volume of air ($\mu\text{g}/\text{m}^3$). PM_{2.5} concentrations can be determined globally through satellite data (Liu et al. 2007; Tian and Chen 2010; van Donkelaar et al. 2010) or at specific locations through direct measurements (Moore et al. 2007; Gummeneni et al. 2011; Lee et al. 2013).

Satellite data allows for global measurements of the light extinction in the atmosphere, this measurement, aerosol optical depth (AOD), has been applied to empirical formulas to determine PM_{2.5} concentrations throughout the world (Koelemeijer et al. 2006; van Donkelaar et al. 2010).

Alternatively, direct concentration measurements can occur at single locations through real-time analysis (Maciejczyk et al. 2004; Yang et al. 2012; Pancras et al. 2013) or gravimetric analysis of PM_{2.5} collected onto filters (Putaud et al. 2004; Clements et al. 2013; Lee et al. 2013). Real-time methods provide temporal data on PM_{2.5} mass concentrations (Gummeneni et al. 2011; Queensland 2013) at locations of interest. Various types of real-time monitors are available for ambient sampling including: the tapered element oscillation microbalance (TEOM) (Allen et al.

1997; Henderson et al. 2007; Gummeneni et al. 2011), aerosol time of flight mass spectrometer (ATOMFS) (Jayne et al. 2000; Qin et al. 2012), beta attenuation monitor (BAM) (Watson et al. 2002; Gobeli et al. 2008), differential mobility particle sizer (DMPS) (Pitz et al. 2001; Aarnio et al. 2005), and aerodynamic particle sizer (APS) (Hitchins et al. 2000; Cabada et al. 2004).

A second method for direct $PM_{2.5}$ concentration measurement is gravimetric analysis of $PM_{2.5}$ collected on filters (Marcazzan et al. 2001; Clements et al. 2013; Lee et al. 2013). Collection occurs through active sampling with a size-selective sampler drawing in a known volume. Two commonly used size-selective air samplers are impactors and cyclones. Impactors operate via the principle of impaction, as air is actively drawn into the sampler it travels through impactor jets that are separated by impaction plates, larger particles have greater inertia and leave the airstream and collect on impaction plates while smaller particles remain in the air flow until they reach the collection filter (Parker and Buchholz 1968). The size-selective capabilities of impactors are determined by the drag force on the particle, the particle momentum, and the transit time across the impactor plate (Hering 2001). By taking into account these factors, the distance between impactor jets and the impaction plate as well as impactor jet width can be modified to be selective for the particle size of interest (Hering 2001), for $PM_{2.5}$ studies the cut-point is set at $2.5\mu m$. Another type of size-selective sampler is cyclones which actively draw in air into a cylindrical or conical chamber with vertical air flow (Abdel-Salam and Dennis 2010). The centrifugal forces of air with increasing velocity in the chamber force particles of larger size and therefore greater inertia to fall from the air stream (Mischler 2013) or impact with the chamber walls (Abdel-Salam and Dennis 2010). Leaving smaller particles, such as those $\leq 2.5\mu m$, to be maintained in the airstream until reaching the collection filter (Abdel-Salam and Dennis 2010). Following collection of $PM_{2.5}$, the mass can be determined from previously

recorded pre-weights of the filter subtracted from the post-collection weight. The concentration then is determined by calculation with the known volume of air that passed over the filter during the sampling period. Once PM_{2.5} concentration is determined at specific locations data can be extrapolated to estimate concentrations in large regions, this practice is commonly done for epidemiology studies to estimate PM_{2.5} exposures (Liao et al. 2006).

Concentration has historically been the predominant form of measurement and therefore government and world agencies have created standards for daily and annual PM_{2.5} concentration. The Environmental Protection Agency (EPA) sets National Ambient Air Quality Standards (NAAQS) with current PM_{2.5} concentrations standards at 12 and 35 µg/m³ for daily and annual, respectively (EPA 2015). The World Health Organization (WHO) concentration standards are 20 and 50 µg/m³ for daily and annual, respectively (WHO 2014). Worldwide, PM_{2.5} concentrations are frequently observed above these standards, in a recent study, the annual average was over seven times the WHO standard (Yang et al. 2012). Domestically, in 2014 over 32 million people lived in counties that were above the EPA PM_{2.5} standards (EPA 2015).

In addition to concentration standards, there is a growing interest in the composition of PM_{2.5} as it is hypothesized to be an important indicator of health outcomes.

1.1.2.2 Composition

PM_{2.5} is mainly comprised of sulfate, nitrate, chloride and ammonia compounds, elemental carbon, metals, and organic carbon (EPA 2015). This complex mixture varies across time and space (Kelly and Fussell 2012) but two components of PM_{2.5} that are consistently of interest are metals and organic carbon (Chellam et al. 2005; Pekey et al. 2013; Rappazzo et al. 2015).

The contribution to PM_{2.5} by metals is a growing area of investigation as there is a wide variety of metals present in PM_{2.5} (Han et al. 2012) and the distribution of metals varies by time (Grieshop et al. 2006), location (Kulshrestha et al. 2009), and season (Lee et al. 2007). Metals are elements that are defined by their high electrical conductivity, common metals observed in ambient PM_{2.5} include: Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sr, Ti, V, and Zn (Chellam et al. 2005). In addition to metals, many inorganic elements are present in PM_{2.5} and analyzed in tandem with metals, inorganic elements in ambient PM_{2.5} include: As, P, S, Si (Huggins et al. 2000; Marcazzan et al. 2001). Measurement of metals and inorganic elements can occur through analysis of PM_{2.5} collected on filters (Huffman et al. 2000; Ren 2009; Choung et al. 2015) and several standard analytical methods are used for analysis. Methods for ambient analysis include: inductively coupled plasma mass spectrometry (ICP-MS) (Pakkanen et al. 2001; Cakmak et al. 2014), inductively coupled plasma optical emission spectroscopy (ICP-OES) (Mateus et al. 2013; Paraskevopoulou et al. 2014), X-ray diffraction (XRD (Huffman et al. 2000; Choung et al. 2015)), X-ray fluorescence (XRF) (Xu et al. 2012; Beelen et al. 2015), and atomic absorption spectroscopy (AAS) (Ren 2009; Lin et al. 2012). Specific quantification methods and principles have been previously detailed for ICP-MS (EPA 1999; ERG 2015), ICP-OES (Hannan 2013), XRD (Mitchell and Perez-Ramirez 2015), XRF (Wirth and Barth 2015), and AAS (EPA 1999).

Organic carbon compounds encompass any components of PM_{2.5} that contain carbon and measurements vary based on temporal trends (Xu et al. 2013), location (Hand et al. 2012), and season (Schauer et al. 2003). Specific organic compounds measured in ambient PM_{2.5} samples include n-Alkanes, steranes/hopanes, and polycyclic aromatic hydrocarbons (PAHs). n-Alkanes are groups of carbon that form a continuous chain (TSTC 2015), steranes/hopanes are organic

compounds that exist in a variety of stereoisomers (TAMU 2015), and PAHs are compounds formed from two or more benzene rings (USGS 2015). PAHs measured in ambient PM_{2.5} include: fluoranthene, pyrene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(ah)anthracene, benzo(ghi)perylene (Chellam et al. 2005). Filter-based collection of ambient PM_{2.5} is used for organics quantification (Huang and Wang 2014; Philip et al. 2014). Two standard analytical methods for organic compounds are solvent extraction gas chromatography mass spectrometry (SE-GC-MS) and thermal desorption gas chromatography mass spectrometry (TD-GC-MS). Detailed methods have previously been reported for SE-GC-MS (Winberry and Jungclaus 1999) and TD-GC-MS (Waterman et al. 2000).

Measurement of ambient PM_{2.5} can occur through analysis of the concentration and composition of PM_{2.5}; these measurements vary temporally and spatially due to several factors including the sources present.

1.1.3 Sources

Sources of PM_{2.5} are a crucial contributing factor to both the concentration and composition of ambient PM_{2.5} (Almeida et al. 2005; Chaloulakou et al. 2005). Importantly, different sources release varying components of PM_{2.5} (Manoli et al. 2002; Saarikoski et al. 2008), of particular focus are the release of organic and metallic compounds. Combustion is the predominant mechanism of PM_{2.5} production and sources of combustion can be either terrestrial or anthropogenic (EPA 2015).

Terrestrial sources of PM_{2.5} include forest fires and volcanoes, both of which are natural forms of combustion that result in particles being released that are $\leq 2.5 \mu\text{m}$. Emissions from

forest fires are dependent on the vegetation and climate but predominantly emissions are PM_{2.5} mass comprised of organic compounds due to the combustion of trees and other plants (Urbanski et al. 2009). Volcanoes are another source of PM_{2.5} unlike forest fires, they mainly emit metals including: Cd, Cs, Cu, K, Mo, Pb, and Se (OSU 2015). Terrestrial sources contribute to PM_{2.5} through both increases in concentration and organic and metallic components the other contributor to PM_{2.5} are anthropogenic sources.

Anthropogenic sources of PM_{2.5} are combustion-related including industrial, residential heating, and traffic sources; these sources dominate PM_{2.5} contribution particularly in highly populated and developing areas (Fuzzi et al. 2015). Industrial sources contribute to PM_{2.5} with primary emissions from coking coal, manufacturing plants, and metallurgic industries (Owoade et al. 2015). Industrial emissions are mainly associated with inorganic elements: S (Owoade et al. 2015) and metals: Al, Cr, Cu, Fe, Na, Ni, Pb, and Zn (Kundu and Stone 2014; Owoade et al. 2015). Residential heating is another source of emissions as the United States utility industry uses over 26 billion liters of residual oil annually (Pattanaik et al. 2012); combustion of this oil creates residual oil fly ash (ROFA). ROFA is comprised of metals: Al, Fe, Ni, and Zn (Roberts et al. 2009). Another option in residential heating is the use of wood stoves and fireplaces, this heating method results in wood smoke. While wood smoke composition varies drastically based on the specific combustion conditions (Kocbach Bolling et al. 2009), generally this source elevates PM_{2.5} concentrations (Zuk et al. 2007) and consists of the inorganic element K (Calloway et al. 1989) and large amounts of organic carbon, specifically PAHs (Danielsen et al. 2011).

The final anthropogenic source is traffic-related emissions of both gasoline and diesel powered vehicles. Traffic-related emissions are generally associated with increased PM_{2.5}

concentration (Charron and Harrison 2005), elemental and organic carbon (Zanobetti et al. 2009), steranes/hopanes (Schauer 2003; Sbihi et al. 2013), and PAHs: benzo(a)pyrene, benzo(e)pyrene, benz(a)anthracene, benzo(ghi)fluoranthene, benzo(ghi)perylene, chrysene, fluorine, and indeno(123-cd)pyrene (Slezakova et al. 2013). Metals have also been well established as a marker for tailpipe emissions and brake and tire wear, frequently associated metals include: Ba, Br, Cu, Pb, and Zn (Lough et al. 2005; Zanobetti et al. 2009). Disentangling the fuel source of the traffic emissions has not been fully established but recent studies are identifying tracers of gasoline and diesel exhaust. Gasoline is associated with organic carbon and Zn (Kundu and Stone 2014). While diesel is associated with elevated elemental carbon in comparison to organic carbon (Kundu and Stone 2014) it also contains specific PAHs: fluoranthene and pyrene (Chellam et al. 2005) as well as metals: Ni, Pb, V (Kundu and Stone 2014) that are not present in notably elevated concentrations in gasoline exhaust.

PM_{2.5} source contribution varies throughout the world as regions have differing terrestrial and anthropogenic sources (Pacyna and Pacyna 2001; van Donkelaar et al. 2010) and therefore differing concentrations of metallic and organic compounds (Manoli et al. 2002; Saarikoski et al. 2008). Importantly, these differences in components coincide with PM_{2.5}-related health effects.

1.1.4 Associated Health Effects

The public health importance of PM_{2.5} exposure has been studied in epidemiology research with the effects on both the respiratory and cardiovascular systems being well established (Pope et al. 1995; Anderson et al. 2012).

The respiratory system was a logical selection to begin understanding the health effects associated with PM_{2.5} as this size fraction of particles is known to be respirable and capable of

entering deep into the lung and into the alveolar region (CCOHS 2015). Frequently connections to PM_{2.5} concentration have been made to both respiratory morbidity and mortality. To determine the effects of PM_{2.5} disease exacerbation, studies have investigated the effect of increases in PM_{2.5} concentration on hospital admissions. Following elevated PM_{2.5} exposure, increases in respiratory-related hospital admissions have been observed in children (Karr et al. 2007; Tecer et al. 2008) as well as individuals with pneumonia (Ilabaca et al. 1999; Neupane et al. 2010) and COPD (Atkinson et al. 2001; Dominici et al. 2006). Additionally PM_{2.5} concentration is associated with development of asthma (EPA 2009), exacerbation of symptoms (Slaughter et al. 2005), and increased medication use (Rabinovitch et al. 2006). Asthma-related hospital admissions (Ito et al. 2007) and emergency department visits (ATSDR 2006) are also associated with PM_{2.5} concentrations. Importantly, associations were found between asthma-related emergency department visits and PM_{2.5} concentrations of 12 µg/m³ (Koenig et al. 1999), the current annual EPA standard (NAAQS 2015). Finally respiratory-related mortality increases (Ostro et al. 2006; Zanobetti and Schwartz 2009) by as much as 8 % (Pope et al. 2002) with 10 µg/m³ changes in PM_{2.5} concentrations.

Beyond respiratory associated health effects from PM_{2.5} exposure, growing epidemiological studies are indicating that PM_{2.5} has adverse effects on other systems, importantly the cardiovascular system. The ability for water soluble as well as insoluble particles of smaller size to enter the extra-pulmonary circulation and transport throughout the body and impact systems including the cardiovascular system has been documented (Kim et al. 2014). Cardiovascular morbidity and mortality are increasingly being associated with PM_{2.5} concentration. PM_{2.5} concentrations are associated with hospital admissions due to myocardial infarction (Peters et al. 2001; Dai et al. 2014) and congestive heart failure (Symons et al. 2006;

Brook et al. 2010). The connection between cardiovascular mortality and PM_{2.5} concentrations is also well established (Dockery et al. 1993; Pope et al. 2004; Crouse et al. 2012) and the risk of death following a cardiovascular event has been observed to be increased by up to 76% with a 10µg/m³ increase in PM_{2.5} (Miller et al. 2007).

Increasing research is indicating that beyond the well-documented effects of PM_{2.5} concentration that the composition of PM_{2.5} has respiratory and cardiovascular impacts, independent of concentration. Recently, biomarkers of inflammation have been studied to evaluate the health effects of components of PM_{2.5} with associations found between systemic inflammation and organics (i.e. high molecular weight PAHs, hopanes) (Delfino et al. 2010). Increased respiratory hospital admissions were linked with elevated levels of metals: Al, As, Cl, Cr, Fe, Ni (Ostro et al. 2009; Zanobetti et al. 2009; Bell et al. 2014) and organics: traffic-related and total organic carbon (Ostro et al. 2009; Peng et al. 2009; Kioumourtzoglou et al. 2013). Similarly, cardiovascular-related hospital admissions were associated with increases in metals: As, Cr, Ni, V (Bell et al. 2009; Zanobetti et al. 2009), groups of combined metals: Cu, Fe, Mn, and Zn (Suh et al. 2011), and organics: hopanes and total organic carbon (Zuo et al. 2002; Tolbert et al. 2007; Zanobetti et al. 2009; Sarnat et al. 2015).

Beyond morbidity associations, components of PM_{2.5} have also been linked to daily, respiratory, and cardiovascular mortality. Associations between daily mortality and inorganic components: S, Si (Ostro et al. 2007; Dai et al. 2014), metals: Al, Ca, Cl, Ni, Zn (Franklin et al. 2008; Cao et al. 2012; Dai et al. 2014), and total organic carbon (Cao et al. 2012; Kim et al. 2015) have been made. Significant associations to respiratory-related mortality have been made to Cl and Ni as well as total organic carbon (Cao et al. 2012). Cardiovascular mortality had similar associations to metals and organics as reported for respiratory mortality (Cao et al. 2012).

Additional associations have recently been made between cardiovascular mortality and PM_{2.5} components, including: inorganic components: S, Si (Ito et al. 2010; Zhou et al. 2011) and metals: Cu, Fe, K, Se, Zn (Ostro et al. 2008; Ito et al. 2011; Zhou et al. 2011).

1.2 PM_{2.5} TOXICOLOGY STUDIES

To establish the biological plausibility of PM_{2.5}-associated health effects as well as elucidate the mechanisms, toxicology studies are routinely conducted (Dellinger et al. 2001; de Kok et al. 2005; Wei et al. 2011; Boogaard et al. 2012; Valavanidis et al. 2013). Research using a variety of *in vitro* and *in vivo* models has been performed to investigate the inflammatory and genotoxic effects of PM_{2.5} exposure. The use of ambient PM_{2.5} in toxicology studies is imperative to capture the complex mixture that exists in the environment. Collection of ambient PM_{2.5} for toxicology studies is predominately performed with concentrated ambient particle systems or filter-based collection methods.

1.2.1 Mechanisms of PM_{2.5}-induced Health Effects

Historically, the health effects of PM_{2.5} exposure have been established in epidemiology research (Pope et al. 1995), but these studies give no indication of biological mechanism involved with these health effects. Toxicology studies are the established method used in the scientific literature to study potential biological mechanisms for these health effects. In recent years, increasing research has pointed to oxidative stress as the predominant mechanism of interest leading to PM_{2.5} health effects. Oxidative stress occurs when the production of reactive oxygen

species (ROS) is elevated beyond the antioxidant defenses present (Boogaard et al. 2012). ROS generation can occur both from the response to PM_{2.5} (de Kok et al. 2006) as well as through direct generation by metallic and organic compounds present in PM_{2.5} (Ghio et al. 2012). Additionally, further production of ROS can occur by inflammatory cells in response to PM_{2.5} (Kelly 2003). ROS can lead to a variety of health impacts, including genotoxicity and inflammation. In order to further understand the mechanisms behind PM_{2.5} health effects, ROS, genotoxicity, and inflammatory responses are investigated in toxicology studies.

1.2.1.1 Reactive Oxygen Species and Oxidative Stress

Because of the importance of ROS in PM_{2.5}-related health effects, ROS is frequently assessed in PM_{2.5} toxicology studies through analysis of ROS generation and oxidative stress markers. Deng et al. analyzed the impact of ambient PM_{2.5} exposure on ROS generation in human lung epithelial cells through studying various time points (4, 12, 24, 48 h) and PM_{2.5} concentrations (25, 50, 100, 200 µg/ml) (2013). Significant increases in ROS, up to 5-fold, above unexposed control cells were observed at 4 and 12 h for all PM_{2.5} concentrations (Deng et al. 2013). Additionally, a 77% increase in ROS production was observed following 24 h of exposure to PM_{2.5} (38 µg/mL) in human endothelial cells (Montiel-Davalos et al. 2010). ROS generation was again observed to be significantly increased above unexposed control cells in human endothelial cells (Wei et al. 2011). In this study, a time point of 6 h was selected and varying PM_{2.5} concentrations (3.8, 19, 76, 190, 380, 1190 µg/ml) were used, following PM_{2.5} exposure, up to 2.8-fold changes above untreated control cells were observed (Wei et al. 2011). The ability for ambient PM_{2.5} to generate ROS has been established and growing research is investigating the impact on ROS generation by varying PM_{2.5}. ROS following PM_{2.5} exposure has been shown

to vary due to the sources present (Zhang et al. 2008), location (Vizcaya-Ruiz et al. 2006; Mirowsky et al. 2015), and season (Daher et al. 2012; Fang et al. 2014) of collection.

Oxidative stress occurs within a cell when the ROS generated is above the production of antioxidants (Boogaard et al. 2012) and common markers of oxidative stress are genes associated with this response or with antioxidant levels. Dieme et al. exposed human bronchial epithelial cells to PM_{2.5} (225 or 900 µg/ml) for various time points (24, 48, 72 h) and measured the oxidative stress marker, malondialdehyde, MDA (2012). Significant increases in MDA were observed only after 24 h of exposure to the higher dose of PM_{2.5} (Dieme et al. 2012). These findings were corroborated in human alveolar epithelial cells where MDA was significantly increased however this observation was at a later time point (72 h) and higher dose (2042 µg/ml) of PM_{2.5} exposure than observed in the aforementioned study (Kouassi et al. 2010).

Beyond markers of damage resulting from oxidative stress, antioxidants can be used as markers since they are essential in the balance with ROS and a change in this balance results in oxidative stress (Boogaard et al. 2012). A significant decrease in the antioxidant ascorbate was reported following 4 h of PM_{2.5} exposure (50 µg/mL) in synthetic respiratory tract lining fluid (Mudway 2004). Another commonly measured antioxidant, glutathione, was significantly reduced in microglia cells following PM_{2.5} exposure (25, 50, 100 µg/ml) for 1.5 h (Sama et al. 2007). Furthermore, the addition of antioxidants to cell culture can mitigate oxidative stress responses induced by PM_{2.5} (Dellinger et al. 2001; Nakayama Wong et al. 2011). ROS has been shown to be generated by PM_{2.5} as measured through its generation, oxidative stress markers, and antioxidant levels. Assessing ROS in PM_{2.5} toxicology studies is essential as it leads to genotoxicity and inflammation.

1.2.1.2 Genotoxicity

PM_{2.5}-induced oxidative stress resulting from elevated ROS generation has multiple consequences in cells. One specific consequence is genotoxicity or damage to DNA (Valavanidis et al. 2013), genotoxicity can be measured through several different markers including: DNA base modifications (Topinka et al. 2011), DNA strand breaks (Shi et al. 2006), and bulky adduct formation (Lepers et al. 2014).

Currently, in PM_{2.5} toxicology studies, the predominant marker of oxidative DNA damage is the base modification, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) (Moller et al. 2014). In addition to being a marker of oxidative damage, 8-oxodG is pre-mutagenic indicator present in carcinogenic cells (Valavanidis et al. 2009). Andre et al. investigated 8-oxodG in human epithelial cells exposed to PM_{2.5} (23.7, 118.6 µg/ml) for varying time points (24, 48, 72 h) (2011). PM_{2.5} was observed to create significant increases in 8-oxodG at all concentrations and time points with a dose dependent response (Andre et al. 2011). The impact of PM_{2.5} composition was documented by Topinka et al. when they studied PM_{2.5} from varying locations (2011). PM_{2.5} from all locations caused oxidative DNA damage but four-fold differences in 8-oxodG were observed, these differences were dependent on the location of PM_{2.5} collection (Topinka et al. 2011).

In addition to DNA base modifications, the ability for PM_{2.5}-induced oxidative stress to cause DNA strand breaks has been investigated. DNA strand breaks are a common marker of oxidative damage (Haney et al. 1999) and response to cell death signals (Gorczyca et al. 1993). A study using human epithelial cells treated with PM_{2.5} (400 µg/ml) for 3 h demonstrated that PM_{2.5} can induce DNA strand breaks and this breaks were highly correlated with ROS generation from PM_{2.5} (Shi et al. 2006). Further evidence supports that PM_{2.5} induced strand breaks are a

result of ROS and subsequent oxidative stress and not a response to cell death signals, as strand breaks occur following PM_{2.5} exposures that do not result in significant cell death (Hsiao et al. 2000). Lastly, the importance of PM_{2.5} composition in DNA strand breaks was observed by de Brito et al. who reported that differences in strand breaks were attributable to variations in PM_{2.5} from different sampling locations (2013).

Finally PM_{2.5} can directly have genotoxic effects when the chemical components of PM_{2.5} directly bind to DNA forming covalent bonds that result in bulky adduct formation (Valavanidis et al. 2013). Bulky adducts can impact DNA base pairing as well as the helical structure of DNA (Hang 2010). Exposure of human epithelial and macrophage cells to PM_{2.5} in the previously described study, Andre et al. demonstrated the ability for PM_{2.5} to cause bulky adducts in both cell lines (2011). Consistent with other genotoxicity markers, bulky adduct formation was dependent on PM_{2.5} composition. Two-fold increases in adduct formation between PM_{2.5} from varying locations was observed in the previously described, Topinka et al. (2011). In addition to location differences, seasonal differences were observed to cause differences in adduct formation with four-fold increases in adduct formation from PM_{2.5} collected at the same sampling location but during different seasons (Lepers et al. 2014).

Genotoxicity induced by PM_{2.5} can result from ROS generation as well as from direct interactions between PM_{2.5} components and DNA. Growing research is highlighting the importance of PM_{2.5} composition on genotoxic effects.

1.2.1.3 Inflammation

One of the most studied mechanisms for the established health effects of PM_{2.5} exposure is inflammation. ROS generation by PM_{2.5} can directly result in increased pulmonary inflammation (de Kok et al. 2006); additionally inflammatory cells can generate ROS and thus

increase oxidative stress during the inflammatory response to PM_{2.5} (Valavanidis et al. 2013). Inflammation is believed to be a key mechanism in respiratory and cardiovascular health impacts of PM_{2.5}, responsible for exacerbation and progression of diseases (Pope et al. 2004). While there is not a PM_{2.5}-specific marker of inflammation, common markers of inflammation used for measurement are white blood cells and cytokines.

White blood cell counts are a common marker of respiratory inflammation and are indicative of systemic inflammatory responses. Collection of bronchoalveolar lavage (BAL) fluid is a technique that provides information on the types of cells and quantity of immune cells and proteins present in the lung (Wuyts et al. 2013). BAL fluid consists of white blood cells (macrophages, neutrophils, lymphocytes) essential to the innate immune response to PM_{2.5} (Alberts et al. 2002). White blood cells secrete small proteins, cytokines, during the inflammatory response in order to effect communication to between cells including macrophages, neutrophils, and lymphocytes. Cytokines encompass a large variety of proteins and are organized based on specific properties including the ability to chemically attract cells (chemokines) and their origins: produced by monocytes (monokine) or leukocytes (interleukins) (Zhang and An 2007). In the lung, cytokines are mainly released from epithelial cells (bronchial and alveolar) and macrophages, the two main cell types involved in the inflammatory response to PM_{2.5} (Hiraiwa and van Eeden 2013). Several cytokines are routinely measured to establish the pro-inflammatory properties of PM_{2.5} including: tumor necrosis factor (TNF) – α (Long et al. 2001; Huang et al. 2003; Jalava et al. 2009; Happonen et al. 2013), macrophage inflammatory protein-2 (MIP-2) (Ning et al. 2000; Jalava et al. 2006; Jalava et al. 2009; Steenhof et al. 2011) , and interleukin (IL)-8 (Lauer et al. 2009; Perrone et al. 2010; Watterson et al. 2012; Akhtar et al.

2014). An essential cytokine in the inflammatory response to PM_{2.5} is interleukin (IL)-6 and PM_{2.5} studies investigating this cytokine are detailed below.

IL-6 is a cytokine that is a well-established marker for systemic inflammation and is therefore frequently measured in epidemiological studies which show strong associations between the cytokine and PM_{2.5} exposure (van Eeden et al. 2005; Thompson et al. 2010). Secretion of IL-6 following PM_{2.5} exposure is predominantly from the abundant white blood cells, macrophages (Wilson et al. 2010). Release of this cytokine is of particular interest because it has been found to be essential during the inflammatory response to PM_{2.5} (Budinger et al. 2011). Due to the importance of IL-6 in the PM_{2.5}-induced inflammation, it is common for toxicology studies to measure IL-6 release and thus literature is available for *in vitro* studies using several different cell types.

Ambient PM_{2.5} has been shown to induce IL-6 release *in vitro* in macrophages (Jalava et al. 2006; Jalava et al. 2009) as well as bronchial (Lauer et al. 2009; Watterson et al. 2012) and alveolar (Perrone et al. 2010) epithelial cells. Jalava et al. measured IL-6 release after 24 h of exposure to ambient PM_{2.5} (15, 50, 150, 300 µg/ml) and found significant increases in IL-6 compared to untreated control cells for all PM_{2.5} concentrations except 15 µg/ml (2006). Interestingly, IL-6 was found to be significantly different based upon the sources present during ambient PM_{2.5} collection (Jalava et al. 2006), again indicating the impact of PM_{2.5} composition on biological responses. Jalava et al. performed subsequent studies to determine IL-6 release by macrophages following 24 h of PM_{2.5} exposure (150 µg/ml) from differing collection locations to investigate the impact of PM_{2.5} composition on IL-6 release (2009).

Bronchial epithelial cells have also been researched to determine IL-6 release in this cell type following exposure to PM_{2.5} (12.5, 25 µg/ml) at various time points (6, 24 h) (Watterson et

al. 2012). All concentrations and time points had significant IL-6 release above untreated control cells (Watterson et al. 2012); this finding contradicts Jalava et al. which did not observe IL-6 release at the lowest PM_{2.5} concentration studied (2006). One rationale for these opposite findings is that the composition of PM_{2.5} varied in the two studies. Additional studies using ambient PM_{2.5} (50 µg/ml) at 18 h found that seasonal differences in PM_{2.5} resulted in differing release of IL-6 (Lauer et al. 2009), further suggesting the importance of PM_{2.5} composition in the inflammatory response.

Finally, IL-6 release has been studied in alveolar epithelial cells by Perrone et al (2010). IL-6 release after 16 h of PM_{2.5} (6.25, 12.5, 25, 50, 100 µg/ml) exposure was significantly increased above untreated cells at all concentrations but release was dependent on the location and therefore composition of PM_{2.5} used for treatment (Perrone et al. 2010). These findings used the lowest PM_{2.5} concentration discussed thus far and significant IL-6 release was observed at this concentration (Perrone et al. 2010) again suggesting that the composition of PM_{2.5} is a critical factor in the ability of PM_{2.5} to induce inflammation.

ROS generation is a key mechanism in PM_{2.5}-related health effects that can lead to genotoxicity and inflammation. An essential inflammatory marker in response to PM_{2.5} is the cytokine IL-6. Importantly, IL-6 release varies due to the composition of PM_{2.5} which can vary due to sources, location, and season. In order to better understand the health effects discussed, ambient PM_{2.5} must be collected for research.

1.2.2 Collection of PM_{2.5} for Toxicology Studies

Use of ambient PM_{2.5} for toxicology studies is essential in further identifying PM_{2.5}-induced health impacts and the underlying mechanisms. How PM_{2.5} is collected for this research is an

important consideration and two methods used are filter-based collection of particles and concentrated ambient particle systems. Both of these methods allow for the use of ambient PM_{2.5} in toxicology studies and the specifics of each method are described in detail below.

1.2.2.1 Filter-based PM_{2.5}

Filter-based collection of PM_{2.5} allows for the use of a variety of sampling equipment and materials as well as flexibility in study design. In order to use the PM_{2.5} for any *in vitro* or *in vivo* experiments following collection, the particles must be removed from the filter, concentrated, and re-suspended into a media appropriate for the exposures; this process will be referred to as “extraction” hereafter (Roper et al. 2015). It is important to note that there is currently no standard protocol for these extractions and that protocols vary greatly between research groups (Mudway 2004; Cavanagh et al. 2009; Huang et al. 2014). Previously reported methods for each extraction step are detailed below followed by key findings that have resulted from filter-based toxicology studies.

Extraction Methods

Extraction is a multistep process starting with filter-based PM_{2.5} and resulting in PM_{2.5} suspended in toxicology media and generally consists of 1) removal 2) concentration and 3) re-suspension (Figure 1). Each step is essential to gather known concentrations of PM_{2.5} prepared for toxicology research.

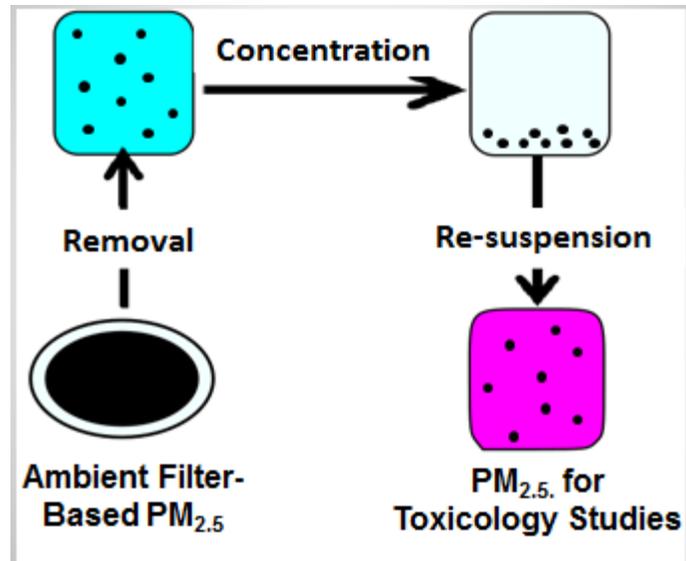


Figure 1: Extraction process schematic

The removal step is the first step of the process and results in the removal of particles from the filter. Methods vary between research groups both in form of removal and solvent type. Frequently implemented removal methods include: probe sonication (Imrich et al. 2000; Mudway 2004; Godri et al. 2011), water bath sonication (Long et al. 2001; Longhin et al. 2013; Janssen et al. 2014), and soxhlet extraction (de Kok et al. 2005; Skarek et al. 2007; Cavanagh et al. 2009). Probe sonicators use ultrasonic frequencies to create microscopic bubbles that violently collapse following formation, releasing large amounts of energy, this process is referred to as cavitation (Sonicators 2015). Use of this system requires a filter to be placed in liquid; the probe is then placed into the liquid and pulsed for various amounts of time depending on the protocol. Sonication times typically remain under 1 minute and are repeated 2 to 3 times (Imrich et al. 2000; Imrich et al. 2007). Routinely, probe sonication is used in conjunction with other removal methods such as vortexing (Mudway 2004; Godri et al. 2011) or water bath sonication (Ning et al. 2000; Seagrave et al. 2006; Van Winkle et al. 2015). A common application for probe sonicators is for the destruction of cell membranes due to the tremendous amount of

energy released during cavitation. While this property is essential for some applications, it creates concern during PM_{2.5} removal as the potential for the release of the filter material in addition to particles arises. Filter material may impact the responses measured in subsequent toxicology research and therefore an additional step of filtering the solution may be used (Bein and Wexler 2014). This additional step of filtering has the ability to alter the biological outcomes as particles may get trapped in the filter and thus are not present in the PM_{2.5} used for toxicology studies.

The principle behind water bath sonication is the same as that for probe sonication (Sonicators 2015) however the intensity of the energy is reduced as the liquid that the filter is placed in is not directly undergoing cavitation rather a glass beaker containing the liquid and filter rests in high purity water that is exposed to the ultrasonic frequencies. The benefit to this removal method is that it is less destructive than probe sonication, eliminating the concern of filter material contamination. Due to the reduced intensity of this method, exposure times to the ultrasonic frequencies are increased compared to probe sonication. Times have a comparatively large range from seconds (Janssen et al. 2014) to 8 h (Rivero et al. 2005). Frequently water bath sonication times are 30 min (Becker et al. 2005; Akhtar et al. 2010; Deng et al. 2013; Kumar et al. 2015) or more (Monn and Becker 1999; Watterson et al. 2007; Watterson et al. 2012). Additionally many protocols use repeated intervals (Perrone et al. 2010; Riva et al. 2011; Mantecca et al. 2012; Huang et al. 2014). Similar to probe sonication, many research groups uses water bath sonication in addition to other methods such as agitation (Schaumann et al. 2004; Valavanidis et al. 2005), vortexing (Cassee et al. 2003; Gerlofs-Nijland et al. 2007), or prior to liquid-liquid extraction (Bein and Wexler 2014; Van Winkle et al. 2015).

The final removal method used by toxicology groups is soxhlet extraction which is a form of solid-liquid extraction that requires a soxhlet apparatus. The apparatus circulates solvent over small pieces of the filter placed in a thimble-holder, as the solvent level rises it is aspirated into a distillation flask and portions of the solid, in this case particles, are removed from the thimble and enter the distillation flask, ultimately resulting in a solvent containing the removed PM_{2.5} (Luque de Castro and Garcia-Ayuso 1998). Soxhlet introduces the aforementioned concern of filter material entering the solution as it requires the filters to be cut into pieces. Although, uncommon compared to sonication removal methods, soxhlet extraction has been utilized for PM_{2.5} removal from filters (de Kok et al. 2005; Skarek et al. 2007).

Each of the removal methods discussed requires a solvent for the particles to enter as they are removed from the filter. A variety of solvents have been used for removal including: water, organic solvents, and toxicological media. Water is a recurrent solvent in removal protocols and is effective in removing up to 75% of total PM_{2.5} mass (Longhin et al. 2013) but only removes water soluble components, leaving non-polar species, including many organics on the filter. Even with the known limitations of using only water as the removal solvent, it is still frequently utilized (Dye et al. 2001; Huang et al. 2003; Baulig et al. 2004; Riva et al. 2011; Deng et al. 2013; Huang et al. 2014). Growing filter-based extraction studies are transitioning from using solely water for extraction to alternative solvents to increase PM_{2.5} recovery.

Removal with organic solvents has been reported to be elevated compared to use of water with efficiencies over 90% (Gerlofs-Nijland et al. 2007) and while water-based removal is still common, there has been an increase in the use of organic solvents in recent years. Typically methanol (Happo et al. 2013; Janssen et al. 2014; Lee et al. 2015) and dichloromethane (Topinka et al. 2011; de Brito et al. 2013) are the solvents selected for removal.

Finally, some removal protocols remove PM_{2.5} directly into the media appropriate for future toxicology studies. Toxicology media used for removal includes: saline (Imrich et al. 2000; Ning et al. 2000; Kumar et al. 2015), PBS (Dellinger et al. 2001; Choi et al. 2004; Van Winkle et al. 2015), and cell culture media (Akhtar et al. 2010; Akhtar et al. 2014). These methods circumvent the concentration and re-suspension steps (described below) but they prevent specific dosing for studies as the removed particles cannot be directly weighed prior to suspension in media. Additionally, these solvents present similar concerns for the removal of all PM_{2.5} constituents as they are frequently water-based.

Following the removal step, PM_{2.5} is suspended in solution; the concentration step removes the solvent, resulting in dry PM_{2.5}. Generally methods used for the concentration step remove the solvent under vacuum (rotary evaporator and lyophilization), evaporation by gas blow down, or with a desiccator. The use of vacuum alters the pressure and allows for an efficient method of solvent removal. Rotary evaporation increases the evaporation speed of the solvent by lowering the pressure (under vacuum) and heating the solution (Calgary 2015). This method has predominantly been used to concentrate particles that are suspended in methanol (Gerlofs-Nijland et al. 2007; Jalava et al. 2009; Happonen et al. 2010; Verma et al. 2012). A second concentration method under vacuum is lyophilization, instead of heating the sample as with rotary evaporation, samples are frozen and solvent is removed through sublimation, the direct transition of a solid to gas, skipping the liquid phase (Labconco 2015). This process is commonly referred to as freeze drying and has been implemented with a variety of solvents including: water (Monn and Becker 1999; Baulig et al. 2004; Geng et al. 2006), PBS (Choi et al. 2004), and combinations of water and organic solvents (Vincent et al. 1997; Becker et al. 2005; Van Winkle et al. 2015).

Aside from use of a vacuum pump, the concentration step can be completed by removing the solvent through the common laboratory techniques of blow down by nitrogen (N₂) gas or by being placed in a desiccator. Blow down by N₂, speeds up the evaporation process by altering the equilibrium between the liquid and gas phases to quickly remove the solvent from the particles (Labconco 2015). Previous studies have used N₂ blow down for the organic solvents methanol (Mudway 2004; Godri et al. 2011; Lee et al. 2015) and dichloromethane (Topinka et al. 2011; Wang et al. 2011; de Brito et al. 2013). Finally, desiccators can be used for the concentration step as the desiccant in a closed chamber removes water vapor from the sample (Camp and Seely 2015). Desiccators have been used for removal of water (Watterson et al. 2009; Gualtieri et al. 2012; Mantecca et al. 2012) from particles. At the end of the concentration step the result is dry PM_{2.5} and mass can be determined.

Following concentration, the dry PM_{2.5} must be re-suspended into a media that is appropriate for the specific toxicology studies being performed. By determining the mass of the dry PM_{2.5}, concentrations can be set at the desired dose when re-suspension occurs. Re-suspension occurs for both *in vitro*: water (Jalava et al. 2006; Jalava et al. 2009) and cell culture media (de Kok et al. 2005; Lauer et al. 2009; Watterson et al. 2009) and *in vivo*: 0.9% saline (Seagrave et al. 2006; Gerlofs-Nijland et al. 2007), PBS (Lee et al. 2015), and water (Schaumann et al. 2004; Happo et al. 2010) experiments.

As there is not a standardized extraction protocol for filter-based studies, procedures vary at each step between research groups, as detailed above. This variation creates a potential bias in that biological outcomes are dependent on the extraction methods selected instead of on the PM_{2.5} exposure.

Findings and Associations using Filter-based Methods

Filter-based extraction toxicology studies are commonly performed to analyze ROS generation/oxidative potential, genotoxicity, and inflammation of PM_{2.5}. Growing efforts are being made to identify components of PM_{2.5} that are most relevant to these responses through connections to characterized PM_{2.5}. In some cases associations are made between biological outcomes and ambient PM_{2.5} measurements, disregarding the compositional changes that may occur throughout the extraction process (Bein and Wexler 2015).

The importance of ROS generation in response to PM_{2.5} has been discussed in detail (Chapter 1.2.1.1) and to determine the components of PM_{2.5} most relevant in ROS generation and oxidative stress associations are made between established markers and ambient measurements of PM_{2.5}. Generation of ROS has been studied and significant correlations have been made to inorganic components: S (Daher et al. 2012) metals: As, Cu, Cr, Fe, Ni, Zn, and total metals (Daher et al. 2012), organic carbon attributable to biomass burning (Hamad et al. 2015), and total organic carbon (Daher et al. 2012). General associations without statistical comparisons have been made between ROS and ambient PM_{2.5} measurements with the metallic and organic contribution to ambient PM_{2.5} (Deng et al. 2013; Longhin et al. 2013) and during different seasons (Gualtieri et al. 2012) being considered to result in differential ROS generation. Additionally, ROS generation was observed to be increased above a benzo(a)pyrene control indicating the ambient mixture produced more ROS than a single constituent (Gualtieri et al. 2012). The oxidative potential of PM_{2.5} has been significantly positively correlated with ambient metals: Cu, Fe, and V (Valavanidis et al. 2005; Janssen et al. 2014) and PAHs: benzo(a)anthracene, benzo(e)pyrene, benzo(a)pyrene, benzo(ghi)perylene (Janssen et al. 2014). Conversely, negative correlations were found between oxidative potential and Ni as well as total

organic carbon (Janssen et al. 2014). Finally, associations between oxidative stress and ambient PM_{2.5} have been made to overall levels of ambient PAHs but no significant correlations were made to specific PAHs (Topinka et al. 2011).

Limited associations between ambient PM_{2.5} and genotoxicity have been made due in part to the comparatively minimal research into PM_{2.5}-induced genotoxic effects. However, a study attempting to make correlations between DNA strand breaks and ambient PAH concentrations found no significant associations (Wang et al. 2011).

Associations between inflammation and ambient PM_{2.5} have thus far been contradictory and predominantly made to cytokines. TNF- α release has been associated with ambient concentration of metals: Al, Co, Cr, Cu, Fe, Mn, Ni, Zn (Huang et al. 2003; Happonen et al. 2010). However, negative associations between TNF- α and metals: As, Cu, Mn, Ni, and V and organic compounds: benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, total organic compounds have also been made (Huang et al. 2003; Happonen et al. 2010). These findings contradict which components are associated with TNF- α release, particularly with discrepancies for metals (Cu, Mn, and Ni) between studies.

IL-8 release was associated with ambient concentrations of specific metals: Ba, Cr, Cu, Fe, Mg, Mn, Ti, and Zn (Huang et al. 2003; Akhtar et al. 2014) and total organic compounds (Akhtar et al. 2014). However studies using the same cell type (human bronchial epithelial) and dosing of PM_{2.5} did not observe a significant increase in IL-8 (Schins et al. 2002; Becker et al. 2005) and no connections to specific components could be made and further implicating the importance of PM_{2.5} composition on biological outcomes.

Finally, IL-6 was positively associated with metals: Al, Co, Cr, Cu, Fe, Mn (Happo et al. 2010). Conversely, negative associations were observed between IL-6 and components of PM_{2.5} including metals: As, Ni, and V and organic compounds: benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, ideno(1,2,3-cd)pyrene, and dibenz(a,h)anthracene (Happo et al. 2010). While studies have shown IL-6 release to be induced by PM_{2.5} exposure *in vitro* (Jalava et al. 2006; Jalava et al. 2009) there are comparable studies that have not observed a significant increase in IL-6 (Osornio-Vargas et al. 2003; Hetland et al. 2005) which contributes to the contradictory findings in filter-based PM_{2.5} research.

Inconsistent associations between toxicology studies along with discrepancies from epidemiology research highlight the potential that filter-based extraction processes impact biological outcomes.

1.2.2.2 Concentrated Ambient Particles (CAPs)

A method for *in vitro* and *in vivo* exposures to ambient PM_{2.5} is the use of concentrated ambient particles (CAPs) which are PM_{2.5} that is highly concentrated above ambient levels to aid in determination of biological impacts. Ambient PM_{2.5} is directly collected from the environment using high volume samplers (5,000 L/min), concentrated, and immediately used for inhalation or *in vitro* exposures (Behbod et al. 2013).

Use of CAPs systems provides a number of advantages that make the systems effective for toxicology research. Firstly, concentrated PM_{2.5} provides higher dosing than present under ambient conditions while maintaining an inhalation exposure. This allows for quicker observations of biological impacts without life time exposure studies or multiple dosing experiments. In some cases, CAPs systems permit the direct determination of PM_{2.5} concentrations used in the toxicology experiment (Devlin et al. 2003; Brook et al. 2009;

Balasubramanian et al. 2013). It should be noted that not all CAPs systems are equipped for this type of characterization and many collect CAPs onto filters that can then be analyzed to determine PM_{2.5} concentrations (Gong et al. 2004; Kodavanti et al. 2005; Ying et al. 2014). Studies using filter-based CAPs for exposures (Goldsmith et al. 1997; Huang et al. 2003) have also occurred and they require extraction for use, this process is detailed in section 1.2.2.1. It has been established that there is not a significant loss of PM_{2.5} components such as metals and organics during the concentration process (Sioutas et al. 1997). This is a key advantage to these systems because the concern of compositional differences from ambient PM_{2.5} is eliminated.

CAPs provide useful data on the health effects of elevated PM_{2.5} concentrations and allow for human, animal, and cell exposures however the designs for concentrating systems do not concentrate the ultrafine fraction of PM_{2.5}. A majority of these smaller particles flow with the major exhaust flow stream and are therefore a concentrated fraction of ultrafine particles does not enter the exposure chamber (Ghio and Huang 2004). This is of particular concern because ultrafine particles alone have been associated with adverse health effects (Knol et al. 2009; Schaumann et al. 2014). Concentrator systems specifically designed to collect ultrafine particles have been created but these lack the fraction of PM_{2.5} that is >.01 μm (Allen et al. 2013). While CAPs systems are typically mounted on rooftops (Gong et al. 2004; Urch et al. 2005; Tong et al. 2015), there is growing research using mobile systems contained in vehicles to have CAPs exposures at selected locations (Kampfrath et al. 2011; Ying et al. 2014). There remains a limitation that only single locations can be studied at a given time, disregarding the spatial (Miller et al. 2007; Krewski et al. 2009) and frequently the seasonal (Becker et al. 2005; Huang et al. 2012) impacts of exposures.

1.3 SCOPE OF DISSERTATION AND STATEMENT OF HYPOTHESIS

While extraction protocols for filter-based ambient $PM_{2.5}$ vary between toxicology research groups, the differences between ambient and extracted $PM_{2.5}$ components have yet to be assessed in the methods currently used. Furthermore, causal constituents of $PM_{2.5}$ are being identified using ambient values with a disregard for the compositional changes that may occur during extraction. Thus far the difference between making associations to biological outcomes based on ambient versus extracted $PM_{2.5}$ has not been established.

Due to this gap in current research, a hypothesis was developed that toxicology studies using filter-based ambient $PM_{2.5}$ are integral in elucidating the mechanisms of $PM_{2.5}$ -induced inflammation which has been well established in epidemiology research however extraction procedures result in $PM_{2.5}$ that is not representative of the ambient filters and these differences impact the identification of $PM_{2.5}$ components associated with respiratory inflammation. This hypothesis will be tested by developing a method to assess compositional differences between ambient and corresponding extracted $PM_{2.5}$ for use in toxicology studies (Chapter 2). Following the determination of the differences between ambient and corresponding extracted $PM_{2.5}$, the impact these differences have on the interpretation of inflammatory responses will be assessed (Chapter 3).

2.0 CHARACTERIZATION OF AMBIENT AND EXTRACTED PM_{2.5} COLLECTED ON FILTERS FOR TOXICOLOGY APPLICATIONS

Adapted from a publication (Roper et al. 2015) in the journal, *Inhalation Toxicology*

2.1 ABSTRACT

Research on the health effects of fine particulate matter (PM_{2.5}) frequently disregards the differences in particle composition between that measured on an ambient filter versus that measured in the corresponding extraction solution used for toxicological testing. This study presents a novel method for characterizing the differences, in metallic and organic species, between the ambient samples and the corresponding extracted solutions through characterization of extracted PM_{2.5} suspended on filters. Removal efficiency was found to be $98.0 \pm 1.4\%$ when measured using pre- and post-removal filter weights, however this efficiency was significantly reduced to $80.2 \pm 0.8\%$ when measured based on particle mass in the extraction solution. Furthermore, only $47.2 \pm 22.3\%$ of metals and $24.8 \pm 14.5\%$ of organics measured on the ambient filter were found in the extraction solution. Individual metallic and organic components were extracted with varying efficiency, with many organics being lost entirely during extraction. Finally, extraction efficiencies of specific PM_{2.5} components were inversely correlated with total mass. This study details a method to assess compositional alterations resulting from extraction of

PM_{2.5} from filters, emphasizing the need for standardized procedures that maintain compositional integrity of ambient samples for use in toxicology studies of PM_{2.5}.

2.2 INTRODUCTION

Ambient fine particulate matter (PM_{2.5}) has long been associated with respiratory and cardiovascular morbidity and mortality (Dockery et al. 1993; Pope et al. 1995; Franklin et al. 2007). Recently, PM_{2.5} and related health effects have been shown to vary across the United States (Bell et al. 2009; Sampson et al. 2013), highlighting the importance of researching the impact of compositional differences in ambient PM_{2.5}. It is particularly important to understand differences in components that are relevant to human health, such as metallic and organic species (Ravindra et al. 2001; Schaumann et al. 2004; Melaku et al. 2008). As epidemiological evidence of PM_{2.5}-associated health effects continues to be strengthened, toxicology studies to understand the mechanisms behind these outcomes and impacts of compositionally differing PM_{2.5} have advanced.

Toxicology studies allow for research into PM_{2.5} health effects while avoiding confounders present in many epidemiological studies, such as lifestyle and occupational factors (Jerrett et al. 2005). In order to capture the compositional complexity of PM_{2.5}, ambient samples must be used; however this requires collection of samples predominantly through concentrator systems or filter-based methods (Ghio and Huang 2004). Concentrated ambient particles (CAPs) provide PM_{2.5} samples that maintain ratios of ambient mixtures while increasing the mass to allow for both *in vitro* and *in vivo* studies (Ghio and Huang 2004). While CAPs provide a number of benefits to research, they require an expensive concentrator system that is fixed at a

single sampling location, lacking the potential to study spatial differences in ambient PM_{2.5} concentration and composition (Matte et al. 2013).

Filter sampling allows for collection of ambient PM_{2.5} that may vary by location and source while utilizing a relatively low-cost method conducive to a variety of air sampling equipment systems (Kundu and Stone 2014). An important consideration is the translation of PM_{2.5} on the filter, to a liquid suspension of PM_{2.5} that can be used in toxicology experiments. This process is integral to maximizing extraction efficiency while maintaining compositional integrity, so that the final extraction solution yields sufficient PM_{2.5} mass that remains representative of ambient PM_{2.5}.

Preparation of PM_{2.5} is typically a multi-step process involving removal from the filter into solution, recovery of dry PM_{2.5}, and re-suspension into media appropriate for the toxicology application. A variety of extraction techniques have been implemented in toxicology research, which differ by the type of filter used for ambient collection, removal procedure and solvent, concentration method, and the media used for re-suspension. Table 1 summarizes a literature review of preparation procedures and emphasizes the variability of extraction methods that have been used.

Table 1: Methods previously implemented for extraction of ambient PM_{2.5} for use in toxicology studies

Removal Method	Solvent	Concentration	Authors
Sonication			
	Water	N/A	(Huang et al. 2003; Schins et al. 2004; Rivero et al. 2005; Riva et al. 2011; Deng et al. 2013)
		Lyophilization	(Vincent et al. 1997; Monn and Becker 1999; Baulig et al. 2004; Geng et al. 2006)
		Desiccator	(Watterson et al. 2007; Gualtieri et al. 2012; Longhin et al. 2013)
		Vacuum Centrifuge	(Huang et al. 2014)
		Dilution	(Schaumann et al. 2004)
		Vacuum and Desiccator	(Valavanidis et al. 2005)
	Methanol	Rotary Evaporator	(Jalava et al. 2006; Gerlofs-Nijland et al. 2007; Jalava et al. 2009; Happo et al. 2010; Verma et al. 2012; Happo et al. 2013; Janssen et al. 2014)
	Toxicology Media	N/A	(Long et al. 2001; Akhtar et al. 2010; Akhtar et al. 2014; Kumar et al. 2015)
	PBS	Lyophilization	(Choi et al. 2004)
Probe Sonication			
	Methanol	N ₂ blow down	(Mudway 2004; Godri et al. 2011)
	Toxicology Media	N/A	(Imrich et al. 2000; Ning et al. 2000)
Soxhlet Extraction			
	DCM	Evaporation	(de Kok et al. 2005)
	And Methanol	Evaporation	(Skarek et al. 2007)
	And Water	Rotary Evaporator	(Cavanagh et al. 2009)
Agitation			
	Water	Lyophilization	(Dye et al. 2001)

Removal method, solvent type, concentration, and authors are listed for toxicological assessments of ambient PM_{2.5} using filter extraction.

Variation in extraction techniques between research groups creates a potential for bias, where findings may be dependent on the extraction procedures used rather than on the characteristics of ambient material (Bein and Wexler 2014). Well-characterized extraction solutions would avoid these biases as the exact concentration and composition of PM_{2.5} used would be known, enabling a more accurate interpretation of exposure studies. Thus far, a limited number of toxicology studies using ambient PM_{2.5} have reported chemical characterization of both metals and organics in extraction solutions (Lauer et al. 2009; Verma et al. 2012; Huang et al. 2014). Here a novel method was developed to measure compositional differences in PM_{2.5} between collected ambient material and the corresponding extraction solutions.

2.3 METHODS

2.3.1 PM_{2.5} Collection

2.3.1.1 Sampling Locations

In winter 2014, PM_{2.5} samples were collected in Pittsburgh, PA at five sampling locations throughout the downtown area including a regional background location in a park 14 km upwind of the downtown area.

2.3.1.2 Sampling Methods

Portable ambient air samplers were deployed approximately 3 m above ground level on metal utility poles and ran for 7 consecutive days at each sampling location. Samplers were enclosed in waterproof cases and equipped with 2.5 µm size-selective Harvard Impactors (HIs)

with 37 mm Teflon™ (PTFE) filters (Pall Corporation, Ann Arbor, MI) or cyclone adapted HIs (Air Diagnostics and Engineering Inc., Harrison, ME) with 37 mm quartz filters (Pall Corporation, Ann Arbor, MI). Vacuum pumps (model PCXR4, SKC Inc., Eighty Four, PA) were calibrated to 4 liters per minute air flow rate (Matte et al. 2013). Quartz filters were pre-baked at 900 °C for 4 h to remove trace organic material.

Four samplers were co-located at each sampling location to provide equivalent samples for ambient characterization as well as for extraction. For the quantification and characterization of ambient material, two samplers per location collected PM_{2.5} on either a PTFE or a quartz filter. For extraction of ambient material into solution, two samplers per location collected PM_{2.5} on PTFE filters.

2.3.1.3 Ambient PM_{2.5} Characterization

PTFE filters were used to determine PM_{2.5} concentrations through gravimetric analysis of filters pre- and post-sampling. Total PM_{2.5} mass was measured on an ultra-microbalance (model XP2U, Mettler Toledo, Columbus, OH) following a 48 h equilibration in a temperature and humidity controlled chamber (20.0 °C and 35% humidity).

Ambient compositional analysis by X-ray fluorescence (XRF) of metals and by thermal desorption gas chromatography mass spectrometry (TD-GC-MS) of organics was performed on PTFE and quartz filters, respectively, at Desert Research Institutes, DRI (Reno, NV). Metals (n=51) and organics (n=34) analyzed are shown in Table 2. Compounds analyzed included 14 of the 16 EPA Priority polycyclic aromatic hydrocarbons (PAHs).

Table 2: Metals and Organics Analyzed

Metals				Organics	
Ag	Cu	Na	Sn	1 methyl phenanthrene	dibenzo[a,h]anthracene
Al	Eu	Nb	Sr	2 methyl phenanthrene	dibenzothiophene
As	Fe	Ni	Ta	9-fluorenone	fluoranthene
Au	Ga	P	Tb	acenaphthene	fluorene
Ba	Hf	Pb	Ti	acenaphthylene	hopanes (n=10)
Br	Hg	Pd	Tl	benzo[a]anthracene	indeno[1,2,3-cd]pyrene
Ca	In	Rb	U	benzo[a]pyrene	phenanthrene
Cd	Ir	S	V	benzo[b]fluoranthene	pyrene
Ce	K	Sb	W	benzo[e]pyrene	steranes (n=4)
Cl	La	Sc	Y	benzo(ghi)fluoranthene	
Co	Mg	Se	Zn	benzo[ghi]perylene	
Cr	Mn	Si	Zr	benzo(jk)fluoranthene	
Cs	Mo	Sm		chrysene	

Metals (n=51) analyzed by X-ray fluorescence and organics (n=34) analyzed by thermal desorption gas chromatography mass spectrometry. All compounds were measured in both ambient and extraction solution samples.

2.3.2 PM_{2.5} Extraction

Refinement of the extraction methods used in this research is detailed in Appendix A.

2.3.2.1 Removal

Following sampling, PTFE filters collected for extraction (n=2 / sampling location) underwent gravimetric analysis, described above, to determine the total PM_{2.5} mass collected. Filters were then placed particle side down in 100 mL glass beakers containing a 9:1 solvent (methanol: sterile Milli-Q water) and sonicated for 2 min at 40 kHz in a waterbath sonicator (Branson Ultrasonics, Danbury, CT). Beakers were sufficiently wide to allow filters to lie flat, avoiding the need to cut filters into pieces. Cutting can intensify release of filter material during sonication, which creates difficulties in post-weighing of filters to determine removal mass. The extracts of the two filters collected from each location were pooled together (Baulig et al. 2004).

After sonication, filters and the beaker were rinsed with methanol to remove any residual particles and all rinses containing PM_{2.5} were stored in a closed 50 mL conical tube at -20 °C until concentration. PTFE filters were left to dry and equilibrate prior to gravimetric analysis for determination of the PM_{2.5} mass removed from each filter. Blank PTFE filters were prepared in the same manner as exposed filters to control for any loss of material throughout the removal process.

2.3.2.2 Concentration

PM_{2.5} suspended in the methanol solution were centrifuged (8,000 g, 15 min) prior to being frozen in liquid nitrogen and concentrated through lyophilization in a 4.5 L bench top freeze dryer (Labconco, Kansas City, MO). Dry concentrated PM_{2.5} samples were stored away from any light sources at -20 °C until further analysis.

2.3.2.3 Re-suspension

Concentrated dry PM_{2.5} samples were re-suspended in a set volume of serum-free Dulbecco's Modified Eagle Medium (DMEM) for future *in vitro* research. Samples were vigorously pipetted and vortexed to distribute PM_{2.5} throughout the media, then immediately prepared for PM_{2.5} characterization. Samples of PM_{2.5} that were removed from the filter, concentrated, and re-suspended in media (hereafter referred to as *extracted samples*) are the most accurate form of PM_{2.5} for characterization of samples used in toxicology research.

2.3.2.4 Extracted PM_{2.5} Characterization

Aliquots of extracted PM_{2.5} samples in DMEM were suspended onto pre-weighed PTFE and quartz filters to allow for gravimetric and chemical analyses comparable to those performed

for ambient samples. Due to the hydrophobic nature of PTFE filters, samples were mixed with methanol and then applied to the filters. PM_{2.5} in solution was left to dry on the filters and then filters were equilibrated for gravimetric analysis. PM_{2.5} mass was determined for extracted samples prior to characterization through XRF and TD-GC-MS analysis (Table 2). Expected masses of all constituents in extracted samples were calculated using the PM_{2.5} mass applied to the filter and ambient composition data. Filters suspended with DMEM-only were weighed and analyzed to allow for blank adjustment of samples due to mass and compositional components present in DMEM.

2.3.3 Statistical Analysis

Statistical analysis for all data was performed with StataSE 13 (StataCorp, LP, College Station, TX) and Prism 6.0 (GraphPad Software, Inc., San Diego, CA). All data were reported as a mean \pm standard deviation (SD). Pearson correlation coefficients were determined between PM_{2.5} mass and specific constituents. Data was analyzed using a one-way analysis of variance (ANOVA) with Bonferroni's test for multiple post-hoc comparisons where appropriate. Where ANOVA indicated significant differences and in all two-group comparisons, differences were investigated using Student's t-test. Differences with p-values < 0.05 were considered significant.

2.4 RESULTS

2.4.1 Sampling Location Differences

Masses for $PM_{2.5}$, metals, and organics were determined for each of the five locations. $PM_{2.5}$ mass was determined at three stages: 1) ambient material collected over the sampling period (“ PM_{amb} ”) 2) recovered material measured based on pre- and post-removal filter weights (“ PM_{rem} ”) and 3) recovered material concentrated and re-suspended into DMEM (“ PM_{ext} ”). Metals and organics masses were determined at stage 1 (“ $metals_{amb}$, $organics_{amb}$ ”) and 3 (“ $metals_{ext}$, $organics_{ext}$ ”). Ambient mass collected varied between sampling sites, but trends between PM_{amb} , PM_{rem} , and PM_{ext} were similar across locations (Figure 2).

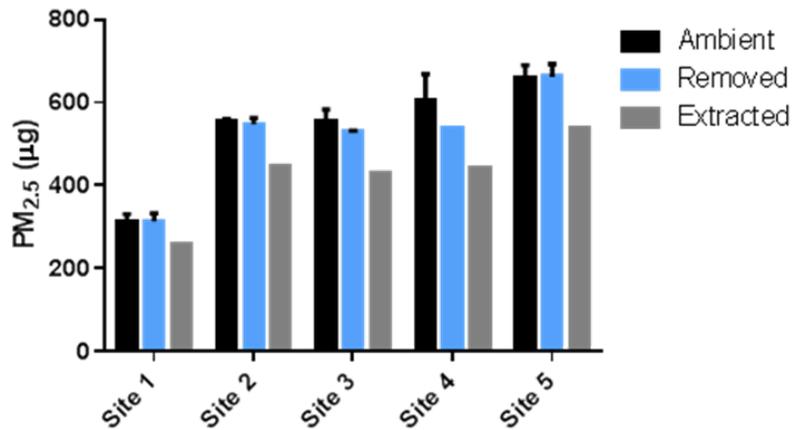


Figure 2: $PM_{2.5}$ across Sampling Sites

Ambient mass, mass following removal from filter via sonication (“removed”), and mass following re-suspension in cell culture media (“extracted”) are displayed for each sampling location in μg ($n=2/\text{site}$ for ambient and removed samples – except for site 4 ($n=1$) due to equipment failure during collection and $n=1/\text{site}$ for extracted samples). Sampling sites are ordered from lowest to highest (1 to 5) ambient $PM_{2.5}$ mass. Data are expressed as means \pm SD.

Metals_{amb} and metals_{ext} (Figure 3A) were calculated by summing the masses of all species analyzed. Metals_{amb} did not correspond to PM_{amb}, yet the highest PM_{amb} sampling location also had the highest metals_{amb}. Metals_{amb} and metals_{ext} also did not trend together indicating that extraction differences between sampling sites impacted metals_{ext}. Interestingly, the lowest metals_{ext} was observed at the location with the highest PM_{amb} and metals_{amb}.

Organics_{amb} and organics_{ext} were quantified by summing all species analyzed and variability was observed between all locations (Figure 3B). Similarly to ambient metals, organics_{amb} did not trend with PM_{amb}. However, unlike metals, the location with the highest organics_{amb} was not from the sampling location with the highest PM_{amb}. All organics_{ext} were less than organics_{amb} and varied between sampling locations independent of PM_{amb}. As with metals_{ext}, the effect of extraction differences between sampling locations was observed in organics_{ext}.

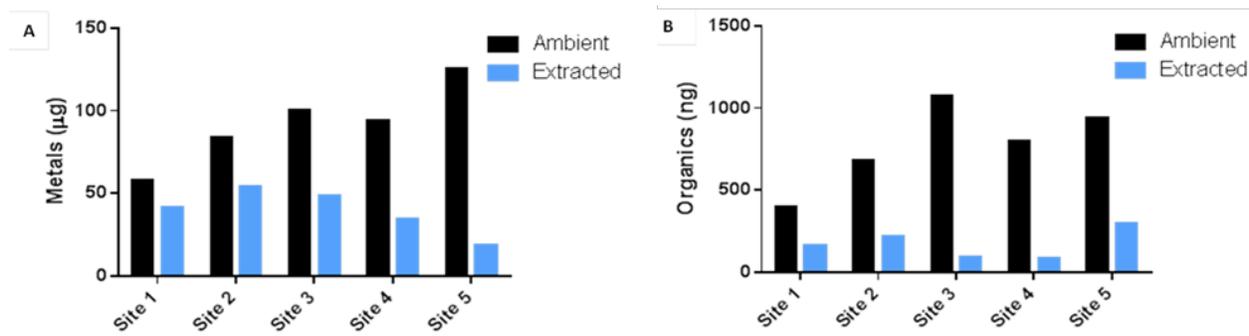


Figure 3: Ambient and Extracted Masses for PM_{2.5} Components

A. Total mass of metals (µg) in ambient samples and corresponding extraction solutions at each sampling site. **B.** Total mass of organics (ng) in ambient samples and corresponding extraction solutions at each sampling site. Site numbering is representative of total ambient PM_{2.5} mass ordering of low to high (1 to 5). Constituents comprising total metals and organics are shown in Table 2.

2.4.2 Extraction Efficiency

Ambient masses of PM_{2.5}, metals, and organics were compared to extracted masses to determine the percent extracted. Removal of total PM_{2.5} was $98.0 \pm 1.4\%$ following sonication; however, extraction efficiencies following concentration and re-suspension in DMEM were found to be substantially lower at $80.2 \pm .8\%$ of PM_{amb} (Figure 4).

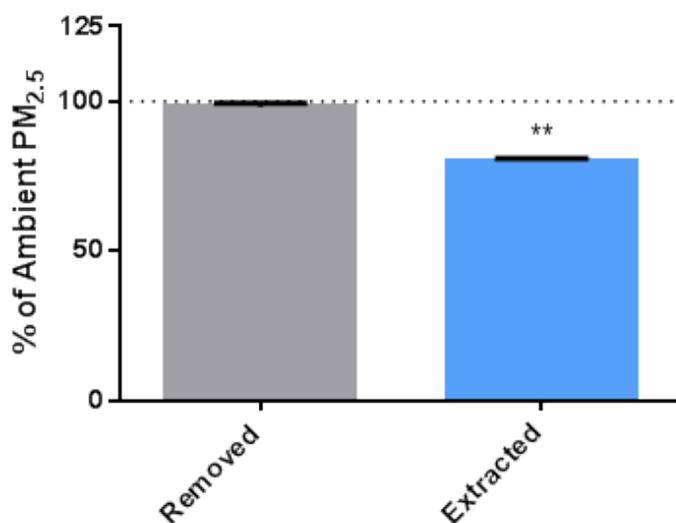


Figure 4: PM_{2.5} Mass Following Removal and Extraction

Mass following removal of PM_{2.5} from filter via sonication and following complete extraction (concentration and re-suspension in cell media) are displayed relative to total ambient PM_{2.5} mass for all sampling locations (n=5). Data are expressed as means \pm SD; **p-value <.001 indicating a statistically significant difference between groups.

Overall extraction efficiency for metals (Table 3) was $47.2 \pm 22.3\%$, with extraction efficiencies for specific metals ranging from 0.7 (for Ce) to 73.4% (for Na). High variability in extraction efficiency was also observed for specific metals between sampling locations (i.e., SD \pm 35.8% for Ni). All averaged extraction efficiencies were less than 100% removal except for three trace metals that were present in DMEM (Ca, Mg, and P); these components were excluded

from calculations of total metals. Contributions to total ambient metals for Ca, Mg, and P were 2.2, 1.1, and 0.0%, respectively.

Extraction efficiencies for organics (Table 3) are displayed for the five compounds detected in both ambient and extracted samples: 1-methyl phenanthrene (1MP), acenaphthylene (Acy), benzo[b]fluoranthene (BbFl), benzo[ghi]perylene (BghiPer), and indeno[1,2,3-cd]pyrene (Ipyr). Variability of efficiency was observed between species (Ipyr to 1MP: 17.0 – 101.5%) as well as between sampling locations for individual species (i.e., BghiPer SD \pm 43.9%). Extraction efficiency for total organics measured was $24.8 \pm 14.5\%$. All hopanes (n=10), steranes (n=4), and other organic compounds (n=15) measured were found at varying concentrations in ambient samples but were below the limit of detection in all extraction solutions, suggesting near complete loss during the extraction process.

Table 3: Extraction Efficiencies

Component		Percent Extracted	SD
PM _{2.5}	Removed	98.0	1.4
	Extracted	80.2	0.8
Metals	Al	40.0	54.8
	Ca	570.4	583.3
	Cd	9.0	20.0
	Ce	27.0	60.3
	Cl	7.9	17.8
	Cr	4.8	10.7
	Cs	10.4	23.3
	Cu	21.4	7.7
	Fe	28.8	9.5
	Mg	227.8	338.5
	Mn	20.7	8.7
	Mo	15.1	12.1
	Na	73.4	49.3
	Ni	26.9	35.8
	P	17128.8	8604.4
	Pb	16.8	11.5
	S	0.7	1.6
	Sn	20.3	45.3
	Sr	3.6	5.1
Zn	6.2	11.7	
Total	47.2	22.3	
Organics	1MP	101.5	23.1
	Acy	31.7	20.9
	BbFl	20.7	19.3
	BghiPer	98.9	43.9
	lpyr	17.0	38.0
	Total	24.8	14.5

Percent extracted with SDs for total PM_{2.5} mass following removal and extraction as well as extracted PM_{2.5} components (metals and organics).

Expected masses for constituents were calculated for extraction samples based upon PM_{2.5} mass and ambient composition. The expected values were compared to actual masses recorded through analysis of extraction solution (Table 4 and Table 5).

Table 4: Expected vs. Actual Metals of Extraction Solution

	Expected (μg)	Actual (μg)
Al	1.3965	0.3545
Ca	1.5980	8.7968
Cd	0.0074	0.0005
Ce	0.1141	0.0379
Cl	15.5670	1.3850
Cr	0.0499	0.0034
Cs	0.0054	0.0028
Cu	0.1621	0.0410
Fe	5.7970	2.0322
Mg	0.7899	1.1810
Mn	0.4285	0.1080
Mo	0.0766	0.0145
Na	21.7918	19.2882
Ni	0.0172	0.0059
P	0.0000	1.7684
Pb	0.1644	0.0340
S	20.3513	0.1847
Sn	0.0065	0.0048
Sr	0.0223	0.0008
Y	0.0066	0.0001
Zn	1.0115	0.0930
Total	69.3641	35.3377

Average expected and actual metals (μg) on filters. Expected metals were calculated using total $\text{PM}_{2.5}$ mass applied to the filter and ambient composition data. Actual values were determined from XRF of extracted solutions.

Table 5: Expected vs. Actual Organics of Extraction Solution

	Expected (ng)	Actual (ng)
Acy	132.9485	36.5572
Ace	0.1473	0.0000
F	0.1520	0.0000
P	2.7877	0.0000
Flu	2.9187	0.0000
Pyr	1.9161	0.0000
9Flo	0.5447	0.0000
DBT	0.0292	0.0000
1MP	0.3019	0.2998
2MP	0.3923	0.0000
Chr	4.6438	0.0000
BbFl	4.0059	0.9423
BjkFl	6.7336	0.0000
BaAnt	2.3504	0.0000
BePyr	2.3839	0.0000
BaPyr	1.6650	0.0000
Ipyr	1.7039	0.2270
DBahAnt	0.2353	0.0000
BghiPer	7.1982	6.8868
BghiFl	0.9941	0.0000
Hopanes	1.9904	0.0000
Steranes	0.1141	0.0000
Total	176.1569	44.9131

Average expected and actual organics (ng) on filters. Expected organics were calculated using total PM_{2.5} mass applied to the filter and ambient composition data. Actual values were determined with TD-GC-MS of extracted samples. Organics analyzed were hopanes (n=10), steranes (n=4), and PAHs (n=20) - abbreviated: acenaphthylene (Acy), acenaphthene (Ace), fluorene (F), phenanthrene (P), fluoranthene (Flu), pyrene (Pyr), 9-fluorenone (9Flo), dibenzothiophene (DBT), 1 methyl phenanthrene (1MP), 2 methyl phenanthrene (2MP), chrysene (Chr), benzo[b]fluoranthene (BbFl), benzo(jk)fluoranthene (BjkFl), benzo[a]anthracene (BaAnt), benzo[e]pyrene (BePyr), benzo[a]pyrene (BaPyr), indeno[1,2,3-cd]pyrene (Ipyr), dibenzo[a,h]anthracene (DBahAnt), benzo[ghi]perylene (BghiPer), and benzo(ghi)fluoranthene (BghiFl).

2.4.3 Relationship of Constituents to Total PM_{2.5}

The relative contribution of measured metals and organics, as a percent of PM_{2.5} mass, was determined for both ambient and extracted samples (Figure 5). In ambient samples, the

contribution of metals to PM_{amb} was 120 times higher than the contribution of organics. However, in extracted samples, the contribution of metals increased to over 705 times higher than organics.

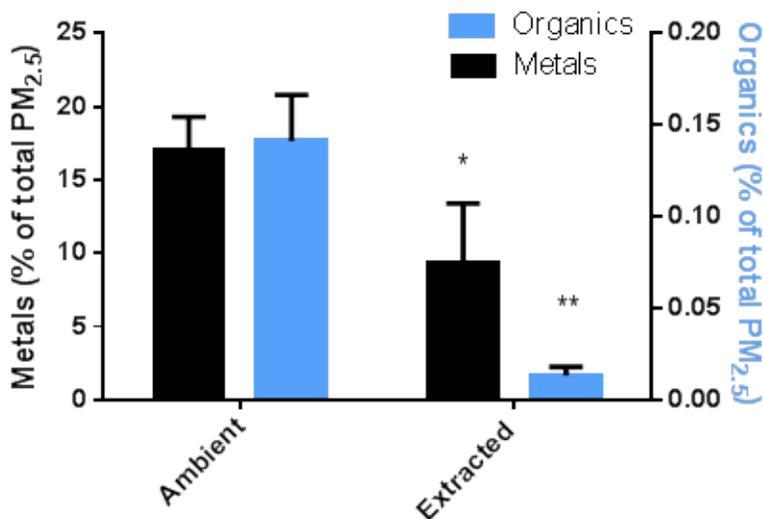


Figure 5: Contribution to Total $PM_{2.5}$ Mass

Percent contribution to $PM_{2.5}$ mass for total metals and organics (sum of constituents listed in Table 2) in ambient samples and extraction solutions. Left y-axis represents percent contribution of metals and the right y-axis indicates organics contribution. Data are expressed as means \pm SD; * p-value $<.05$ and **p-value $<.001$, indicating a statistically significant difference between ambient and extracted samples.

2.4.4 Correlations

2.4.4.1 Ambient to Extracted

Correlations of $PM_{2.5}$ to metal and organic components were calculated to compare how $PM_{2.5}$ mass and constituents were related for both ambient and extracted samples (Table 6). Statistically significant positive correlations were observed between PM_{amb} and Fe and Zn, while none of the extracted constituents exhibited a statistically significant correlation with PM_{ext} . Marked differences were observed between ambient and extracted correlations of constituents to

PM_{2.5} mass (reported as ambient : extracted) for metals (0.911 : -0.219), organics (0.805 : -0.083), and a number of specific constituents including Al (0.967 : -0.299), Cr (0.725 : -0.350), Zn (0.975 : -0.166), and Acy (0.734 : -0.243).

To determine how specific components of PM_{2.5} related between ambient and extracted samples, correlation coefficients were determined for each component (Table 6). Several extracted constituent values were negatively correlated with ambient measurements of the same constituent, including: total metals, total organics, and specific components such as Al, S, and Sr. Positive correlations were observed for a number of components including: Cs, Fe, Mn, Sn, 1MP, and BbFl. Statistically significant positive correlations between ambient and extracted values were present for PM_{2.5} mass and Tb.

2.4.4.2 Extraction Percent to Ambient Characteristics

Correlations between calculated extraction percentages of specific components to total PM_{2.5} were made to investigate potential trends in extraction efficiency based upon PM_{amb} (Table 6). The percent of total mass extracted was significantly positively correlated with PM_{amb}. While both the percent of extracted metals and organics were negatively correlated with PM_{amb}. These correlations suggest that as ambient PM_{2.5} mass increases, the efficiency of extraction for total metals and organics decreases. Similar trends were seen in individual constituents including: Al, Cu, Fe, Pb, and Acy. However, not all components measured had negative correlations between extraction efficiency and total PM_{2.5}, these included: Cd, Cr, Sn, and BbFl.

Table 6: Correlations of PM_{2.5}

Component	Amb to Ext	PM _{2.5} mass to constituents			
		Amb	Ext	% Ext	
PM _{2.5}	.975*			-.273	
Metals	Al	-.203	.967	-.299	-.717
	Ca	.005	.925	-.530	-.836
	Cd	.459	.426	.350	.513
	Ce	.218	-.322	-.215	.068
	Cl	.079	.931	-.215	.068
	Cr	.189	.725	-.350	.513
	Cs	.696	.068	-.003	.335
	Cu	.554	.947	.263	-.744
	Fe	.708	.993*	.502	-.571
	Mg	.115	-.005	-.808	-.125
	Mn	.571	.940	.474	-.175
	Mo	-.003	.507	-.744	-.590
	Na	-.639	.707	.051	.033
	Ni	.045	.951	.048	.184
	P	N/A	N/A	-.098	-.928
	Pb	.504	.736	.213	-.643
	S	-.189	.858	-.215	.068
Sn	.809	-.081	.350	.513	
Sr	-.427	.809	-.555	-.528	
Tb	1.000*	.079	.635	.079	
Zn	.312	.975*	-.166	.108	
Total	-.610	.911	-.219	-.815	
Organics	1MP	.752	.365	.812	-.123
	Acy	-.231	.734	-.243	-.616
	BbFl	.570	.953	.670	.467
	BghiPer	.139	.630	.652	.279
	Ipyr	.078	.579	.635	.079
	Total	-.083	.805	-.083	-.522

Pearson's correlation coefficients are presented for total PM_{2.5} and constituents (metals and organics) between ambient ("amb") and extraction solution ("ext") samples as well as PM_{2.5} mass to: ambient values, extraction solution values, and percent extracted. * p-value <.05, indicating a statistically significant correlation.

2.5 DISCUSSION

2.5.1 PM_{2.5} Mass

The percent of mass removed via sonication was consistent across sampling locations, which is a characteristic necessary to avoid a methods bias. Less inter-filter variability was seen in removal

efficiency than has previously been reported, where efficiency ranged from 59 to 95% (Imrich et al. 2000). Increased consistency in removal efficiency in this research is likely due to more deliberate selection of solvents based on anticipated chemical characteristics of PM_{2.5} components, as well as refinement of sonication methods. Extraction protocols using water as the sole solvent are common, and while effective for removal of water soluble components and approximately 75% of PM_{2.5} mass, water is not effective for extraction of non-polar species, including many organics compounds (Hawthorne et al. 2000; Watterson et al. 2007; Longhin et al. 2013). It should be noted that a portion of studies neglect to report removal percentages, limiting inter-method comparisons. Based solely on mass removal from the filter, the methods outlined here maintained a high PM_{2.5} yield and were consistent between filters and sampling locations.

The significant positive correlation between ambient and extracted PM_{2.5} mass suggests that ambient mass loadings do not impact the extraction of total PM_{2.5}. Consistent extraction independent of mass makes the outlined methods translatable to many regions and sampling time-scales. The methods are also effective in reducing release of filter material into the extracted solutions, as no significant loss of mass was observed with blank sonicated filters. The lack of observed fiber loss is likely due to a combination of the filter type used and decreased time and intensity of sonication. Previous methods have utilized potentially destructive probe sonication or extended sonication times, which can necessitate filtering of samples to remove fibers, but also introduces a potential loss of PM_{2.5} (Godri et al. 2011; Riva et al. 2011; Huang et al. 2014; Van Winkle et al. 2015).

2.5.2 Components of PM_{2.5}

Differences in extraction efficiency between total PM_{2.5} and constituents of PM_{2.5} demonstrate a key limitation of filter extraction methods, discussed below. Importantly, a vast majority of the PAHs (15 of the 20 analyzed), hopanes, and steranes were not extracted at any of the locations. While loss in organics was not unexpected due to the volatility of the compounds (EPA 2014), quantifying the shift from ambient contributions is useful to establish differences from filter samples.

A number of individual components, total organics, and total metals were inversely related to corresponding ambient masses, a result of decreased extraction efficiencies as mass increased. Ideally, ambient and extracted components would be equally correlated to total mass, indicating that composition of extracted PM_{2.5} was similar to that of ambient PM_{2.5}. However, positive correlations in ambient samples alone suggest that the relative composition of the ambient material is changed during the extraction process. Additionally, as ambient PM_{2.5} mass increases, the percentage of metals and organics extracted decreases.

2.5.3 Loss between Removal and Concentration

Translating the removal solution into dry particulate material is an imperative step before re-suspension into cell culture media to create an extraction solution for toxicology experiments. In this study, this process created a significant loss of mass and presumably loss of compounds that were volatile or soluble in the removal solution (9:1 methanol in water). However, characterization was only performed following re-suspension in cell culture media; additional characterizations of the removal and extraction solutions could identify at what point in the

process the losses occurred. Better characterization of these losses is essential to accurate research, as extraction percentages are frequently reported as total mass removed from filter, without consideration of losses that occur during the subsequent preparation steps.

2.5.4 Impacts on Toxicology Applications

Studies using ambient PM_{2.5} extracted from filters are an integral component for assessing biological impacts of particulate matter both *in vitro* and *in vivo*. In some cases, responses are correlated to ambient concentrations, without regard for changes that occur during the extraction process (de Kok et al. 2005). The shift in relative contributions of specific components to total PM_{2.5} demonstrates that the resultant extraction solution in this work is not representative of the ambient mixtures. Recently, different extraction methods were found to result in distinct biological impacts (Van Winkle et al. 2015). Identifying the specific components of PM_{2.5} that are not representatively extracted by protocols can suggest mechanisms responsible for the varying biological responses. In this work we identified the loss of numerous health-relevant compounds including: Cr, Fe, Ni, Pb, Zn and 10 of the 16 EPA Priority PAHs (Chen and Lippmann 2009). There is a need to further understand what effects these losses have on subsequent toxicology analyses.

Extraction efficiencies of components of PM_{2.5} were shown in this research to vary between sampling locations. Similar investigations found that extraction efficiencies were influenced by the source mixture and therefore composition of PM_{2.5} (Bein and Wexler 2014). These findings are particularly important for studies examining multiple sampling locations or the impacts of mixed sources. With inconsistent extraction efficiency, the variation of ambient samples will be obscured or lost, and toxicology results will not be representative of the actual

exposure of interest. An additional concern is the finding that extraction efficiencies of metals and organics are inversely related to total PM_{2.5} loadings. High mass loadings are necessary in toxicology studies to provide adequate material for exposures, but it is ineffective to collect such loadings when they decrease the yield of metal and organic species.

2.5.5 Limitations

In this study, extraction was performed on samples with spatially varying ambient PM_{2.5} concentration and composition. The impacts of temporal and seasonal variation in PM_{2.5} composition on extraction efficiencies were not investigated here, but future research in these areas would strengthen the correspondence to studies using temporally variant ambient samples. Furthermore, results from this study are only generalizable to the methods utilized, and efficiencies will differ based upon the extraction procedures employed (Bein and Wexler 2015). In this study, PM_{2.5} was re-suspended into cell culture media; to accurately determine how composition may be impacted by resuspension in different toxicology medium (ie. saline, PBS, or water) further studies should be conducted. However, based on this and previous works, it is clear that complete extraction has not been achieved using any current methodology; therefore, extraction solutions will differ compositionally from ambient source material, and the issues highlighted by this extraction protocol are likewise of concern with other methods (Happo et al. 2010; Akhtar et al. 2014).

Characterization of PM_{2.5} was performed only on ambient material and final extraction solutions; thus, changes in composition during the intermediate stages are unknown. Identifying specific steps in extraction procedure that are most impactful on the recovery of PM_{2.5} components would help to establish refined procedures that maintain ambient compositions.

While this work has begun to uncover compositional differences, it examined only a subset of the key components of PM_{2.5} other substantial contributors to mass including inorganic ions and total elemental and organic carbon would further elucidate the compositional changes following extraction.

2.6 CONCLUSION

This research has outlined a method for the extraction of PM_{2.5} from filter samples, which was effective in high mass recovery while maintaining filter integrity. Comparison of ambient and extracted samples suggests that the method was more effective in recovering metals in the extraction solution compared to organics. To the authors' knowledge, only one study has performed a well-characterized extraction solution analysis, and this research highlighted the variance in extraction of components of PM_{2.5} based on the extraction procedures implemented (Bein and Wexler 2015). However, this current work is the first to compare components measured in PM_{2.5} filter extract with those measured on collocated ambient filters from multiple sampling locations. The narrow understanding of alteration to PM_{2.5} composition as a result of extraction is a limitation that persists throughout a vast majority of toxicology research using PM_{2.5} collected on filters. Further awareness of the underlying mechanisms for the observed compositional shifts, in addition to the adoption of standardized extraction techniques that more efficiently extract all components of PM_{2.5}, would allow for biological impact studies that are more readily translatable to ambient exposures, and would facilitate comparisons between studies.

3.0 IMPACT OF ESTABLISHED DIFFERENCES BETWEEN AMBIENT AND CORRESPONDING EXTRACTED PM_{2.5} ON INTERPRETATION OF INFLAMMATORY RESPONSES

Adapted from a publication submitted to the journal, Atmospheric Environment

3.1 ABSTRACT

Filter-based toxicology studies have been conducted to establish the biological plausibility of the well-established health impacts associated with fine particulate matter (PM_{2.5}) exposure. Ambient PM_{2.5} collected on filters is extracted into solution for toxicology applications but frequently characterization is nonexistent or only performed on filter-based PM_{2.5}, without consideration of compositional differences that occur during the extraction processes. To date, the impact of making associations to measured components in ambient instead of extracted PM_{2.5} has not been investigated. Filter-based PM_{2.5} was collected at locations (n=5) with each sample containing sufficient material to allow for detailed characterization of both ambient and extracted PM_{2.5}. Alveolar macrophages (AMJ2-C11) were exposed (3, 24, and 48h) to PM_{2.5} and the pro-inflammatory cytokine interleukin (IL)-6 was measured. IL-6 release differed significantly between PM_{2.5} collected from different locations in an urban area; surprisingly, IL-6 release was found to be highest following treatment with PM_{2.5} from the lowest ambient concentration

location. IL-6 was negatively correlated with the sum of ambient metals analyzed, as well as with concentrations of specific constituents which have been previously associated with respiratory health effects. However, positive correlations of IL-6 with extracted concentrations indicated that the negative associations between IL-6 and ambient concentrations do not accurately represent the relationship between inflammation and PM_{2.5} exposure. Additionally, seven organic compounds had significant associations with IL-6 release when ambient concentrations were considered, but were not detected in the extracted solution at all. Basing inflammatory associations on ambient concentrations that are not necessarily representative of *in vitro* exposures creates misleading results, and this study highlights the importance of characterizing extraction solutions to conduct accurate health impact research.

3.2 INTRODUCTION

Exposure to ambient fine particulate matter (PM_{2.5}) has been associated with morbidity and mortality related to respiratory inflammation (Abbey et al. 1995; Dominici et al. 2006; Tecer et al. 2008; Ostro et al. 2009). Associations have also been found between adverse respiratory health outcomes and specific constituents of PM_{2.5}, such as metallic and organic species, independent of total PM_{2.5} concentration (Mar et al. 2000; Ostro et al. 2007; Ostro et al. 2009). To identify the mechanisms of ambient PM_{2.5}-induced respiratory illnesses and establish the biological plausibility of epidemiological findings, toxicology studies have employed filter-based collection of ambient PM_{2.5} (Choi et al. 2004; Sawyer et al. 2010; Kumar et al. 2015).

Through the collection of ambient PM_{2.5} on filters, toxicology studies can be conducted to identify associations between PM_{2.5} components and biological outcomes. This practice can

identify specific components that are most impactful through investigation of the genotoxicity (Dellinger et al. 2001; Topinka et al. 2011; Wang et al. 2011), mutagenicity (de Kok et al. 2005; Skarek et al. 2007; de Brito et al. 2013), oxidative potential (Godri et al. 2011; Gualtieri et al. 2012; Janssen et al. 2014), and inflammatory impacts (Happo et al. 2010; Riva et al. 2011; Akhtar et al. 2014; Kumar et al. 2015). In these previous studies, the complex mixture of PM_{2.5} present on the ambient filters was characterized and associations were made to the biological responses observed. The practice of basing association of components present on the ambient filter and not on the extraction solution, disregards compositional changes that may occur during the extraction process to prepare filter-based PM_{2.5} for toxicology research.

Recently, complications of extraction have been demonstrated, where total PM_{2.5} mass extracted can remain high (80%), while less efficient extraction and even complete loss of health-relevant components of PM_{2.5} (i.e. benzo[a]pyrene, Ni, pyrene) can occur (Roper et al. 2015). Extraction efficiencies can vary based upon the extraction method implemented, as well as the characteristics of collected PM_{2.5} (i.e. source contributions, mass loadings) (Bein and Wexler 2014; Roper et al. 2015). The lack of a standardized extraction protocol is of further concern, as it results in methods that vary between research groups and further inhibit cross-study comparisons.

While growing research has begun to characterize extracted PM_{2.5} components for associations to oxidative potential (Verma et al. 2012), genotoxic (Danielsen et al. 2011), mutagenic (Cavanagh et al. 2009), and inflammatory (Van Winkle et al. 2015) outcomes, associations are still routinely made to ambient values. Additionally, the resultant discrepancies in associations between using ambient or extracted values has yet to be evaluated. Enhanced understanding of how ambient samples translate to extracted solutions – and how this can impact

interpretation of biological outcomes – will ultimately enable the identification of the causal components of PM_{2.5}-associated health effects.

In this study, the release of the pro-inflammatory cytokine, interleukin (IL)-6, was assessed in alveolar macrophages (AM) following exposure to PM_{2.5} samples that were extracted from ambient filters. Importantly, this study was designed to establish the need for well-characterized extraction solutions when identifying causal components. Associations between IL-6 release and PM_{2.5} were made using both ambient and corresponding extracted PM_{2.5} measurements to determine if findings were dependent on the stage at which PM_{2.5} was characterized.

3.3 METHODS

3.3.1 PM_{2.5} Collection

Sampling and extraction methods have been described in detail previously (Roper et al. 2015). Briefly, portable ambient air samplers were deployed approximately 3 m above ground level on metal utility poles for 7 consecutive days. Four sampling locations were distributed throughout downtown Pittsburgh, PA, and one additional site was located in a park 14.5 km upwind of the urban area. Harvard Impactors (HI) and cyclone adapted HIs (Air Diagnostics and Engineering Inc., Harrison, ME) served as size-segregators, collecting PM_{2.5} on 37 mm Teflon™ (PTFE) filters and 37 mm quartz filters, respectively (Pall Corporation, Ann Arbor, MI). Four samplers were co-located at each location: two filters (one PTFE, one quartz) were collected for the

characterization of ambient PM_{2.5}, and two filters (both PTFE) were collected for extraction and subsequent toxicology research with *in vitro* exposures.

3.3.2 PM_{2.5} Extraction

Following sampling, PTFE filters collected for *in vitro* exposures underwent gravimetric analysis on an ultra-microbalance (model XP2U, Mettler Toledo, Columbus, OH) following a 48 h equilibration in a temperature and humidity controlled chamber (20.0 °C and 35% humidity). Filters were weighed pre- and post-sampling to determine ambient PM_{2.5} mass collected. Using two PTFE filters per sampling location to ensure adequate mass for exposures, samples were sonicated in a 9:1 solution of methanol in sterile Milli-Q water. The resultant PM_{2.5} suspensions were concentrated through lyophilization and stored at -20 °C. PTFE filters underwent gravimetric analysis following sonication to determine the mass removed from each filter. In preparation for *in vitro* analysis, PM_{2.5} from each sampling location was re-suspended in serum-free Dulbecco's Modified Eagle Medium (DMEM). In order to maintain both concentration and compositional differences between locations, PM_{2.5} from each sampling location was re-suspended in an equal volume of DMEM, which was the volume required in order to translate PM_{2.5} mass from the lowest ambient concentration to an extraction concentration of 70 µg/mL. This concentration has been previously established to induce the release of cytokines (Becker et al. 2003; Sawyer et al. 2010); all extracted sample concentrations remained lower than thresholds previously observed to induce cell death (Schins et al. 2002). Aliquots of extracted samples were re-deposited onto PTFE and quartz filters and were characterized according to the same procedures as ambient PM_{2.5}, described below.

3.3.3 PM_{2.5} Characterization

PM_{2.5} concentrations were calculated via gravimetric analysis of PTFE filters pre- and post-deployment. Ambient and extracted PM_{2.5} composition was determined via X-ray fluorescence (XRF) analysis of inorganic species (from PTFE filter) and thermal desorption gas chromatography mass spectrometry (TD-GC-MS) analysis of organics (from quartz filter), at Desert Research Institutes, DRI (Reno, NV). Inorganic species, referred to hereafter as “metals” (n=51) and organics (n=34) analyzed are listed in Table 7: List of Constituents Analyzed.

Table 7: List of Constituents Analyzed

Metals				Organics	
Ag	Cu	Na	Sn	1-methylphenanthrene (1MP)	dibenzo[a,h]anthracene (DBahA)
Al	Eu	Nb	Sr	2-methylphenanthrene (2MP)	dibenzothiophene (DBT)
As	Fe	Ni	Ta	9-fluorenone (9Flo)	fluoranthene (Flu)
Au	Ga	P	Tb	acenaphthene (Ace)	fluorine (F)
Ba	Hf	Pb	Ti	acenaphthylene (Acy)	hopanes (n=10)
Br	Hg	Pd	Tl	benzo[a]anthracene (BaAnt)	indeno[1,2,3-cd]pyrene (Ipyr)
Ca	In	Rb	U	benzo[a]pyrene (BaPyr)	phenanthrene (P)
Cd	Ir	S	V	benzo[b]fluoranthene (BbFl)	pyrene (Pyr)
Ce	K	Sb	W	benzo[e]pyrene (BePyr)	steranes (n=4)
Cl	La	Sc	Y	benzo(ghi)fluoranthene (BghiFl)	
Co	Mg	Se	Zn	benzo(ghi)perylene (BghiPer)	
Cr	Mn	Si	Zr	benzo(jk)fluoranthene (BjkFl)	
Cs	Mo	Sm		chrysene (Chr)	

3.3.4 *In Vitro* PM_{2.5} Exposure

Mouse alveolar macrophage cells, AMJ2-C11 (American Type Culture Collection (ATCC), Rockville, MD) were cultured in DMEM supplemented with 5% fetal bovine serum (FBS), 4 mM L-glutamine, 1.5 g / L sodium bicarbonate, 4.5 g/L glucose, 5 mM HEPES, 50 U/mL penicillin, and 50 µg/mL streptomycin, following the ATCC protocol.

Cells were seeded at 2.5×10^5 onto 6 well plates and underwent a 2 h starvation at 37°C in a 5%CO₂ / 95% air atmosphere. Immediately following starvation, all wells were exposed to PM_{2.5} suspended in serum-free DMEM or controls for 3, 24, or 48 h. Control cells received: media alone, media containing lipopolysaccharide (LPS) to control for potential endotoxin contamination (Tager et al. 2010), or media containing extracts of unexposed filters to control for filter material released during sonication. LPS contamination of PM_{2.5} samples was quantified using a chromogenic endotoxin quantitation kit (Pierce LAL Chromogenic Endotoxin Quantitation Kit, Thermo Scientific, Pittsburgh, PA) and LPS-control cells were treated with the highest LPS concentration detected from previous samples collected in Pittsburgh, PA (0.174 EU/mL). Duplicate wells for each sample/control were exposed at each time point.

3.3.5 Post-Exposure Analysis

Following exposure (3, 24, or 48h) plates were vigorously shaken and cell media was collected. Total cell counts (Z1 Coluter Particle Counter, Beckman Coulter Inc., Brea, CA) were recorded; media was then centrifuged at 14,000 g for 5 min. Supernatants were collected and stored at -80°C until analysis.

IL-6 concentrations were measured in duplicate for all cell supernatants following manufacturer's instructions for an enzyme-linked immunosorbent assay (ELISA) specific for mouse IL-6 (R&D Systems, Minneapolis, MN).

3.3.6 Statistical Analysis

Statistical analysis for all data was performed with StataSE 13 (StataCorp, LP, College Station, TX) and Prism 6.0 (GraphPad Software, Inc., San Diego, CA). All data is reported as a mean \pm standard error of the mean (SEM). Data obtained for IL-6 concentrations between treatments and controls was analyzed using a one-way analysis of variance (ANOVA) with Bonferroni's test for multiple post-hoc comparisons where appropriate. Pearson's correlation coefficients were calculated for IL-6 concentrations to both ambient and extracted components of PM_{2.5}. Differences with p-values < 0.05 were considered significant.

3.4 RESULTS

3.4.1 IL-6 Response Following PM_{2.5} Exposure

Sampling locations (Sites 1 to 5) were ordered lowest to highest with respect to ambient PM_{2.5} concentration, without consideration of composition or extracted concentration. IL-6 release from AMs following exposure to PM_{2.5} from each location was measured relative to media controls at 3, 24, and 48 h (Figure 6). Equipment failure during the sampling period resulted in reduced collection of PM_{2.5} at Site 4, and IL-6 was only evaluated at 3 h post exposure. IL-6 release following treatment with PM_{2.5} from sites with the highest ambient concentrations (Sites 3-5) was not significantly different from the control at any time point. In contrast, PM_{2.5} from the site with the lowest ambient concentration (Site 1) induced a significant increase in IL-6 release at 24 and 48 h. IL-6 release in response to PM_{2.5} from Site 2 was significantly higher than both

the control and PM_{2.5} from other sampling locations following 24 h of exposure. A significant prolonged release of IL-6 (up to 48 h) was only observed following exposure to PM_{2.5} from Site 1.

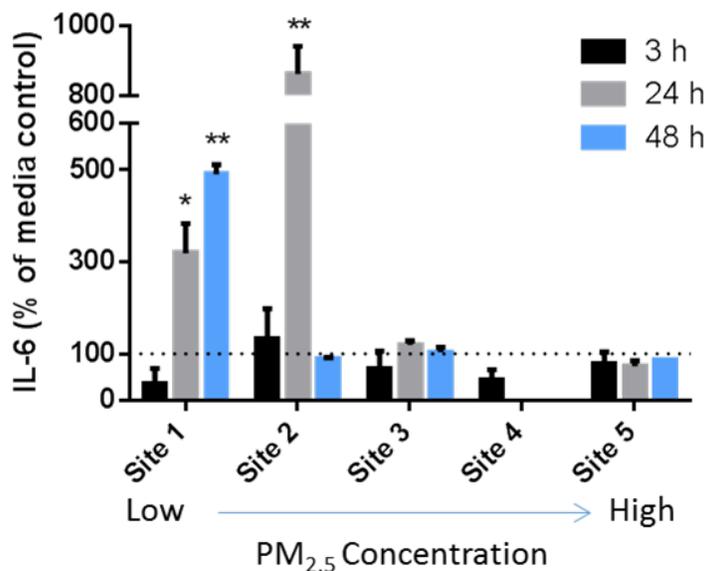


Figure 6: IL-6 Concentrations Following PM_{2.5} Exposure

IL-6 concentrations (% of media control) measured by ELISA method in AMJ2-C11 cell supernatants following PM_{2.5} exposure of 3, 24, and 48 h from varying ambient samples (n=2 / site and time). Sampling locations are ordered from low to high (1 to 5) ambient PM_{2.5} concentrations. Doses (µg/mL) for cell exposure were: 71.9 (Site 1), 125.1 (Site 2), 121.4 (Site 3), 122.6 (Site 4), 151.2 (Site 5). Results are presented as mean ± SEM with * and ** indicating statistically significant difference from control (p<.05 and .001, respectively). “x” indicates time points missing from site 4 due to equipment failure during ambient PM_{2.5} collection.

In order to determine the impact of ambient LPS present in PM_{2.5} on the release of IL-6, the percent change between LPS induced IL-6 and concentrations from cells treated with PM_{2.5} was calculated for each time point. At 3 h, all PM_{2.5} induced expression of IL-6 was below the response observed in the LPS treated cells (expression levels ranged between sampling locations from 18.3 to 78.8% below the LPS control). At 24 and 48 h following PM_{2.5}, all sampling

locations resulted in elevated levels of IL-6 compared to the LPS control (6.8 to 998.9% above at 24 h and 40.6 to 364.6% above at 48 h). The initial impact of LPS was evident in the pro-inflammatory release at 3 h but following a prolonged exposure (24 and 48 h), all PM_{2.5} samples induced expression of IL-6 above levels due to LPS treatment alone. Sonicated blank filters that acted as an extraction methods control did not show a significant difference in IL-6 release from media controls at any of the time points.

Total cells increased for all treatments and controls over time with no visible indication of cell death except for a duplicate of AMs exposed to PM_{2.5} from Site 2 for 48 h. This duplicate was observed to have a reduced cell count and visual signs of cell death while the complementary sample had consistent cell counts to all other groups, IL-6 was only measured in the Site 2 sample without reduced cell counts.

3.4.2 Ambient vs. Extracted Comparisons

Detailed comparisons of the composition of ambient and corresponding extracted PM_{2.5} have been previously reported (Roper et al. 2015) for the samples used in this study. Briefly, total PM_{2.5} mass recovery was high while a significant loss of metals and organics was observed for all sampling locations, with highest losses observed as ambient PM_{2.5} concentration increased. Ambient and extracted values for select metals (Table 8) and organics (Table 9) display the differences in contributions of components of PM_{2.5} following extraction from filters. Loss of the contribution to PM_{2.5} mass of components occurred across all sampling sites in the extracted samples compared to ambient samples for all components measured except: Al, Ce, Na, 1MP, Ipyr, and BghiFl. These six components had observed mass ratios that increased at one or more sampling locations following extraction.

Table 8: Ambient and Extracted Metals (ng / ug PM_{2.5}) for each Sampling Location

Site		Al	Cd	Ce	Cl	Cr	Cu	Fe	Mn	Na	Ni	Pb	S	Sr	Zn	Total
1	Amb	0.50	0.00	0.71	8.88	0.15	0.21	5.79	0.24	79.06	0.04	0.32	73.89	0.05	1.75	189.73
	Ext	0.60	0.00	0.00	0.00	0.00	0.07	2.49	0.06	43.78	0.00	0.10	0.00	0.00	0.00	161.18
2	Amb	2.93	0.00	0.32	38.92	0.11	0.44	15.53	0.99	48.49	0.04	0.39	45.53	0.05	2.75	151.71
	Ext	3.61	0.00	0.52	19.03	0.00	0.15	7.44	0.42	67.52	0.04	0.13	2.06	0.01	0.91	120.21
3	Amb	3.99	0.05	0.01	41.96	0.10	0.42	13.36	0.91	44.66	0.04	0.52	45.70	0.06	2.32	170.37
	Ext	0.00	0.00	0.00	0.00	0.00	0.10	5.37	0.25	57.30	0.01	0.13	0.00	0.00	0.06	111.37
4	Amb	3.65	0.00	0.17	28.50	0.14	0.32	13.68	1.37	41.11	0.04	0.28	39.37	0.04	2.32	144.26
	Ext	0.00	0.00	0.00	0.00	0.00	0.10	3.01	0.22	68.39	0.00	0.00	0.00	0.00	0.03	78.41
5	Amb	4.09	0.03	0.31	50.81	0.11	0.44	16.18	1.19	54.68	0.04	0.40	46.83	0.05	2.51	196.23
	Ext	0.00	0.00	0.00	0.00	0.03	0.06	4.71	0.25	0.00	0.01	0.06	0.00	0.00	0.03	35.38

Ambient (“Amb”) and extracted (“Ext”) metals (ng / ug PM_{2.5}) determined through gravimetric analysis of PM_{2.5} mass and metals characterization by XRF. “Total” refers to sum of all components analyzed (n=51).

Table 9: Ambient Organics (pg / ug PM_{2.5}) for each Sampling Location

Site		Acy	Pyr	1MP	Chr	BbFl	BjkFl	Ba-Ant	Be-Pyr	Ba-Pyr	lpyr	Bghi-Fl	Hop-anes	Total
1	Amb	955.96	20.61	3.83	31.54	30.81	69.50	13.93	16.55	11.16	11.52	54.06	3.95	1300.76
	Ext	658.20	0.00	5.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.48	0.00	641.26
2	Amb	784.48	25.81	2.56	49.39	33.94	65.41	24.86	25.62	18.74	18.69	73.68	20.33	1230.16
	Ext	430.50	0.00	2.67	0.00	14.69	0.00	0.00	0.00	0.00	0.00	89.79	0.00	489.91
3	Amb	1464.59	23.12	2.45	39.25	34.55	53.51	19.76	19.41	14.65	13.69	51.63	16.07	1827.10
	Ext	140.95	0.00	2.43	0.00	18.71	0.00	0.00	0.00	0.00	15.79	89.52	0.00	211.39
4	Amb	914.71	13.49	1.58	33.45	30.83	48.22	16.16	18.20	10.80	16.10	70.76	14.77	1231.76
	Ext	194.88	0.00	2.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	37.66	0.00	190.08
5	Amb	1189.79	0.00	2.36	32.47	31.32	43.89	18.24	16.02	11.38	8.90	42.13	19.42	1476.44
	Ext	489.52	0.00	2.82	0.00	10.55	0.00	0.00	0.00	0.00	0.00	74.54	0.00	548.96

Ambient (“Amb”) and extracted (“Ext”) metals (ng / ug PM_{2.5}) determined through gravimetric analysis of PM_{2.5} mass and organics characterization by TD-GC-MS. “Total” refers to sum of all organic components analyzed (n=34).

Additional information on ambient characterization data for all sampling locations is provided in Appendix B.

Release of IL-6 was correlated to both mass as well as specific constituents. The inflammatory marker was poorly correlated with total PM_{2.5} (<0.184) across all sampling locations at 3 and 24h, for both ambient and extracted concentrations. After 48h of exposure, IL-6 was negatively correlated with ambient (-.592) and extracted (-.581) concentrations (p=.043).

and .048, respectively). The increased IL-6 concentrations following exposure to PM_{2.5} from sampling Site 1, discussed above, are consistent with these results.

The trends between IL-6 release and sums of total metals (Table 10) and organics (Table 11) analyzed is reported for both ambient and extracted measurements at all time points. At 3 h, no correlation was observed for total metals or organics in either ambient or extracted samples. At 24 h, there was a negative correlation between total ambient metals and IL-6 concentrations ($p=.006$), whereas no such relationship was seen for total extracted metals. Ambient metals and organics were both observed to have a negative relationship with IL-6 release. Conversely, extracted metals and organics both showed a positive relationship with IL-6 release (Figure 7). At 48 h, total metals were slightly positively correlated to inflammation for both ambient and extracted measurements. Total organics correlations again differed, with a slight negative relationship with ambient and a slight positive relationship with extracted measurements.

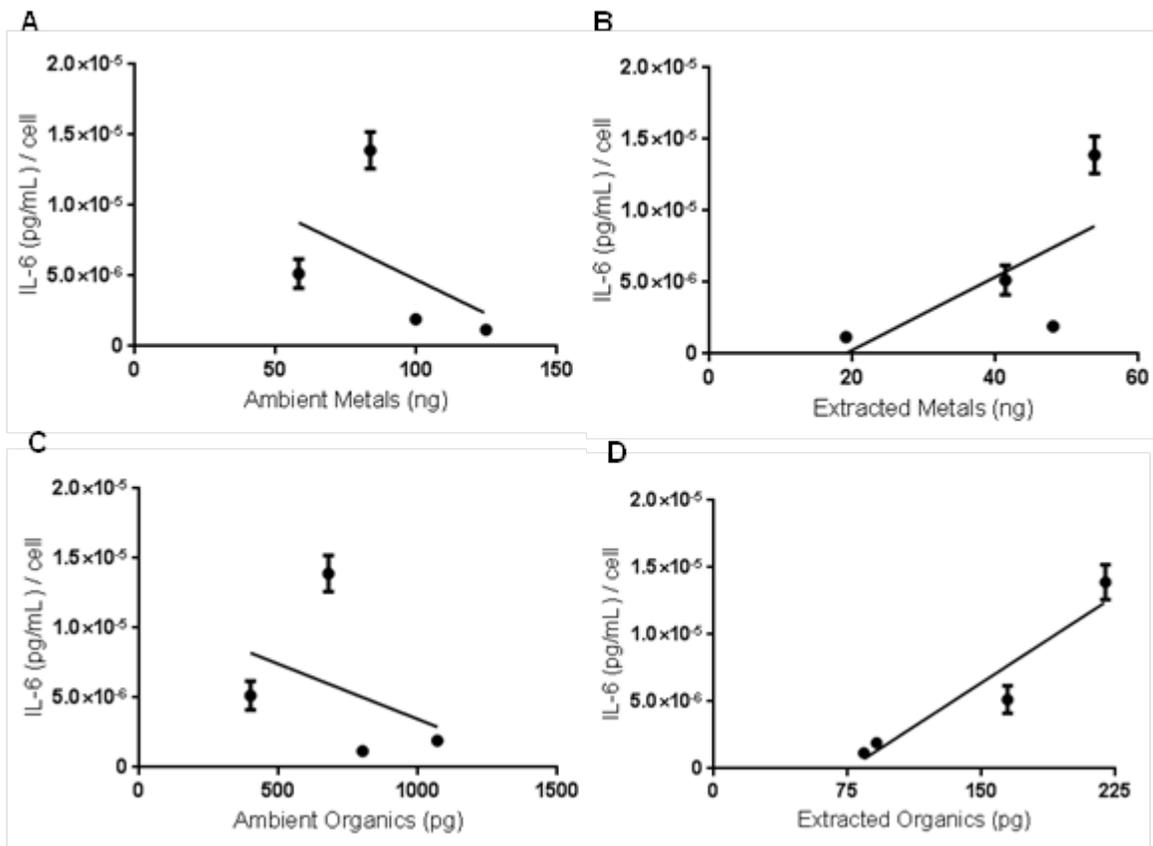


Figure 7: Scatterplots of IL-6 Release by Constituents

Scatterplots of IL-6 release from each sampling location by constituents following 24 h of $PM_{2.5}$ exposure. IL-6 [(pg/mL)/cell] release per total ambient (A) and extracted (B) metals (ng) or total ambient (C) and extracted (D) organics (pg). Totals refer to the sum of all constituents measured (Table 1), metals (n=51) and organics (n=34). Results are presented as mean \pm SEM (n=2 / site).

The correlations between IL-6 concentrations and the proportional mass of individual metals (ng / μg $PM_{2.5}$, Table 10) and organics (pg / μg $PM_{2.5}$, Table 11) were calculated for all sampling locations at each time point following exposure using both ambient and extracted values. Normalization to total $PM_{2.5}$ mass was utilized to eliminate the impact of differing doses between sampling locations (70 – 151 $\mu g/mL$).

At 3 h, specific extracted metals were positively related to IL-6 releases, including: Ce, Mn, Ni, and Zn and these relationships were not observed when using ambient measurements.

Interestingly, at 24 h, significant correlations between Ni and IL-6 were observed, with inverse relationships when using ambient (-.856, p=.002) and extracted (.857, p=.002) measurements. Positive relationships were present with extracted measurements of Al, Ce, Cl, Cs, S, Zn (p=<.001) and Cu, Fe, and Mn (p=.002, .045, and .049, respectively). These relationships were not observed in the ambient metals, with the exception of Cs (p=<.001). At 48 h, negative relationships of specific ambient metals were observed Al, Cl, Cu, Fe, Mn, and Zn (p=.045, .040, .035, .039, .040, and .050, respectively). Positive correlations were present for the extracted metals, Ca, Mg, Mo, and Y (p=.042, .035, .049, .036, respectively).

Table 10: Pearson’s Correlations of Metals to IL-6 Release

	3 h		24 h		48 h	
	Am	Ex	Am	Ex	Am	Ex
Al	.075	.385	-.016	.961 [▲]	-.586 [*]	-.091
Ca	.195	-.118	-.070	-.346	-.543	.594 [*]
Cd	-.057	.007	-.519	-.372	-.395	-.218
Ce	-.034	.404	-.025	.945 [▲]	.532	-.215
Cl	.231	.404	.068	.945 [▲]	-.598 [*]	-.215
Cr	-.226	.007	-.089	-.372	.574 [*]	-.218
Cs	.404	.239	.945 [*]	.891 [▲]	-.215	-.325
Cu	.323	.323	.242	.852 [*]	-.611 [*]	-.281
Fe	.282	.429	.269	.642 [*]	-.602 [*]	-.540
Mg	-.226	-.254	-.749 [*]	-.239	.465	.610 [*]
Mn	.072	.419	.158	.634 [*]	-.599 [*]	-.544
Mo	.035	-.223	.129	-.206	.547	.579 [*]
Na	-.121	.066	-.259	.603	.585 [*]	-.002
Ni	-.242	.437	-.856 [*]	.857 [*]	.378	-.381
Pb	.119	.245	-.133	.534	-.456	-.057
S	-.145	.404	-.246	.945 [▲]	.608 [*]	-.215
Sn	-.166	-.007	-.489	-.372	.482	-.218
Sr	-.010	.286	-.590	.739 [*]	-.147	.292
Y	.100	-.208	.299	-.226	.513	.609 [*]
Zn	.386	.409	.490	.934 [▲]	-.576 [*]	-.252
Total	-.123	-.015	-.796 [*]	.198	.323	.470

Correlations of IL-6 release at 3, 24, and 48 h to both ambient (Am) and extracted (Ex) metal constituents. “Total” refers to the sum of all metals (n=51) analyzed in Table 7. *, [▲] indicate a statistically significant correlation with p<.05 and <.001, respectively.

At 3 h, positive correlations were observed for individual ambient organic components: Ace, Chr, BaAnt, and BaPyr. However, none of these constituents were present in extracted samples; Acy, 1MP, BbFl, Ipyr, and BghiPer were the only organics detected following extraction. At 24 h, individual ambient organics had positive correlations with IL-6 including: Ace ($p < .001$), Flu ($p = .018$), Chr ($p = .003$), BaAnt ($p = .028$), BePyr ($p < .001$), BaPyr ($p = .004$), DBahA ($p = .022$), BghiPer ($p < .001$), and BghiFl ($p = .035$). These correlations were not observed for extracted organics, and a majority of the compounds were not even present in extracted samples. In contrast to ambient associations, extracted Ipyr had a slight negative association with IL-6. The negative association between IL-6 and ambient Acy was not observed in the extracted samples where nearly no correlation was observed. At 48 h, negative relationships were observed with ambient measurements of fluorine and hopanes ($p = .043$ and $.038$, respectively); while these compounds were not detected in the extracted samples and therefore no correlation was observed.

Table 11: Pearson’s Correlations of Organics to IL-6 Release

	3 h		24 h		48 h	
	Am	Ex	Am	Ex	Am	Ex
Acy	-.153	.064	-.678*	.001	-.266	.453
Ace	.404	-	.945 [▲]	-	-.215	-
F	.323	-	.279	-	-.590*	-
P	.158	-	.193	-	.516	-
Flu	.279	-	.722*	-	-.001	-
Pyr	.146	-	.514	-	.109	-
9Flo	-.161	-	-.236	-	.608*	-
DBT	-.063	-	-.295	-	-.267	-
1MP	-.008	-.215	-.145	-.220	.607*	.603*
2MP	.296	-	.536	-	-.502	-
Chr	.401	-	.825*	-	-.389	-
BbFl	.271	.280	.410	.232	-.447	-.557
BjkFl	.145	-	.412	-	.461	-
BaAnt	.432	-	.687*	-	-.519	-
BePyr	.381	-	.881 [▲]	-	-.317	-
BaPyr	.400	-	.814*	-	-.387	-
Ipyr	.235	-.063	.866*	-.295	-.211	-.267
DBahA	.218	-	.709*	-	-.406	-
BghiPer	.172	.329	.923 [▲]	.386	-.032	-.569
BghiFl	.369	-	.667*	-	-.528	-
Hopanes	.337	-	.335	-	-.603*	-
Steranes	.247	-	.203	-	-.509	-
Total	-.085	.124	-.533	.046	-.303	.405

Correlations of IL-6 release at 3, 24, and 48 h to both ambient (Am) and extracted (Ex) organic constituents. “Total” refers to the sum of all organics (n=34) analyzed in Table 1. – indicates a missing value due to the constituent not being present in the extracted solution. *, [▲] indicate a statistically significant correlation with $p < .05$ and $< .001$, respectively.

3.5 DISCUSSION

IL-6 release following exposure to ambient PM_{2.5} has been well-documented in cells involved in the respiratory inflammatory response: macrophages (Becker et al. 2005; Jalava et al. 2006), alveolar epithelial cells (Hetland et al. 2004; Shang et al. 2013), and bronchial epithelial cells (Watterson et al. 2007; Lauer et al. 2009). Our findings corroborate previous time-course

experiments in which significant release of IL-6 after PM_{2.5} exposure was observed at longer time points (more than 3 h) (Watterson et al. 2012). Interestingly, not all sampling locations induced release of IL-6 above controls and the locations which had significant increases in IL-6 were from sites with the lowest ambient concentrations. Based on previous epidemiology research (Ruckerl et al. 2006; Villeneuve et al. 2015), these locations would be hypothesized to induce the lowest response. While elevated release of a pro-inflammatory mediator with increasing ambient concentrations was not observed in this study, previous *in vitro* exposures have found similar results. IL-6 was not observed to have a significant increase compared to controls following exposure to PM_{2.5} from an urban sampling location with high vehicular traffic (Perrone et al. 2010). Findings of mutagenic effects of PM_{2.5} have been similarly unexpected: for example, Zhao et al. (2002) found that the sampling location selected as the “clean” control site, free of any major industrial sources, caused elevated mutagenic potency compared to sampling locations with vehicular and industrial sources. When interpreting these findings, an important factor to consider is that conclusions are drawn from ambient concentrations prior to extraction, and the differences in PM_{2.5} following extraction are a potential cause for discrepancies with epidemiology research.

The inconsistency of these results with current epidemiological findings was also observed for specific constituents of PM_{2.5}. Metals established to be associated with health effects including Ni, S, and Sr (Bernstein et al. 2004; Schwarze et al. 2006), had negative correlations with IL-6 expression when using ambient values but significant positive correlations when using extracted PM_{2.5} values, highlighting the potential for misinterpretation of biological outcomes in filter-based toxicology research. Of the organics analyzed, seven were found to have significant correlations to IL-6 expression, but were not detectable in the extracted PM_{2.5}.

Significant or complete losses in both metals (i.e. Cu, Cr, Fe, Ni, Zn) and organics (i.e. benzo[a]pyrene, benzo[e]pyrene, benzo[ghi]perylene, pyrene) occur when preparing ambient PM_{2.5} using the extraction methods detailed (Roper et al. 2015). Ambient values of these constituents have previously been used to make associations to inflammation (Huang et al. 2003; Akhtar et al. 2014) and oxidative potential (Valavanidis et al. 2005; Janssen et al. 2014). This study demonstrated that based solely on ambient measurements, the associations between IL-6 and PM_{2.5} constituents made are inaccurate and may indicate health relevancy of compounds, while associations made using data from extracted PM_{2.5} do not support this conclusion. When correlating IL-6 expression to extracted values, positive correlations were observed for both total metals and total organics concentrations, as anticipated from established epidemiology research (Schaumann et al. 2004; Delfino et al. 2010). Characterization of extracted PM_{2.5} solution allows for measurements that are representative of the exposure and avoids inaccurate associations between constituents and inflammatory responses.

Frequently, biological assays are performed using PM_{2.5} that has not been characterized in either ambient or extracted form (Akhtar et al. 2010; Deng et al. 2013; Happo et al. 2013; Jeong et al. 2014). This practice avoids misidentification of causal constituents but disregards compositional variability altogether, despite the establishment of PM_{2.5} composition as a driver of health effects, independent of concentration (Mar et al. 2000; Ostro et al. 2010). Furthermore, use of uncharacterized PM_{2.5} hinders the ultimate goal of identifying key constituents responsible for health effects. Limited toxicology studies using filter-based extraction methods have characterized samples following partial or complete extraction procedures (Jalava et al. 2009; Huang et al. 2014). These studies allow for connections to be made to components or groups of components in PM_{2.5} that are the predominant factors in PM_{2.5}-associated health outcomes.

Awareness of the need for research to base associations on extracted values of PM_{2.5} will facilitate accurate determination of associations to corroborate epidemiological findings and ultimately to identify the mechanisms of PM_{2.5}-related health effects.

While this preliminary research raised potential issues of not using properly characterized PM_{2.5}, there are a number of limitations that must be considered for future research. First, the dose ($\mu\text{g PM}_{2.5} / \text{mL}$) varied between sampling locations and cells were therefore exposed to varying concentrations of ambient PM_{2.5}. All concentrations were within a range commonly utilized for *in vitro* studies, and while direct measurements of cell death were not recorded, cell counts increased over time at all locations indicating proliferation continued and was more substantial than cell death. These doses were selected to preserve both compositional and concentration differences between sampling locations and in all data analysis, constituents were normalized to total PM_{2.5}. While this study explored effects on a single inflammatory marker, PM_{2.5} has been shown to induce a robust release of numerous cytokines and markers of oxidative stress (Mitschik et al. 2008; Araujo 2010; Anderson et al. 2012). Additionally, the macrophage cell line utilized is reflective of responses specific to that line, and as such is not predictive of the response by other cell types or systemic responses. Future studies using equivalent doses of PM_{2.5} in other cells essential to the inflammatory response to PM_{2.5} (epithelial cells or co-culture systems) or *in vivo* models with the measurement of multiple inflammatory markers would provide further information on the inflammatory response to PM_{2.5}, as well as further demonstrate the importance of well-characterized samples when making associations with these cell types.

3.6 CONCLUSION

PM_{2.5} toxicology research has previously made associations between biological responses and ambient measurements, disregarding subsequent changes due to extraction methods. This study has shown that associations made to ambient PM_{2.5} are not comparable to those made to extracted PM_{2.5}. This misrepresentation is due to compositional alterations in PM_{2.5} that occur during extraction of PM_{2.5} from a filter. Recent studies have investigated the potential consequences of different filter extraction methods on biological impacts of PM_{2.5}; marked differences in inflammatory gene expression was observed and found to be dependent on the extraction method utilized (Van Winkle et al. 2015). In conjunction with the current study, these results demonstrate the importance of extraction methods as well as the importance of characterizing PM_{2.5} composition when studying toxicological outcomes. Increased understanding of the impacts of PM_{2.5} on inflammatory responses is essential to create targeted interventions relevant to human health; it is imperative that such interventions be based on accurate associations made using well-characterized PM_{2.5} following extraction.

4.0 CONCLUSIONS

Exposure to outdoor air pollution is a global public health concern as it caused 3.7 million premature deaths in 2012 and is established as carcinogenic to humans (WHO 2014). Approximately one half of the global urban population is exposed to PM_{2.5} concentrations that are at least 2.5 times above the WHO annual standard of 10 µg/m³ (Thomas and Osseiran 2014) and associations between ambient PM_{2.5} and adverse respiratory (Tecer et al. 2008; Ostro et al. 2009) and cardiovascular (Zanobetti et al. 2009; Pope et al. 2011) effects are well-documented. Beyond concentration, growing research is establishing that PM_{2.5} composition is critical in human health outcomes. Only a single constituent of PM_{2.5} currently has a standard with the EPA (EPA 2014) even though human health effects have been observed and are associated with specific metallic and organic components (Ostro et al. 2010). The need for regulations for components of PM_{2.5} is evident based upon epidemiology research but toxicology studies in this field will establish the biological plausibility of these findings and support targeted regulations to protect human health.

This research investigated the common practice of extraction of filter-based PM_{2.5} for toxicology studies. Characterization of both ambient and extracted PM_{2.5} was conducted to determine compositional differences between the two samples. Additionally, an *in vitro* assessment of PM_{2.5}-induced release of a pro-inflammatory cytokine was measured. Associations were made between the cytokine and constituents of PM_{2.5} as measured in both the ambient and

extracted samples to determine if differences occurred in the associations due to compositional changes in the PM_{2.5}.

Successful development of a method to compare filter-based ambient and extracted PM_{2.5}: Thus far, a study comparing extensive characterization of metals and organic compounds between filter-based and corresponding extracted PM_{2.5} has not been conducted. A method was developed to investigate this comparison as extraction of filter-based PM_{2.5} is common practice in toxicology studies and it is essential to determine if these studies are truly representative of ambient PM_{2.5} composition. This research was successful in developing a method to compare ambient and extracted PM_{2.5} through identical characterization analysis procedures. The findings were that a significant loss of both metallic and organic species with a shift in contribution of constituents between the ambient and extracted samples.

A number of metals associated with adverse health outcomes were lost during the extraction process including: Cr, Fe, Ni, and Pb. Chromium exposure is a risk factor for various acute respiratory effects such as wheezing and coughing (EPA 2000), chronic exposure is an established risk factor for lung cancer as observed in occupational exposure studies (Gibb et al. 2000; Halasova et al. 2009). Exposure to Fe present in ambient PM_{2.5} has been associated with respiratory hospital admissions (Ostro et al. 2009) and cardiovascular (Ito et al. 2011) mortality. Exposure to ambient concentrations of Ni has also been associated adverse health effects including increased daily mortality (Cao et al. 2012) and respiratory (Zanobetti and Schwartz 2009) and cardiovascular (Bell et al. 2009) hospital admissions.

One of the inorganic elements measured in this study was sulfur, S, which is important due to the health effects and increased mortality (Pope and Dockery 2006) associated with sulfate. The method for analysis in this study, XRF, quantifies all S species present, including

sulfate, organosulfate, methanesulfonates, and sulfite (Neubauer et al. 1996; Surratt et al. 2008). Significant loss of S was observed in this research and this could be due in part to a high contribution of organosulfates in the region (Shakya and Peltier 2015), these sulfur species would be soluble in the removal solvent and therefore may have been lost during the concentration step of the extraction process.

Significant or nearly complete loss was observed during the extraction process for many organic components including 10 of the 16 EPA Priority PAHs. PAHs listed on the EPA's priority list are compounds known to be persistent in the environment and toxic (Bojes and Pope 2007). Many PAH's including BaPyr are known carcinogens (IARC 2015), this PAH was not detectable in the extracted solution. Several other PAHs that are classified as probably (DBahA) or possibly (BaAnt, BjkFl, Chr) carcinogenic to humans (IARC 2015) were also not detectable in the extracted PM_{2.5}.

Increased loss of components of PM_{2.5} was observed as total PM_{2.5} mass increased. This finding is of particular concern because many toxicology studies collect PM_{2.5} at sampling locations of high PM_{2.5} concentration to provide adequate material for exposures. Collection of such loadings for biological outcome research is not representative of PM_{2.5} from the sampling locations as the yield of metallic and organic species is decreased. Of further concern, these compounds are established to be health relevant. A hypothesized reason for the decreased extraction at sites of increased mass is that the high concentration locations had a greater contribution of traffic and diesel exhaust. Previously, diesel exhaust has been observed to extract from filters at reduced percentages compared to other sources and is hypothesized to be due to an increased elemental carbon contact that is more strongly bound to the filter material (Pleil 2014).

Loss of these compounds during extraction could in part explain the results in previous filter-based PM_{2.5} studies that did not observe significant increases in cytokines including TNF- α (Mantecca et al. 2012), IL-8 (Schins et al. 2002; Becker et al. 2005), and IL-6 (Monn and Becker 1999; Hetland et al. 2005). If the metallic and organic species that induce inflammation are not present in the prepared toxicology media, the anticipated release of cytokines would not occur. Additionally, reduced concentrations of the constituents in the extracted solutions may attenuate the biological responses and thus underestimate the response induced by ambient PM_{2.5}.

As losses were observed in many PM_{2.5} constituents related to human health effects, the question can be raised about the validity of using filter-based extractions for toxicology tests. Because of the variable and complex mixtures of ambient PM_{2.5}, there is obvious importance of using ambient filter-based samples. These samples capture the compositional differences of PM_{2.5} which cannot be recreated using single constituents or fixed particle concentrator systems. However, the preparation of ambient PM_{2.5} for toxicology research detailed alters the components present and is no longer representative of ambient mixtures. These findings lead to the question of what impact these differences have on toxicology research.

Extraction of PM_{2.5} impacts the interpretation of constituents associated with the release of an inflammatory marker: Filter-based PM_{2.5} toxicology research continues to make associations between ambient measurements and biological outcomes (Perrone et al. 2010; Riva et al. 2011; Akhtar et al. 2014; Kumar et al. 2015), due to the identification of compositional alterations in extracted PM_{2.5} in Chapter 2, a study was designed to determine if these alterations impacted the associations that are routinely made. This research found that associations between PM_{2.5} constituents and release of the cytokine, IL-6, by alveolar macrophages varied greatly

based upon the values of PM_{2.5} (ambient or extracted) used in the associations. When using ambient constituent measurements, significant associations between IL-6 release and 11 PAHs were made, however these PAHs were not detected in the extraction solution. Additionally, significant negative associations were made between IL-6 release and metals (Al, Cu, Fe, Mg, Ni, Zn, and total metals) using ambient measurements, these associations contradict associations made with extracted values as well as previous research.

In addition to this research, previous filter-based studies have found negative associations between inflammatory markers and ambient values of PM_{2.5} components: Cu, Fe, Ni, Zn, and PAHs (Huang et al. 2003; Happonen et al. 2010; Lee et al. 2015). In particular for this study, ambient values of Ni had a significant negative association to IL-6 release; however, Ni has been established to induce inflammation in toxicology studies using CAPs (Saldiva et al. 2002) and inflammation-related disorders in epidemiology research (Lippmann et al. 2006). Importantly, when using the extracted solution values of Ni present, significant positive associations were observed, in line with the previous toxicity assessment of Ni (Chen and Lippmann 2009). This study has highlighted the need to characterize extraction solutions of PM_{2.5} to allow for the accurate interpretation of constituent connections to biological outcomes.

Currently, there is a need to establish the health-relevant components of PM_{2.5} to more adequately create standards beyond just PM_{2.5} concentration. More targeted regulations will better protect human health and toxicology studies are required to accurately create constituent specific regulations. However there is a concern when filter-based toxicology studies draw conclusions about the biological impact of PM_{2.5} constituents using ambient measurements. The concern with these studies is that the compositional alterations that occur during current filter

extraction methods create misleading associations between biological outcomes and the constituents of interest.

Future directions: Previous filter-based toxicology studies have made associations between biological outcomes and ambient measurements of PM_{2.5} constituents (Valavanidis et al. 2005; Akhtar et al. 2014; Janssen et al. 2014). This practice disregards the potential alterations that occur during filter extraction. This study has demonstrated that a significant loss of metals and organic compounds occurs during extraction of PM_{2.5} from filters but the exact point in the extraction process that losses occur has not been established. It is hypothesized that losses are predominantly from removal of the solvent during the concentration step as constituents that are soluble would remain in solution and the concentrated dry PM_{2.5} would be predominantly insoluble particles. To test this hypothesis, characterization of the solution following the removal step should be performed to determine the components present compared to those present after concentration.

Furthermore, this study identified the misinterpretation of causal constituents of IL-6 release when using ambient instead of extracted PM_{2.5} values. Additional research measuring other health outcome indicators and in different model systems should be conducted to determine if similar misinterpretations occur. Additional health outcome indicators include markers of inflammation (cytokines, total cell counts), ROS (oxidative potential, generation of ROS, markers of oxidative stress), and genotoxicity (bulky adduct formation, DNA strand breaks). As PM_{2.5} toxicology has previously been performed in a variety of cell types and *in vivo* systems it is imperative that similar experiments to those detailed in this study occur to identify the impacts in each model.

An important future study that evolved from these findings is to determine the difference in response between an ambient exposure to constituents and an exposure to extracted PM_{2.5}. The hypothesis for this investigation is that the release of IL-6 is attenuated when using extracted values of PM_{2.5} compared to ambient levels of constituents. A study design to test this hypothesis would involve extensive ambient characterization of PM_{2.5} samples, this mixture would then be re-created using purchased chemical constituents in the proportions established from the ambient concentrations. The IL-6 release would be measured in alveolar macrophage cells following treatment with 1) re-created ambient conditions PM_{2.5} or 2) extracted PM_{2.5} using filter-based samples and the extraction processes used in this work. An alternative to this exposure design would be to compare a CAPs exposure to an exposure of equivalent PM_{2.5} mass but from a filter sample that was extracted. This future research would help better establish if current filter-based toxicology studies are underrepresenting the health effects of ambient PM_{2.5} exposures.

Due to current disregard for the compositional changes occurring during extraction, it is essential that a standardized extraction protocol is created for PM_{2.5} toxicology studies. Creation of a standardized protocol, particularly one designed to reduce the loss of constituents, would reduce the current concern of a methods bias on the toxicology outcomes (Van Winkle et al. 2015). Also, a standardized protocol would allow for inter-study comparability that is currently a major limitation when reviewing literature on filter-based studies.

Finally, the possibility for alternative methods to filter extraction should be considered for future research. Thus far, there have been a limited number of studies using alternative methods, these methods include: placing filters directly into cell culture media and collecting PM_{2.5} onto non-filter substrates. The practice of placing the filter into cell culture media has been used for PM_{2.5} research (Pavagadhi et al. 2013) however several concerns exist about this

method and it is not a common method for toxicology studies. The first concern is that PM_{2.5} exposure is dominated by the particles that were collected most recently on the filter as they are in the most direct contact with the cell culture media. The cells would only be exposed to components soluble in the media as other components would remain on the filter. The use of alternative collection substrates such as foils (Kroll et al. 2013) is a promising alternative to filter collection as it allows for gravimetric analysis of collected particles and they can be directly re-suspended into toxicology media. While this collection substrate is not as prominent in the literature as standard filters, it is a potential alternative that should be considered for future research if filter-based extraction methods are not improved and standardized.

APPENDIX A: EXTRACTION METHODS REFINEMENT

Prior to the refined extraction methods used in this study (Chapters 2 and 3), several different removal methods based on previous literature (Table 1) were explored to determine the method that resulted in the most efficient mass removal. These studies were done without consideration of compositional components and based solely on total PM_{2.5} mass. Filters (cellulose, PTFE-coated, and PTFE) were used for ambient PM_{2.5} collection using size-selective Harvard Impactors, as described in Chapter 2, at the intersection of the Hot Metal Bridge and Second Ave., Pittsburgh, PA throughout 2012 and 2013.

Removal efficiencies with standard deviations were reported for various filter types, removal methods, and removal solvents (Table 14). All efficiencies were adjusted for loss of mass from blank sonicated filters that underwent identical procedures, collected PM_{2.5} filters (n=5/ removal method) and blank filters (n=5/removal method). The first removal method, probe sonication, was performed at 20 kHz with a probe sonicator (3 x 10s). Waterbath sonication was performed as previously described in Chapter 2.

Table 12: Removal efficiencies

	Probe Sonication	Waterbath Sonication
Water	PTFE-coated: 24.2 ± 20.8% Cellulose: 8.8 ± 16.6%	
0.9% Saline		PTFE-coated: 45.7 ± 19.3% Cellulose: 48.9 ± 33.5%
Methanol		PTFE: 89.9 ± 4.4%
Methanol:Water (9:1)		PTFE: 98.3 ± 1.4%

Based on the removal efficiencies of total PM_{2.5} mass, the method selected for all subsequent studies was sonication using a waterbath sonicator in a 9:1 methanol/water solvent. This method had the highest removal efficiency with the lowest variability between samples and was not observed to be destructive to the filter itself.

APPENDIX B: AMBIENT CHARACTERIZATION DATA

Detailed characterization of ambient PM_{2.5} through concentration and analysis of metallic and organic species is provided for each sampling location in this appendix. Detailed description of collection and characterization methods is provided in Chapter 2.

Maps created in geographic information systems (GIS, ArcGIS 10, ESRI, Redlands, CA) for each of the five sampling locations for ambient concentrations of PM_{2.5} concentration (Figure 8), total metals (Figure 9), and total organics (Figure 10). The term “total” refers to the sum of all compounds analyzed for metals and organics, n=51 and 34, respectively (Table 2).

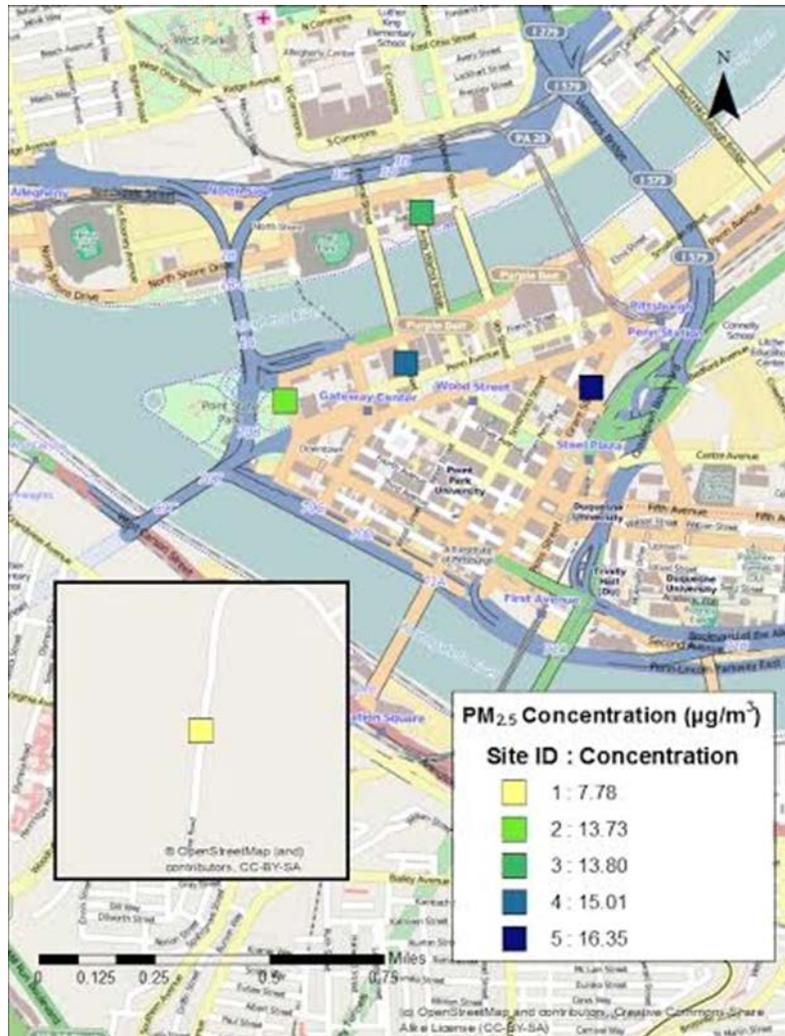


Figure 8: Map of Ambient PM_{2.5} Concentrations

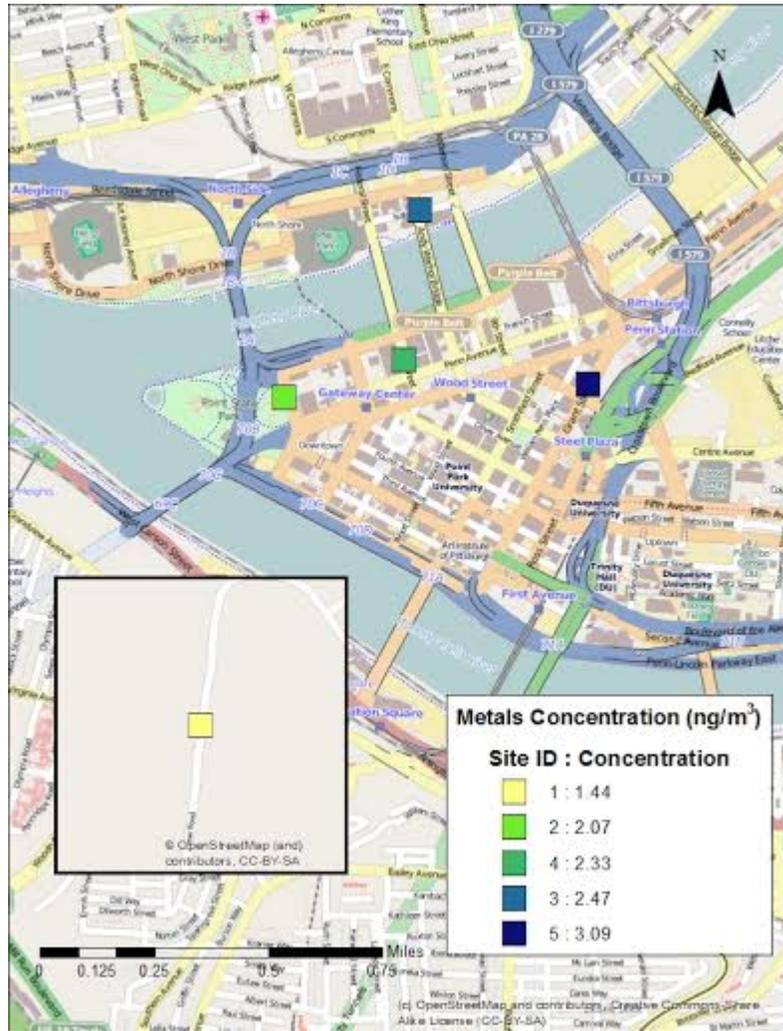


Figure 9: Map of Ambient Metals Concentrations

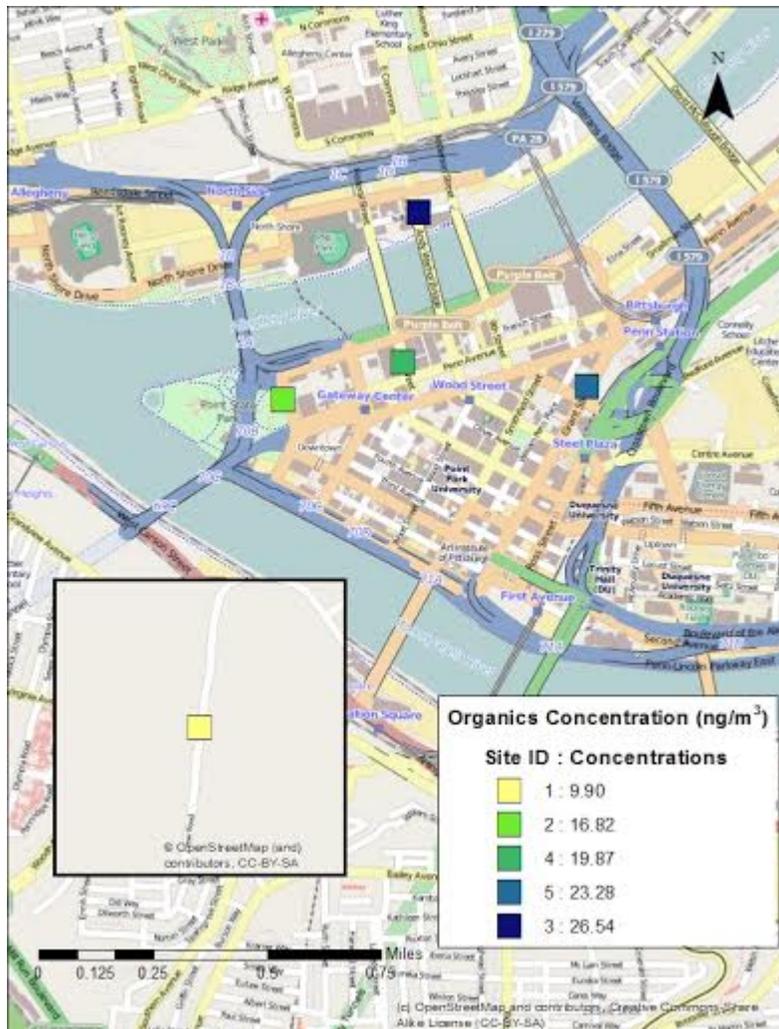


Figure 10: Map of Ambient Organics Concentrations

The concentrations of individual metals (Table 12) and organics (Table 13) are provided for each of the five sampling locations.

Table 13: Ambient Metals Concentrations (ng/m³) for Sampling Locations

	Al	Ba	Ca	Cd	Ce	Cl	Cr	Cs	Cu	Fe	Mg	Mn	Mo	Na	Ni	P	Pb	S	Sn	Sr	Y	Zn	Total Metals
1	3.83	0.00	20.11	0.00	5.38	67.56	1.15	0.00	1.58	44.04	33.14	1.81	2.12	601.57	0.34	0.00	2.40	562.27	0.37	0.41	0.33	13.33	1443.65
2	40.14	0.00	51.33	0.00	4.32	532.26	1.53	0.83	5.97	212.42	0.00	13.53	2.87	663.15	0.50	0.00	5.31	622.69	0.00	0.68	0.42	37.55	2074.93
3	57.88	1.46	56.00	0.66	0.21	609.48	1.38	0.00	6.14	194.07	29.86	13.24	2.04	648.60	0.62	0.00	7.60	663.78	0.00	0.93	0.18	33.65	2474.49
4	58.86	0.00	48.28	0.00	2.75	459.81	2.20	0.00	5.21	220.63	3.52	22.07	2.49	663.15	0.61	0.00	4.50	635.10	0.00	0.68	0.10	37.44	2327.02
5	64.46	0.00	77.24	0.50	4.85	801.17	1.73	0.00	6.92	255.11	54.02	18.71	2.59	862.15	0.67	0.00	6.36	738.44	0.59	0.85	0.00	39.51	3094.14

Table 14: Ambient Organics Concentrations (pg/m³) for Sampling Locations

	Acy	Ace	F	P	Flu	Pyr	9Flo	DBT	1MP	2MP	Chr	BbFl	BjkFl	BaAnt	BePyr	BaPyr	lpyr	DBahA	BghiPer	BghiFl	Hop-anes	Ster-anes	Total Organics
1	7274.02	0.00	0.00	237.40	201.23	156.80	80.19	0.00	29.14	23.21	240.01	234.42	528.81	105.97	125.94	84.92	87.66	0.00	411.34	46.50	30.08	0.00	9897.63
2	10729.27	76.94	26.56	371.94	406.53	353.00	51.43	0.00	35.06	56.82	675.47	464.18	894.64	339.97	350.35	256.27	255.55	49.73	1007.77	129.96	278.00	15.30	16824.75
3	21271.49	0.00	30.30	369.11	395.85	335.85	54.10	16.81	35.65	58.58	570.06	501.85	777.13	286.92	281.98	212.71	198.84	35.23	749.88	112.66	233.39	8.11	26536.51
4	14755.13	0.00	0.00	202.25	227.49	217.55	43.47	0.00	25.55	18.82	539.60	497.38	777.90	260.75	293.63	174.28	259.73	49.33	1141.42	133.42	238.21	13.48	19869.39
5	18760.15	0.00	24.28	321.65	346.26	0.00	62.32	0.00	37.27	52.99	512.04	493.80	691.98	287.56	252.65	179.40	140.40	0.00	664.27	122.22	306.18	24.56	23279.96

BIBLIOGRAPHY

- Aarnio, P., T. Yli-Tuomi, et al. (2005). "The concentrations of and exposure to fine particles (PM_{2.5}) in the Helsinki subway system." Atmospheric Environment **39**(28): 5059-5066.
- Abbey, D. E., B. E. Ostro, et al. (1995). "Chronic respiratory symptoms associated with estimated long-term ambient concentrations of fine particulates less than 2.5 microns in aerodynamic diameter (PM_{2.5}) and other air pollutants." Journal of exposure analysis and environmental epidemiology **5**(2): 137-159.
- Abdel-Salam, M. and J. H. Dennis. (2010). "Review of aerosol sampling methods and introduction of a new low cost aerosol sampler." Aerosol Sampler Retrieved October 29, 2015, 2015, from http://www.ids-environment.com/common/paper/paper_25/salam_dennis_aerosol_monitoring.pdf.
- Akhtar, U. S., R. D. McWhinney, et al. (2010). "Cytotoxic and proinflammatory effects of ambient and source-related particulate matter (PM) in relation to the production of reactive oxygen species (ROS) and cytokine adsorption by particles." Inhalation toxicology **22 Suppl 2**: 37-47.
- Akhtar, U. S., N. Ratstogi, et al. (2014). "The combined effects of physicochemical properties of size-fractionated ambient particulate matter on in vitro toxicity in human A549 lung epithelial cells." Toxicology Reports **1**: 145-156.
- Alberts, B., A. Johnson, et al. (2002). Molecular Biology of the Cell. 4th edition. Innate Immunity New York, Garland Science.
- Allen, G., C. Sioutas, et al. (1997). "Evaluation of the TEOM(R) method for measurement of ambient particulate mass in urban areas." Journal of the Air & Waste Management Association **47**(6): 682-689.
- Allen, J. L., K. Conrad, et al. (2013). "Developmental exposure to concentrated ambient particles and preference for immediate reward in mice." Environmental Health Perspectives **121**(1): 32-38.
- Almeida, S. M., C. A. Pio, et al. (2005). "Source apportionment of fine and coarse particulate matter in a sub-urban area at the Western European Coast." Atmospheric Environment **39**(17): 3127-3138.
- Anderson, J. O., J. G. Thundiyil, et al. (2012). "Clearing the air: a review of the effects of particulate matter air pollution on human health." Journal of medical toxicology : official journal of the American College of Medical Toxicology **8**(2): 166-175.
- Andre, V., S. Billet, et al. (2011). "Mutagenicity and genotoxicity of PM_{2.5} issued from an urbano-industrialized area of Dunkerque (France)." Journal of applied toxicology : JAT **31**(2): 131-138.
- Araujo, J. A. (2010). "Particulate air pollution, systemic oxidative stress, inflammation, and atherosclerosis." Air quality, atmosphere, & health **4**(1): 79-93.

- Atkinson, R. W., H. R. Anderson, et al. (2001). "Acute effects of particulate air pollution on respiratory admissions: results from APHEA 2 project. Air Pollution and Health: a European Approach." American journal of respiratory and critical care medicine **164**(10 Pt 1): 1860-1866.
- ATSDR (2006). A study of ambient air contaminants and asthma in New York City: Part A and B. A. f. T. S. a. D. Registry. Atlanta.
- Balasubramanian, P., M. P. Sirivelu, et al. (2013). "Differential effects of inhalation exposure to PM_{2.5} on hypothalamic monoamines and corticotrophin releasing hormone in lean and obese rats." Neurotoxicology **36**: 106-111.
- Baulig, A., J. J. Poirault, et al. (2004). "Physicochemical characteristics and biological activities of seasonal atmospheric particulate matter sampling in two locations of Paris." Environmental science & technology **38**(22): 5985-5992.
- Becker, S., L. A. Dailey, et al. (2005). "Seasonal variations in air pollution particle-induced inflammatory mediator release and oxidative stress." Environmental Health Perspectives **113**(8): 1032-1038.
- Becker, S., S. Mundandhara, et al. (2005). "Regulation of cytokine production in human alveolar macrophages and airway epithelial cells in response to ambient air pollution particles: further mechanistic studies." Toxicology and Applied Pharmacology **207**(2 Suppl): 269-275.
- Becker, S., J. M. Soukup, et al. (2003). "Response of human alveolar macrophages to ultrafine, fine, and coarse urban air pollution particles." Experimental Lung Research **29**(1): 29-44.
- Beelen, R., G. Hoek, et al. (2015). "Natural-cause mortality and long-term exposure to particle components: an analysis of 19 European cohorts within the multi-center ESCAPE project." Environmental Health Perspectives **123**(6): 525-533.
- Behbod, B., B. Urch, et al. (2013). "Endotoxin in concentrated coarse and fine ambient particles induces acute systemic inflammation in controlled human exposures." Occupational and environmental medicine **70**(11): 761-767.
- Bein, K. J. and A. S. Wexler (2014). "A high-efficiency, low-bias method for extracting particulate matter from filter and impactor substrates." Atmospheric Environment **90**: 87-95.
- Bein, K. J. and A. S. Wexler (2015). "Compositional variance in extracted particulate matter using different filter extraction techniques." Atmospheric Environment **107**: 24-34.
- Bell, M., K. Ebisu, et al. (2009). "Hospital Admissions and Chemical Composition of Fine Particulate Matter (PM_{2.5}) for 106 US Counties." Epidemiology **20**(6): S29-S29.
- Bell, M. L., K. Ebisu, et al. (2014). "Associations of PM_{2.5} constituents and sources with hospital admissions: analysis of four counties in Connecticut and Massachusetts (USA) for persons \geq 65 years of age." Environmental Health Perspectives **122**(2): 138-144.
- Bell, M. L., K. Ebisu, et al. (2009). "Hospital admissions and chemical composition of fine particle air pollution." American journal of respiratory and critical care medicine **179**(12): 1115-1120.
- Bernstein, J. A., N. Alexis, et al. (2004). "Health effects of air pollution." The Journal of allergy and clinical immunology **114**(5): 1116-1123.
- Bojes, H. K. and P. G. Pope (2007). "Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at oil exploration and production sites in Texas." Regulatory toxicology and pharmacology : RTP **47**(3): 288-295.

- Boogaard, H., N. A. Janssen, et al. (2012). "Contrasts in oxidative potential and other particulate matter characteristics collected near major streets and background locations." Environmental Health Perspectives **120**(2): 185-191.
- Brook, R. D., S. Rajagopalan, et al. (2010). "Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association." Circulation **121**(21): 2331-2378.
- Brook, R. D., B. Urch, et al. (2009). "Insights into the Mechanisms and Mediators of the Effects of Air Pollution Exposure on Blood Pressure and Vascular Function in Healthy Humans." Hypertension **54**(3): 659-667.
- Budinger, G. R., J. L. McKell, et al. (2011). "Particulate matter-induced lung inflammation increases systemic levels of PAI-1 and activates coagulation through distinct mechanisms." PloS one **6**(4): e18525.
- Cabada, J. C., S. Rees, et al. (2004). "Mass size distributions and size resolved chemical composition of fine particulate matter at the Pittsburgh supersite." Atmospheric Environment **38**(20): 3127-3141.
- Cakmak, S., R. Dales, et al. (2014). "Metal composition of fine particulate air pollution and acute changes in cardiorespiratory physiology." Environmental pollution **189**: 208-214.
- Calgary, U. o. (2015). "Rotary Evaporation: The "Rotovap"." Organic Laboratory Techniques 8, from <http://www.chem.ucalgary.ca/courses/351/laboratory/rotavap.pdf>.
- Calloway, C. P., S. Li, et al. (1989). "A refinement of the potassium tracer method for residential wood smoke." Atmospheric Environment **23**(1): 67-69.
- Camp, U. and O. Seely. (2015). "Proper Use of a Desiccator." from <http://www.csudh.edu/oliver/che230/labmanual/desiccat.htm>.
- Cao, J., H. Xu, et al. (2012). "Fine particulate matter constituents and cardiopulmonary mortality in a heavily polluted Chinese city." Environmental Health Perspectives **120**(3): 373-378.
- Cassee, F. R., P. H. Fokkens, et al. (2003). Respiratory Allergy and Inflammation Due to Ambient Particles (RAIAP). Bilthoven, RIVM.
- Cavanagh, J. A., K. Trought, et al. (2009). "Exploratory investigation of the chemical characteristics and relative toxicity of ambient air particulates from two New Zealand cities." The Science of the total environment **407**(18): 5007-5018.
- CCOHS. (2015). "How Do Particulates Enter the Respiratory System?", from http://www.ccohs.ca/oshanswers/chemicals/how_do.html.
- Chaloulakou, A., P. Kassomenos, et al. (2005). "Particulate matter and black smoke concentration levels in central Athens, Greece." Environment International **31**(5): 651-659.
- Charron, A. and R. M. Harrison (2005). "Fine (PM_{2.5}) and coarse (PM_{2.5-10}) particulate matter on a heavily trafficked London highway: sources and processes." Environmental science & technology **39**(20): 7768-7776.
- Chellam, S., P. Kulkarni, et al. (2005). "Emissions of organic compounds and trace metals in fine particulate matter from motor vehicles: a tunnel study in Houston, Texas." Journal of the Air & Waste Management Association **55**(1): 60-72.
- Chen, H. and M. S. Goldberg (2009). "The effects of outdoor air pollution on chronic illnesses." McGill journal of medicine : MJM : an international forum for the advancement of medical sciences by students **12**(1): 58-64.
- Chen, L. C. and M. Lippmann (2009). "Effects of metals within ambient air particulate matter (PM) on human health." Inhalation toxicology **21**(1): 1-31.

- Choi, J. H., J. S. Kim, et al. (2004). "Comparative study of PM2.5 - and PM10 - induced oxidative stress in rat lung epithelial cells." Journal of veterinary science **5**(1): 11-18.
- Choung, S., J. Oh, et al. (2015). "Comparison of physicochemical properties between fine (PM) and coarse airborne particles at cold season in Korea." The Science of the total environment **541**: 1132-1138.
- Clements, A. L., B. Buzcu-Guven, et al. (2013). "Role of particulate metals in heterogenous secondary sulfate formation." Atmospheric Environment **75**: 233-240.
- Crouse, D. L., P. A. Peters, et al. (2012). "Risk of nonaccidental and cardiovascular mortality in relation to long-term exposure to low concentrations of fine particulate matter: a Canadian national-level cohort study." Environmental Health Perspectives **120**(5): 708-714.
- Daher, N., A. Ruprecht, et al. (2012). "Characterization, sources and redox activity of fine and coarse particulate matter in Milan, Italy." Atmospheric Environment **49**: 130-141.
- Dai, L., A. Zanobetti, et al. (2014). "Associations of Fine Particulate Matter Species with Mortality in the United States: A Multicity Time-Series Analysis." Environmental Health Perspectives **122**(8): 837-842.
- Danielsen, P. H., P. Moller, et al. (2011). "Oxidative stress, DNA damage, and inflammation induced by ambient air and wood smoke particulate matter in human A549 and THP-1 cell lines." Chemical research in toxicology **24**(2): 168-184.
- de Brito, K. C., C. T. de Lemos, et al. (2013). "Comparative genotoxicity of airborne particulate matter (PM2.5) using Salmonella, plants and mammalian cells." Ecotoxicology and environmental safety **94**: 14-20.
- de Kok, T. M., H. A. Driecce, et al. (2006). "Toxicological assessment of ambient and traffic-related particulate matter: a review of recent studies." Mutation research **613**(2-3): 103-122.
- de Kok, T. M., J. G. Hogervorst, et al. (2005). "Genotoxicity and physicochemical characteristics of traffic-related ambient particulate matter." Environmental and molecular mutagenesis **46**(2): 71-80.
- Delfino, R. J., N. Staimer, et al. (2010). "Associations of primary and secondary organic aerosols with airway and systemic inflammation in an elderly panel cohort." Epidemiology **21**(6): 892-902.
- Delfino, R. J., N. Staimer, et al. (2010). "Association of biomarkers of systemic inflammation with organic components and source tracers in quasi-ultrafine particles."
- Dellinger, B., W. A. Pryor, et al. (2001). "Role of free radicals in the toxicity of airborne fine particulate matter." Chemical research in toxicology **14**(10): 1371-1377.
- Deng, X., W. Rui, et al. (2013). "PM2.5 induces Nrf2-mediated defense mechanisms against oxidative stress by activating PIK3/AKT signaling pathway in human lung alveolar epithelial A549 cells." Cell biology and toxicology **29**(3): 143-157.
- Deng, X., F. Zhang, et al. (2013). "PM2.5-induced oxidative stress triggers autophagy in human lung epithelial A549 cells." Toxicology in vitro : an international journal published in association with BIBRA **27**(6): 1762-1770.
- Devlin, R. B., A. J. Ghio, et al. (2003). "Elderly humans exposed to concentrated air pollution particles have decreased heart rate variability." The European respiratory journal. Supplement **40**: 76s-80s.

- Dieme, D., M. Cabral-Ndior, et al. (2012). "Relationship between physicochemical characterization and toxicity of fine particulate matter (PM_{2.5}) collected in Dakar city (Senegal)." Environmental Research **113**: 1-13.
- Dockery, D. W., C. A. Pope, 3rd, et al. (1993). "An association between air pollution and mortality in six U.S. cities." The New England Journal of Medicine **329**(24): 1753-1759.
- Dockery, D. W., C. A. Pope, et al. (1993). "An Association between Air-Pollution and Mortality in 6 United-States Cities." New England Journal of Medicine **329**(24): 1753-1759.
- Dominici, F., R. D. Peng, et al. (2006). "Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases." JAMA : the journal of the American Medical Association **295**(10): 1127-1134.
- Dye, J. A., J. R. Lehmann, et al. (2001). "Acute pulmonary toxicity of particulate matter filter extracts in rats: coherence with epidemiologic studies in Utah Valley residents." Environmental Health Perspectives **109 Suppl 3**: 395-403.
- EPA (1999). Determination of metals in ambient particulate matter using atomic absorption (AA) spectroscopy. Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air. Cincinnati.
- EPA (1999). Determination of metals in ambient particulate matter using inductively coupled plasma/mass spectrometry (ICP/MS). Compendium of methods for determination of inorganic compounds in ambient air. E. P. Agency. Cincinnati.
- EPA. (2000). "Chromium Compounds." Technology Transfer Network - Air Toxics Web Site Retrieved October 3, 2015, from <http://www3.epa.gov/airtoxics/hlthef/chromium.html>.
- EPA (2009). Integrated Science Assessment for Particulate Matter.
- EPA. (2014). "National Ambient Air Quality Standards (NAAQS)." Air and Radiation, from <http://www.epa.gov/air/criteria.html>.
- EPA. (2014). "Semi-Volatile Organic Compounds." Mid-Atlantic Brownfields & Land Revitalization, from <http://www.epa.gov/reg3hwmd/bf-lr/regional/analytical/semi-volatile.htm>.
- EPA. (2015). "Air Quality Trends." Retrieved September 29, 2015, 2015, from <http://www3.epa.gov/airtrends/aqtrends.html>.
- EPA. (2015). "Basic Information." Particulate Matter (PM) Retrieved September 30, 2015, from <http://www3.epa.gov/pm/basic.html>.
- EPA. (2015). "National Ambient Air Quality Standards." Technology Transfer Network Retrieved September 29, 2015, from <http://www3.epa.gov/ttn/naqs/criteria.html>.
- EPA. (2015). "Particulate Matter." Extramural Research Retrieved September 30, 2015, from <http://www.epa.gov/ncer/science/pm/>.
- ERG (2015). Standard operating procedure for the determination of lead in PM₁₀ by inductively coupled plasma mass spectrometry (ICP-MS) with Hot Block dilute acid and hydrogen peroxide filter extraction. I. Eastern Research Group. Morrisville
- Fang, T., V. Verma, et al. (2014). "A semi-automated system for quantifying the oxidative potential of ambient particles in aqueous extracts using the dithiothreitol (DTT) assay: results from the Southeastern Center for Air Pollution and Epidemiology (SCAPE)." Atmospheric Measurement Techniques Discussions **7**(7): 7245-7279.
- Franklin, M., P. Koutrakis, et al. (2008). "The role of particle composition on the association between PM_{2.5} and mortality." Epidemiology **19**(5): 680-689.

- Franklin, M., A. Zeka, et al. (2007). "Association between PM_{2.5} and all-cause and specific-cause mortality in 27 US communities." Journal of exposure science & environmental epidemiology **17**(3): 279-287.
- Fuzzi, S., U. Baltensperger, et al. (2015). "Particulate matter, air quality and climate: lessons learned and future needs." Atmospheric chemistry and physics **15**: 8217-8299.
- Geng, H., Z. Meng, et al. (2006). "In vitro responses of rat alveolar macrophages to particle suspensions and water-soluble components of dust storm PM_{2.5}." Toxicology in vitro : an international journal published in association with BIBRA **20**(5): 575-584.
- Gerlofs-Nijland, M. E., J. A. Dormans, et al. (2007). "Toxicity of coarse and fine particulate matter from sites with contrasting traffic profiles." Inhalation toxicology **19**(13): 1055-1069.
- Ghio, A. J., M. S. Carraway, et al. (2012). "Composition of air pollution particles and oxidative stress in cells, tissues, and living systems." Journal of toxicology and environmental health. Part B, Critical reviews **15**(1): 1-21.
- Ghio, A. J. and Y. C. Huang (2004). "Exposure to concentrated ambient particles (CAPs): a review." Inhalation toxicology **16**(1): 53-59.
- Gibb, H. J., P. S. Lees, et al. (2000). "Lung cancer among workers in chromium chemical production." American journal of industrial medicine **38**(2): 115-126.
- Gobeli, D., H. Scholoesser, et al. (2008). Met One Instruments BAM-1020 Beta Attenuation Mass Monitor US-EPA PM_{2.5} Federal Equivalent Method Field Test Results. M. O. I. Incorporated. Grants Pass.
- Godri, K. J., R. M. Harrison, et al. (2011). "Increased oxidative burden associated with traffic component of ambient particulate matter at roadside and urban background schools sites in London." PloS one **6**(7): e21961.
- Goldsmith, C. A., C. Frevert, et al. (1997). "Alveolar macrophage interaction with air pollution particulates." Environmental Health Perspectives **105 Suppl 5**: 1191-1195.
- Gong, H., Jr., W. S. Linn, et al. (2004). "Altered heart-rate variability in asthmatic and healthy volunteers exposed to concentrated ambient coarse particles." Inhalation toxicology **16**(6-7): 335-343.
- Gorczyca, W., J. Gong, et al. (1993). "Detection of DNA strand breaks in individual apoptotic cells by the in situ terminal deoxynucleotidyl transferase and nick translation assays." Cancer research **53**(8): 1945-1951.
- Grieshop, A. P., E. M. Lipsky, et al. (2006). "Fine particle emission factors from vehicles in a highway tunnel: effects of fleet composition and season." Atmospheric Environment **40**: S287-298.
- Gualtieri, M., E. Longhin, et al. (2012). "Gene expression profiling of A549 cells exposed to Milan PM_{2.5}." Toxicology Letters **209**(2): 136-145.
- Gummeneni, S., Y. Bin Yusup, et al. (2011). "Source apportionment of particulate matter in the ambient air of Hyderabad city, India." Atmospheric Research **101**(3): 752-764.
- Halasova, E., T. Matakova, et al. (2009). "Human lung cancer and hexavalent chromium exposure." Neuro endocrinology letters **30 Suppl 1**: 182-185.
- Hamad, S. H., M. M. Shafer, et al. (2015). "Seasonal trends in the composition and ROS activity of fine particulate matter in Baghdad, Iraq." Atmospheric Environment **100**: 102-110.
- Han, I., J. N. Mihalic, et al. (2012). "Assessment of heterogeneity of metal composition of fine particulate matter collected from eight U.S. counties using principal component analysis." Journal of the Air & Waste Management Association **62**(7): 773-782.

- Hand, J. L., B. A. Schichtel, et al. (2012). "Seasonal composition of remote and urban fine particulate matter in the United States." Journal of geophysical research. Atmospheres : JGR **117**(D5).
- Haney, J. T., Jr., T. H. Connor, et al. (1999). "Detection of ozone-induced DNA single strand breaks in murine bronchoalveolar lavage cells acutely exposed in vivo." Inhalation toxicology **11**(4): 331-341.
- Hang, B. (2010). "Formation and repair of tobacco carcinogen-derived bulky DNA adducts." Journal of nucleic acids **2010**: 709521.
- Hannan, J. (2013). US EPA Method 200.7 using the Thermo Scientific iCAP 7600 ICP-OES Duo. T. Scientific.
- Happo, M., A. Markkanen, et al. (2013). "Seasonal variation in the toxicological properties of size-segregated indoor and outdoor air particulate matter." Toxicology in vitro : an international journal published in association with BIBRA **27**(5): 1550-1561.
- Happo, M. S., M. R. Hirvonen, et al. (2010). "Seasonal variation in chemical composition of size-segregated urban air particles and the inflammatory activity in the mouse lung." Inhalation toxicology **22**(1): 17-32.
- Hawthorne, S. B., C. B. Grabanski, et al. (2000). "Comparisons of soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recovery, selectivity and effects on sample matrix." Journal of chromatography. A **892**(1-2): 421-433.
- Henderson, S. B., B. Beckerman, et al. (2007). "Application of land use regression to estimate long-term concentrations of traffic-related nitrogen oxides and fine particulate matter." Environmental science & technology **41**(7): 2422-2428.
- Hering, S. V. (2001). Impactors, Cyclones, and Other Inertial and Gravitational Collectors. Air Sampling Instruments, American Conference of Governmental and Industrial Hygienists: 279-289.
- Hetland, R. B., F. R. Cassee, et al. (2005). "Cytokine release from alveolar macrophages exposed to ambient particulate matter: heterogeneity in relation to size, city and season." Particle and Fibre Toxicology **2**: 4.
- Hetland, R. B., F. R. Cassee, et al. (2004). "Release of inflammatory cytokines, cell toxicity and apoptosis in epithelial lung cells after exposure to ambient air particles of different size fractions." Toxicology in vitro : an international journal published in association with BIBRA **18**(2): 203-212.
- Hiraiwa, K. and S. F. van Eeden (2013). "Contribution of lung macrophages to the inflammatory responses induced by exposure to air pollutants." Mediators of inflammation **2013**: 619523.
- Hitchins, J., L. Morawska, et al. (2000). "Concentrations of submicrometre particles from vehicle emissions near a major road." Atmospheric Environment **34**(1): 51-59.
- Hsiao, W. L., Z. Y. Mo, et al. (2000). "Cytotoxicity of PM(2.5) and PM(2.5--10) ambient air pollutants assessed by the MTT and the Comet assays." Mutation research **471**(1-2): 45-55.
- Huang, L. and G. Wang (2014). "Chemical characteristics and source apportionment of atmospheric particles during heating period in Harbin, China." Journal of environmental sciences **26**(12): 2475-2483.

- Huang, Q., J. Zhang, et al. (2014). "Effects of water soluble PM2.5 extracts exposure on human lung epithelial cells (A549): A proteomic study." Journal of applied toxicology : JAT **34**(6): 675-687.
- Huang, S. L., M. K. Hsu, et al. (2003). "Effects of submicrometer particle compositions on cytokine production and lipid peroxidation of human bronchial epithelial cells." Environmental Health Perspectives **111**(4): 478-482.
- Huang, W., J. Cao, et al. (2012). "Seasonal variation of chemical species associated with short-term mortality effects of PM(2.5) in Xi'an, a Central City in China." American Journal of Epidemiology **175**(6): 556-566.
- Huang, Y. C., A. J. Ghio, et al. (2003). "The role of soluble components in ambient fine particles-induced changes in human lungs and blood." Inhalation toxicology **15**(4): 327-342.
- Huffman, G. P., F. E. Huggins, et al. (2000). "Characterization of fine particulate matter produced by combustion of residual fuel oil." Journal of the Air & Waste Management Association **50**(7): 1106-1114.
- Huggins, F. E., N. Shah, et al. (2000). "XAFS spectroscopic characterization of elements in combustion ash and fine particulate matter." Fuel Processing Technology **65**(66): 203-218.
- IARC. (2015). "International Agency for Research on Cancer." Retrieved October 3, 2015, from <http://www.iarc.fr/index.php>.
- Ilabaca, M., I. Olaeta, et al. (1999). "Association between levels of fine particulate and emergency visits for pneumonia and other respiratory illnesses among children in Santiago, Chile." Journal of the Air & Waste Management Association **49**(9 Spec No): 154-163.
- Imrich, A., Y. Ning, et al. (2000). "Insoluble components of concentrated air particles mediate alveolar macrophage responses in vitro." Toxicology and Applied Pharmacology **167**(2): 140-150.
- Imrich, A., Y. Ning, et al. (2007). "Alveolar macrophage cytokine response to air pollution particles: oxidant mechanisms." Toxicology and Applied Pharmacology **218**(3): 256-264.
- Imrich, A., Y. Y. Ning, et al. (2000). "Insoluble components of concentrated air particles mediate alveolar macrophage responses in vitro." Toxicology and Applied Pharmacology **167**(2): 140-150.
- Ito, K., R. Mathes, et al. (2010). "Fine Particulate Matter Constituents Associated with Cardiovascular Hospitalizations and Mortality in New York City." Environmental Health Perspectives.
- Ito, K., R. Mathes, et al. (2011). "Fine particulate matter constituents associated with cardiovascular hospitalizations and mortality in New York City." Environmental Health Perspectives **119**(4): 467-473.
- Ito, K., G. D. Thurston, et al. (2007). "Characterization of PM2.5, gaseous pollutants, and meteorological interactions in the context of time-series health effects models." Journal of Exposure Science and Environmental Epidemiology **17**: S45-S60.
- Jalava, P. I., M. R. Hirvonen, et al. (2009). "Associations of urban air particulate composition with inflammatory and cytotoxic responses in RAW 246.7 cell line." Inhalation toxicology **21**(12): 994-1006.

- Jalava, P. I., R. O. Salonen, et al. (2006). "In vitro inflammatory and cytotoxic effects of size-segregated particulate samples collected during long-range transport of wildfire smoke to Helsinki." Toxicology and Applied Pharmacology **215**(3): 341-353.
- Janssen, N. A., A. Yang, et al. (2014). "Oxidative potential of particulate matter collected at sites with different source characteristics." The Science of the total environment **472**: 572-581.
- Jayne, J. T., D. C. Leard, et al. (2000). "Development of an aerosol mass spectrometer for size and composition analysis of submicron particles." Aerosol Science and Technology **33**(1-2): 49-70.
- Jeong, S. C., C. Y. Shin, et al. (2014). "Gene expression profiling of human alveolar epithelial cells (A549 cells) exposed to atmospheric particulate matter 2.5 collected from Seoul, Korea." Molecular and cellular toxicology **10**: 361-368.
- Jerrett, M., R. T. Burnett, et al. (2005). "Spatial analysis of air pollution and mortality in Los Angeles." Epidemiology **16**(6): 727-736.
- Kampfath, T., A. Maiseyeu, et al. (2011). "Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways." Circulation Research **108**(6): 716-726.
- Karr, C., T. Lumley, et al. (2007). "Effects of subchronic and chronic exposure to ambient air pollutants on infant bronchiolitis." American Journal of Epidemiology **165**(5): 553-560.
- Kelly, F. J. (2003). "Oxidative stress: its role in air pollution and adverse health effects." Occupational and environmental medicine **60**(8): 612-616.
- Kelly, F. K. and J. C. Fussell (2012). "Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter." Atmospheric Environment **60**: 504-526.
- Kim, J. W., S. Park, et al. (2014). "The role of air pollutants in initiating liver disease." Toxicological research **30**(2): 65-70.
- Kim, S. Y., S. J. Dutton, et al. (2015). "The short-term association of selected components of fine particulate matter and mortality in the Denver Aerosol Sources and Health (DASH) study." Environmental health : a global access science source **14**: 49.
- Kioumourtzoglou, M. A., A. Zanobetti, et al. (2013). "The effect of primary organic particles on emergency hospital admissions among the elderly in 3 US cities." Environmental health : a global access science source **12**(1): 68.
- Knol, A. B., J. J. de Hartog, et al. (2009). "Expert elicitation on ultrafine particles: likelihood of health effects and causal pathways." Particle and Fibre Toxicology **6**: 19.
- Kobach Bolling, A., J. Pagels, et al. (2009). "Health effects of residential wood smoke particles: the importance of combustion conditions and physicochemical particle properties." Particle and Fibre Toxicology **6**: 29.
- Kodavanti, U. P., M. C. Schladweiler, et al. (2005). "Consistent pulmonary and systemic responses from inhalation of fine concentrated ambient particles: roles of rat strains used and physicochemical properties." Environmental Health Perspectives **113**(11): 1561-1568.
- Koelemeijer, R. B. A., C. D. Homan, et al. (2006). "Comparison of spatial and temporal variations of aerosol optical thickness and particulate matter over Europe." Atmospheric Environment **40**: 5304.
- Koenig, J. Q., G. Norris, et al. (1999). "An association between asthma emergency department visits for children and fine particles in Seattle." American journal of respiratory and critical care medicine **159**(3): A773-A773.

- Kouassi, K. S., S. Billet, et al. (2010). "Oxidative damage induced in A549 cells by physically and chemically characterized air particulate matter (PM_{2.5}) collected in Abidjan, Cote d'Ivoire." Journal of applied toxicology : JAT **30**(4): 310-320.
- Krewski, D., M. Jerrett, et al. (2009). "Extended follow-up and spatial analysis of the American Cancer Society study linking particulate air pollution and mortality." Research report(140): 5-114; discussion 115-136.
- Kroll, A., J. K. Gietl, et al. (2013). "In vitro toxicology of ambient particulate matter: correlation of cellular effects with particle size and components." Environmental toxicology **28**(2): 76-86.
- Kulshrestha, A., P. G. Satsangi, et al. (2009). "Metal concentration of PM(2.5) and PM(10) particles and seasonal variations in urban and rural environment of Agra, India." The Science of the total environment **407**(24): 6196-6204.
- Kumar, R. K., A. M. Shadie, et al. (2015). "Differential injurious effects of ambient and traffic-derived particulate matter on airway epithelial cells." Respirology **20**(1): 73-79.
- Kundu, S. and E. A. Stone (2014). "Composition and sources of fine particulate matter across urban and rural sites in the Midwestern United States." Environmental science. Processes & impacts **16**(6): 1360-1370.
- Labconco (2015). A Guide To Freeze Drying for the Laboratory Labconco.
- Labconco (2015). RapidVap N2 and N2/48 Dry Evaporation Systems. Kansas City
- Lauer, F. T., L. A. Mitchell, et al. (2009). "Temporal-spatial analysis of US-Mexico border environmental fine and coarse PM air sample extract activity in human bronchial epithelial cells." Toxicology and Applied Pharmacology **238**(1): 1-10.
- Lauer, F. T., L. A. Mitchell, et al. (2009). "Temporal-spatial analysis of U.S.-Mexico border environmental fine and coarse PM air sample extract activity in human bronchial epithelial cells." Toxicology and Applied Pharmacology **238**(1): 1-10.
- Lee, C. S. L., X. D. Li, et al. (2007). "Heavy metals and Pb isotopic composition of aerosols in urban and suburban areas of Hong Kong and Guangzhou, South China - Evidence of the long-range transport of air contaminants." Atmospheric Environment **41**(2): 432-447.
- Lee, J. H., J. H. Jeong, et al. (2013). "Toxic Trace and Earth Crustal Elements of Ambient PM_{2.5} Using CCT-ICP-MS in an Urban Area of Korea." Environmental Engineering Research **18**(1): 3-8.
- Lee, K. Y., J. J. Cao, et al. (2015). "Inhibition of the WNT/B-catenin pathway by fine particulate matter in haze: Roles of metals and polycyclic aromatic hydrocarbons." Atmospheric Environment **109**: 118-129.
- Lepers, C., V. Andre, et al. (2014). "Xenobiotic metabolism induction and bulky DNA adducts generated by particulate matter pollution in BEAS-2B cell line: geographical and seasonal influence." Journal of applied toxicology : JAT **34**(6): 703-713.
- Liao, D., D. J. Pequet, et al. (2006). "GIS approaches for the estimation of residential-level ambient PM concentrations." Environmental Health Perspectives **114**(9): 1374-1380.
- Lin, H. P., X. Y. Wu, et al. (2012). "Analysis of atmospheric particles and heavy metals character at the area of Lanzhou City in summer and winter." China Environmental Science **32**: 810-815.
- Lippmann, M., K. Ito, et al. (2006). "Cardiovascular effects of nickel in ambient air." Environmental Health Perspectives **114**(11): 1662-1669.
- Liu, Y., P. Koutrakis, et al. (2007). "Estimating fine particulate matter component concentrations and size distributions using satellite-retrieved fractional aerosol optical depth: Part 1 -

- Method development." Journal of the Air & Waste Management Association **57**(11): 1351-1359.
- Long, C. M., H. H. Suh, et al. (2001). "A pilot investigation of the relative toxicity of indoor and outdoor fine particles: in vitro effects of endotoxin and other particulate properties." Environmental Health Perspectives **109**(10): 1019-1026.
- Longhin, E., J. A. Holme, et al. (2013). "Cell cycle alterations induced by urban PM2.5 in bronchial epithelial cells: characterization of the process and possible mechanisms involved." Particle and Fibre Toxicology **10**: 63.
- Lough, G. C., J. J. Schauer, et al. (2005). "Emissions of metals associated with motor vehicle roadways." Environmental science & technology **39**(3): 826-836.
- Luque de Castro, M. D. and L. E. Garcia-Ayuso (1998). "Soxhlet extraction of solid materials: an outdated technique with a promising innovative future." Analytica Chimica Acta **369**(1-2): 1-10.
- Maciejczyk, P., J. H. Offenberg, et al. (2004). "Ambient pollutant concentrations measured by a mobile laboratory in South Bronx, NY." Atmospheric Environment **38**(31): 5283-5294.
- Manoli, E., D. Voutsas, et al. (2002). "Chemical characterization and source identification/apportionment of fine and coarse air particles in Thessaloniki, Greece." Atmospheric Environment **36**(6): 949-961.
- Mantecca, P., M. Gualtieri, et al. (2012). "Adverse Biological Effects of Milan Urban PM Looking for Suitable Molecular Markers of Exposure." Chemical Industry & Chemical Engineering Quarterly **18**(4): 635-641.
- Mar, T. F., G. A. Norris, et al. (2000). "Associations between air pollution and mortality in Phoenix, 1995-1997." Environmental Health Perspectives **108**(4): 347-353.
- Marcazzan, G. M., S. Vaccaro, et al. (2001). "Characterisation of PM10 and PM2.5 particulate matter in the ambient air of Milan (Italy)." Atmospheric Environment **35**(27): 4639-4650.
- Mateus, V. L., I. L. G. Monteiro, et al. (2013). "Study of the chemical composition of particulate matter from the Rio de Janeiro metropolitan region, Brazil, by inductively coupled plasma-mass spectrometry and optical emission spectrometry." Spectrochimica Acta Part B-Atomic Spectroscopy **86**: 131-136.
- Matte, T. D., Z. Ross, et al. (2013). "Monitoring intraurban spatial patterns of multiple combustion air pollutants in New York City: Design and implementation." Journal of Exposure Science and Environmental Epidemiology **23**(3): 223-231.
- Melaku, S., V. Morris, et al. (2008). "Seasonal variation of heavy metals in ambient air and precipitation at a single site in Washington, DC." Environmental pollution **155**(1): 88-98.
- Miller, K. A., D. S. Siscovick, et al. (2007). "Long-term exposure to air pollution and incidence of cardiovascular events in women." The New England Journal of Medicine **356**(5): 447-458.
- Mirowsky, J. E., L. Jin, et al. (2015). "In vitro and in vivo toxicity of urban and rural particulate matter from California." Atmospheric Environment **103**: 256-262.
- Mischler, S. E. (2013). A multistage cyclone array for the collection of size-segregated silica aerosols to test the hypothesis that ultrafine crystalline silica particles are more efficient in their activation of macrophages. PhD, University of Pittsburgh.
- Mitchell, S. and J. Perez-Ramirez (2015). X-ray diffraction. West Chester University. I. f. C. a. Bioengineering. Zurich.
- Mitschik, S., R. Schierl, et al. (2008). "Effects of particulate matter on cytokine production in vitro: a comparative analysis of published studies." Inhalation toxicology **20**(4): 399-414.

- Moller, P., P. H. Danielsen, et al. (2014). "Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles." Mutation research. Reviews in mutation research **762**: 133-166.
- Monn, C. and S. Becker (1999). "Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine (PM_{2.5}) and coarse particles (PM_{10-2.5}) in outdoor and indoor air." Toxicology and Applied Pharmacology **155**(3): 245-252.
- Montiel-Davalos, A., J. Ibarra-Sanchez Mde, et al. (2010). "Oxidative stress and apoptosis are induced in human endothelial cells exposed to urban particulate matter." Toxicology in vitro : an international journal published in association with BIBRA **24**(1): 135-141.
- Moore, D. K., M. Jerrett, et al. (2007). "A land use regression model for predicting ambient fine particulate matter across Los Angeles, CA." Journal of Environmental Monitoring **9**(3): 246-252.
- Mudway, I. S. (2004). Comparing the Toxicity of Particulate Matter (PM) Collected by Different Samplers. London, King's College London.
- NAAQS. (2015). "National Ambient Air Quality Standards (NAAQS)." from <http://www.epa.gov/ttn/naaqs/criteria.html>.
- Nakayama Wong, L. S., H. H. Aung, et al. (2011). "Fine particulate matter from urban ambient and wildfire sources from California's San Joaquin Valley initiate differential inflammatory, oxidative stress, and xenobiotic responses in human bronchial epithelial cells." Toxicology in vitro : an international journal published in association with BIBRA **25**(8): 1895-1905.
- Neubauer, K. R., S. T. Sum, et al. (1996). "Sulfur speciation in individual aerosol particles." Journal of geophysical research. Atmospheres : JGR **100**(D13): 18700-18707.
- Neupane, B., M. Jerrett, et al. (2010). "Long-term exposure to ambient air pollution and risk of hospitalization with community-acquired pneumonia in older adults." American journal of respiratory and critical care medicine **181**(1): 47-53.
- Ning, Y., A. Imrich, et al. (2000). "Alveolar macrophage cytokine production in response to air particles in vitro: role of endotoxin." Journal of toxicology and environmental health. Part A **59**(3): 165-180.
- Osornio-Vargas, A. R., J. C. Bonner, et al. (2003). "Proinflammatory and cytotoxic effects of Mexico City air pollution particulate matter in vitro are dependent on particle size and composition." Environmental Health Perspectives **111**(10): 1289-1293.
- Ostro, B., R. Broadwin, et al. (2006). "Fine particulate air pollution and mortality in nine California counties: results from CALFINE." Environmental Health Perspectives **114**(1): 29-33.
- Ostro, B., W. Y. Feng, et al. (2007). "The effects of components of fine particulate air pollution on mortality in California: results from CALFINE." Environmental Health Perspectives **115**(1): 13-19.
- Ostro, B., M. Lipsett, et al. (2010). "Long-term exposure to constituents of fine particulate air pollution and mortality: results from the California Teachers Study." Environmental Health Perspectives **118**(3): 363-369.
- Ostro, B., L. Roth, et al. (2009). "The effects of fine particle components on respiratory hospital admissions in children." Environmental Health Perspectives **117**(3): 475-480.
- Ostro, B. D., W. Y. Feng, et al. (2008). "The impact of components of fine particulate matter on cardiovascular mortality in susceptible subpopulations." Occupational and environmental medicine **65**(11): 750-756.

- OSU. (2015). "Average Compositions and Trace Gases." Volcano World Retrieved September 30, 2015, from <http://volcano.oregonstate.edu/average-compositions-and-trace-gases>.
- Owoade, K. O., P. K. Hopke, et al. (2015). "Chemical compositions and source identification of particulate matter (PM_{2.5} and PM_{2.5-10}) from a scrap iron and steel smelting industry along the Ife-Ibadan highway, Nigeria." Atmospheric Pollution Research **6**: 107-119.
- Pacyna, J. M. and E. G. Pacyna (2001). "An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide." Environmental Reviews **9**(4): 269-298.
- Pakkanen, T. A., K. Loukkola, et al. (2001). "Sources and chemical composition of atmospheric fine and coarse particles in the Helsinki area." Atmospheric Environment **35**(32): 5381-5391.
- Pancras, J. P., M. S. Landis, et al. (2013). "Source apportionment of ambient fine particulate matter in Dearborn, Michigan, using hourly resolved PM chemical composition data." Science of The Total Environment **448**: 2-13.
- Paraskevopoulou, D., E. Liakakou, et al. (2014). Long-term measurement of aerosol chemical composition in Athens, Greece. European Geosciences Union General Assembly Vienna.
- Park, J. H. (2015). "Impactors and Particle Size Distribution." Retrieved September 30, 2015, from [http://www.industrialventilation.net/ClassDownloads/IHS_725/L11%20Impactor%20Data%20Reduction/Impactor_Park%20\(1\).pdf](http://www.industrialventilation.net/ClassDownloads/IHS_725/L11%20Impactor%20Data%20Reduction/Impactor_Park%20(1).pdf).
- Parker, G. W. and H. Buchholz (1968). Size classification of submicron particles by a low-pressure cascade impactor. R. C. Division.
- Pattanaik, S., F. E. Huggins, et al. (2012). "Chemical speciation of Fe and Ni in residual oil fly ash fine particulate matter using X-ray absorption spectroscopy." Environmental science & technology **46**(23): 12927-12935.
- Pavagadhi, S., R. Betha, et al. (2013). "Physicochemical and toxicological characteristics of urban aerosols during a recent Indonesian biomass burning episode." Environmental science and pollution research international **20**(4): 2569-2578.
- Pekey, H., B. Pekey, et al. (2013). "Source Apportionment of Personal Exposure to Fine Particulate Matter and Volatile Organic Compounds using Positive Matrix Factorization." Water Air and Soil Pollution **224**: 1403.
- Peng, R. D., M. L. Bell, et al. (2009). "Emergency admissions for cardiovascular and respiratory diseases and the chemical composition of fine particle air pollution." Environmental Health Perspectives **117**(6): 957-963.
- Perrone, M. G., M. Gualtieri, et al. (2010). "Seasonal variations in chemical composition and in vitro biological effects of fine PM from Milan." Chemosphere **78**(11): 1368-1377.
- Peters, A., D. W. Dockery, et al. (2001). "Increased particulate air pollution and the triggering of myocardial infarction." Circulation **103**(23): 2810-2815.
- Philip, S., R. V. Martin, et al. (2014). "Global chemical composition of ambient fine particulate matter for exposure assessment." Environmental science & technology **48**(22): 13060-13068.
- Pitz, M., W. G. Kreyling, et al. (2001). "Change of the ambient particle size distribution in East Germany between 1993 and 1999." Atmospheric Environment **35**(25): 4357-4366.
- Pleil, J. D. (2014). Extraction Poster at ISES. C. Roper. Research Triangle Park.

- Pope, C. A., 3rd, R. T. Burnett, et al. (2002). "Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution." JAMA : the journal of the American Medical Association **287**(9): 1132-1141.
- Pope, C. A., 3rd, R. T. Burnett, et al. (2004). "Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease." Circulation **109**(1): 71-77.
- Pope, C. A., 3rd, R. T. Burnett, et al. (2011). "Lung cancer and cardiovascular disease mortality associated with ambient air pollution and cigarette smoke: shape of the exposure-response relationships." Environmental Health Perspectives **119**(11): 1616-1621.
- Pope, C. A., 3rd and D. W. Dockery (2006). "Health effects of fine particulate air pollution: lines that connect." Journal of the Air & Waste Management Association **56**(6): 709-742.
- Pope, C. A., M. J. Thun, et al. (1995). "Particulate Air-Pollution as a Predictor of Mortality in a Prospective-Study of Us Adults." American journal of respiratory and critical care medicine **151**(3): 669-674.
- Putaud, J. P., F. Raes, et al. (2004). "A European aerosol phenomenology-2: chemical characteristics of particulate matter at kerbside, urban, rural and background sites in Europe." Atmospheric Environment **38**(16): 2579-2595.
- Qin, X., K. A. Pratt, et al. (2012). "Seasonal comparisons of single-particle chemical mixing state in Riverside, CA." Atmospheric Environment **59**: 587-596.
- Queensland. (2013). "Tapered element oscillating microbalance." Collecting and measuring airborne particles Retrieved September 29, 2015, 2015, from <https://www.qld.gov.au/environment/pollution/monitoring/air-pollution/oscillating-microbalance/>.
- Rabinovitch, N., M. Strand, et al. (2006). "Particulate levels are associated with early asthma worsening in children with persistent disease." American journal of respiratory and critical care medicine **173**(10): 1098-1105.
- Rappazzo, K. M., J. L. Daniels, et al. (2015). "Exposure to Elemental Carbon, Organic Carbon, Nitrate, and Sulfate Fractions of Fine Particulate Matter and Risk of Preterm Birth in New Jersey, Ohio, and Pennsylvania (2000-2005)." Environmental Health Perspectives **123**(10): 1059-1065.
- Ravindra, A. K. Mittal, et al. (2001). "Health risk assessment of urban suspended particulate matter with special reference to polycyclic aromatic hydrocarbons: a review." Reviews on environmental health **16**(3): 169-189.
- Ren, S. L. (2009). Study on Pollution Characteristics of Heavy Metal in PM10 and PM2.5 in Urumqi. Master's, Xinjiang Agricultural University.
- Riva, D. R., C. B. Magalhaes, et al. (2011). "Low dose of fine particulate matter (PM2.5) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice." Inhalation toxicology **23**(5): 257-267.
- Rivero, D. H., S. R. Soares, et al. (2005). "Acute cardiopulmonary alterations induced by fine particulate matter of Sao Paulo, Brazil." Toxicological sciences : an official journal of the Society of Toxicology **85**(2): 898-905.
- Roberts, J. R., S. H. Young, et al. (2009). "The soluble nickel component of residual oil fly ash alters pulmonary host defense in rats." Journal of Immunotoxicology **6**(1): 49-61.
- Roper, C., L. G. Chubb, et al. (2015). "Characterization of ambient and extracted PM2.5 collected on filters for toxicology applications." Inhalation toxicology.

- Ruckerl, R., A. Ibal-Mulli, et al. (2006). "Air pollution and markers of inflammation and coagulation in patients with coronary heart disease." American journal of respiratory and critical care medicine **173**(4): 432-441.
- Saarikoski, S., H. Timonen, et al. (2008). "Sources of organic carbon in fine particulate matter in northern European urban air." Atmospheric chemistry and physics **8**: 6281-6295.
- Saldiva, P. H., R. W. Clarke, et al. (2002). "Lung inflammation induced by concentrated ambient air particles is related to particle composition." American journal of respiratory and critical care medicine **165**(12): 1610-1617.
- Sama, P., T. C. Long, et al. (2007). "The cellular and genomic response of an immortalized microglia cell line (BV2) to concentrated ambient particulate matter." Inhalation toxicology **19**(13): 1079-1087.
- Sampson, P. D., M. Richards, et al. (2013). "A regionalized national universal kriging model using Partial Least Squares regression for estimating annual PM2.5 concentrations in epidemiology." Atmospheric Environment **75**: 383-392.
- Sarnat, S. E., A. Winquist, et al. (2015). "Fine particulate matter components and emergency department visits for cardiovascular and respiratory diseases in the St. Louis, Missouri-Illinois, metropolitan area." Environmental Health Perspectives **123**(5): 437-444.
- Sawyer, K., S. Mundandhara, et al. (2010). "The effects of ambient particulate matter on human alveolar macrophage oxidative and inflammatory responses." Journal of toxicology and environmental health. Part A **73**(1): 41-57.
- Sbihi, H., J. R. Brook, et al. (2013). "A new exposure metric for traffic-related air pollution? An analysis of determinants of hopanes in settled indoor house dust." Environmental health : a global access science source **12**: 48.
- Schauer, C., R. Niessner, et al. (2003). "Polycyclic aromatic hydrocarbons in urban air particulate matter: decadal and seasonal trends, chemical degradation, and sampling artifacts." Environmental science & technology **37**(13): 2861-2868.
- Schauer, J. J. (2003). "Evaluation of elemental carbon as a marker for diesel particulate matter." Journal of exposure analysis and environmental epidemiology **13**(6): 443-453.
- Schaumann, F., P. J. Borm, et al. (2004). "Metal-rich ambient particles (particulate matter 2.5) cause airway inflammation in healthy subjects." American journal of respiratory and critical care medicine **170**(8): 898-903.
- Schaumann, F., C. Fromke, et al. (2014). "Effects of ultrafine particles on the allergic inflammation in the lung of asthmatics: results of a double-blinded randomized cross-over clinical pilot study." Particle and Fibre Toxicology **11**: 39.
- Schins, R., A. Knaapen, et al. (2002). "Cytotoxic and Inflammatory Effects of Coarse and Fine Particulate Matter in Macrophages and Epithelial Cells." The Annals of occupational hygiene **46**: 203-206.
- Schins, R. P., J. H. Lightbody, et al. (2004). "Inflammatory effects of coarse and fine particulate matter in relation to chemical and biological constituents." Toxicology and Applied Pharmacology **195**(1): 1-11.
- Schwarze, P. E., J. Ovrevik, et al. (2006). "Particulate matter properties and health effects: consistency of epidemiological and toxicological studies." Human & experimental toxicology **25**(10): 559-579.
- Seagrave, J., J. D. McDonald, et al. (2006). "Lung toxicity of ambient particulate matter from southeastern U.S. sites with different contributing sources: relationships between composition and effects." Environmental Health Perspectives **114**(9): 1387-1393.

- Shakya, K. M. and R. E. Peltier (2015). "Non-sulfate sulfur in fine aerosols across the United States: Insight for organosulfate prevalence." Atmospheric Environment **100**: 159-166.
- Shang, Y., L. Fan, et al. (2013). "Genotoxic and inflammatory effects of organic extracts from traffic-related particulate matter in human lung epithelial A549 cells: the role of quinones." Toxicology in vitro : an international journal published in association with BIBRA **27**(2): 922-931.
- Shi, T., R. Duffin, et al. (2006). "Hydroxyl-radical-dependent DNA damage by ambient particulate matter from contrasting sampling locations." Environmental Research **101**(1): 18-24.
- Sioutas, C., P. Koutrakis, et al. (1997). "Harvard/EPA ambient fine particle concentrators for human and animal exposures." Journal of Aerosol Science **28**: 1057-1061.
- Skarek, M., J. Janosek, et al. (2007). "Evaluation of genotoxic and non-genotoxic effects of organic air pollution using in vitro bioassays." Environment International **33**(7): 859-866.
- Slaughter, J. C., E. Kim, et al. (2005). "Association between particulate matter and emergency room visits, hospital admissions and mortality in Spokane, Washington." Journal of exposure analysis and environmental epidemiology **15**(2): 153-159.
- Slezakova, K., D. Castro, et al. (2013). "Impact of vehicular traffic emissions on particulate-bound PAHs: Levels and associated health risks." Atmospheric Research **127**: 141-147.
- Sonicators, Q. (2015). Ultrasonic Liquid Processors. Newtown.
- Steenhof, M., I. Gosens, et al. (2011). "In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands is associated with PM composition, size fraction and oxidative potential--the RAPTES project." Particle and Fibre Toxicology **8**: 26.
- Suh, H. H., A. Zanobetti, et al. (2011). "Chemical properties of air pollutants and cause-specific hospital admissions among the elderly in Atlanta, Georgia." Environmental Health Perspectives **119**(10): 1421-1428.
- Surratt, J. D., Y. Gomez-Gonzalez, et al. (2008). "Organosulfate formation in biogenic secondary organic aerosol." The journal of physical chemistry. A **112**(36): 8345-8378.
- Symons, J. M., L. Wang, et al. (2006). "A case-crossover study of fine particulate matter air pollution and onset of congestive heart failure symptom exacerbation leading to hospitalization." American Journal of Epidemiology **164**(5): 421-433.
- Tager, I. B., F. W. Lurmann, et al. (2010). "Temporal and spatial patterns of ambient endotoxin concentrations in Fresno, California." Environmental Health Perspectives **118**(10): 1490-1496.
- TAMU. (2015). "Biomarkers." from http://www-odp.tamu.edu/publications/207_IR/chap_10/c10_5.htm.
- Tecer, L. H., O. Alagha, et al. (2008). "Particulate matter (PM(2.5), PM(10-2.5), and PM(10)) and children's hospital admissions for asthma and respiratory diseases: a bidirectional case-crossover study." Journal of toxicology and environmental health. Part A **71**(8): 512-520.
- Thomas, G. and N. Osseiran. (2014). "Air quality deteriorating in many of the world's cities." Media Centre Retrieved October 4, 2015, from <http://www.who.int/mediacentre/news/releases/2014/air-quality/en/>.
- Thompson, A. M., A. Zanobetti, et al. (2010). "Baseline repeated measures from controlled human exposure studies: associations between ambient air pollution exposure and the systemic inflammatory biomarkers IL-6 and fibrinogen." Environmental Health Perspectives **118**(1): 120-124.

- Tian, J. and D. M. Chen (2010). "A semi-empirical model for predicting hourly ground-level fine particulate matter (PM_{2.5}) concentration in southern Ontario from satellite remote sensing and ground-based meteorological measurements." Remote Sensing of Environment **114**(2): 221-229.
- Tolbert, P. E., M. Klein, et al. (2007). "Multipollutant modeling issues in a study of ambient air quality and emergency department visits in Atlanta." Journal of exposure science & environmental epidemiology **17 Suppl 2**: S29-35.
- Tong, H., A. G. Rappold, et al. (2015). "Dietary Supplementation with Olive Oil or Fish Oil and Vascular Effects of Concentrated Ambient Particulate Matter Exposure in Human Volunteers." Environmental Health Perspectives.
- Topinka, J., P. Rossner, Jr., et al. (2011). "DNA adducts and oxidative DNA damage induced by organic extracts from PM_{2.5} in an acellular assay." Toxicology Letters **202**(3): 186-192.
- TSTC. (2015). "Chapter 3 - Alkanes." Organic Chemistry **1**, from <http://www.chemtech.org/cn/cn2323/2323-3.htm>.
- Urbanski, S. P., W. M. Hao, et al. (2009). Chemical Composition of Wildland Fire Emissions. Developments in Environmental Science. A. Bytnerowicz, M. Arbaugh, A. Riebau and C. Anderson, Elsevier. **8**.
- Urch, B., F. Silverman, et al. (2005). "Acute blood pressure responses in healthy adults during controlled air pollution exposures." Environmental Health Perspectives **113**(8): 1052-1055.
- USGS. (2015). "Polycyclic aromatic hydrocarbons (PAHs)." Environmental Health - Toxic Substances, from <http://toxics.usgs.gov/definitions/pah.html>.
- Valavanidis, A., T. Vlachogianni, et al. (2009). "8-hydroxy-2'-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis." Journal of environmental science and health. Part C, Environmental carcinogenesis & ecotoxicology reviews **27**(2): 120-139.
- Valavanidis, A., T. Vlachogianni, et al. (2013). "Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms." International journal of environmental research and public health **10**(9): 3886-3907.
- Valavanidis, A., T. Vlahoyianni, et al. (2005). "Comparative study of the formation of oxidative damage marker 8-hydroxy-2'-deoxyguanosine (8-OHdG) adduct from the nucleoside 2'-deoxyguanosine by transition metals and suspensions of particulate matter in relation to metal content and redox reactivity." Free radical research **39**(10): 1071-1081.
- van Donkelaar, A., R. V. Martin, et al. (2010). "Global estimates of ambient fine particulate matter concentrations from satellite-based aerosol optical depth: development and application." Environmental Health Perspectives **118**(6): 847-855.
- van Eeden, S. F., A. Yeung, et al. (2005). "Systemic response to ambient particulate matter: relevance to chronic obstructive pulmonary disease." Proceedings of the American Thoracic Society **2**(1): 61-67.
- Van Winkle, L. S., K. Bein, et al. (2015). "Biological Dose Response to PM_{2.5}: Effect of Particle Extraction Method on Platelet and Lung Responses." Toxicological sciences : an official journal of the Society of Toxicology **143**(2): 349-359.
- Verma, V., R. Rico-Martinez, et al. (2012). "Contribution of water-soluble and insoluble components and their hydrophobic/hydrophilic subfractions to the reactive oxygen

- species-generating potential of fine ambient aerosols." Environmental science & technology **46**(20): 11384-11392.
- Villeneuve, P. J., S. A. Weichenthal, et al. (2015). "Long-term Exposure to Fine Particulate Matter Air Pollution and Mortality Among Canadian Women." Epidemiology **26**(4): 536-545.
- Vincent, R., P. Goegan, et al. (1997). "Regulation of promoter-CAT stress genes in HepG2 cells by suspensions of particles from ambient air." Fundamental and applied toxicology : official journal of the Society of Toxicology **39**(1): 18-32.
- Vizcaya-Ruiz, A. D., M. E. Gutierrez-Castillo, et al. (2006). "Characterization and in vitro biological effects of concentrated particulate matter from Mexico City." Atmospheric Environment **40**: S583-S592.
- Wang, W., N. Jariyasopit, et al. (2011). "Concentration and photochemistry of PAHs, NPAHs, and OPAHs and toxicity of PM_{2.5} during the Beijing Olympic Games." Environmental science & technology **45**(16): 6887-6895.
- Waterman, D., B. Horsfield, et al. (2000). "Quantification of polycyclic aromatic hydrocarbons in the NIST standard reference material (SRM1649A) urban dust using thermal desorption GC/MS." Analytical Chemistry **72**(15): 3563-3567.
- Watson, J. G., J. C. Chow, et al. (2002). "Particle size relationships at the Fresno supersite." Journal of the Air & Waste Management Association **52**(7): 822-827.
- Watterson, T. L., B. Hamilton, et al. (2009). "Urban particulate matter causes ER stress and the unfolded protein response in human lung cells." Toxicological sciences : an official journal of the Society of Toxicology **112**(1): 111-122.
- Watterson, T. L., B. Hamilton, et al. (2012). "Urban particulate matter activates Akt in human lung cells." Archives of toxicology **86**(1): 121-135.
- Watterson, T. L., J. Sorensen, et al. (2007). "Effects of PM_{2.5} collected from Cache Valley Utah on genes associated with the inflammatory response in human lung cells." Journal of toxicology and environmental health. Part A **70**(20): 1731-1744.
- Wei, H., D. Wei, et al. (2011). "Oxidative stress induced by urban fine particles in cultured EA.hy926 cells." Human & experimental toxicology **30**(7): 579-590.
- WHO. (2014). "Ambient (outdoor) air quality and health." Media centre Retrieved September 29, 2015, from <http://www.who.int/mediacentre/factsheets/fs313/en/>.
- WHO. (2015). "Ambient Air Pollution." Global Health Observatory (GHO) data Retrieved October 28, 2015, 2015, from http://www.who.int/gho/phe/outdoor_air_pollution/en/.
- Wilson, D. W., H. H. Aung, et al. (2010). "Exposure of mice to concentrated ambient particulate matter results in platelet and systemic cytokine activation." Inhalation toxicology **22**(4): 267-276.
- Winberry, W. T. and G. Jungclaus (1999). Determination of polycyclic aromatic hydrocarbons (PAHs) in ambient air using gas chromatography/mass spectrometry. Compendium of methods for the determination of toxic organic compounds in ambient air. EPA.
- Wirth, K. and A. Barth. (2015, August 20, 2015). "X-Ray Fluorescence." Geochemical Instrumentation and Analysis Retrieved September 30, 2015, from http://serc.carleton.edu/research_education/geochemsheets/techniques/XRF.html.
- Wuyts, W. A., C. Dooms, et al. (2013). "The clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease." American journal of respiratory and critical care medicine **187**(7): 777.

- Xu, H. M., J. J. Cao, et al. (2012). "Lead concentrations in fine particulate matter after the phasing out of leaded gasoline in Xi'an, China." Atmospheric Environment **46**: 217-224.
- Xu, H. M., J. Tao, et al. (2013). "Characteristics of fine particulate non-polar organic compounds in Guangzhou during the 16th Asian Games: Effectiveness of air pollution controls." Atmospheric Environment **76**: 94-101.
- Yang, C. X., X. W. Peng, et al. (2012). "A time-stratified case-crossover study of fine particulate matter air pollution and mortality in Guangzhou, China." International archives of occupational and environmental health **85**(5): 579-585.
- Yang, L., X. Zhou, et al. (2012). "Airborne fine particulate pollution in Jinan, China: Concentrations, chemical compositions and influence on visibility impairment." Atmospheric Environment **55**: 506-514.
- Ying, Z., X. Xu, et al. (2014). "Long-term exposure to concentrated ambient PM_{2.5} increases mouse blood pressure through abnormal activation of the sympathetic nervous system: a role for hypothalamic inflammation." Environmental Health Perspectives **122**(1): 79-86.
- Zanobetti, A., M. Franklin, et al. (2009). "Fine particulate air pollution and its components in association with cause-specific emergency admissions." Environmental health : a global access science source **8**: 58.
- Zanobetti, A. and J. Schwartz (2009). "The effect of fine and coarse particulate air pollution on mortality: a national analysis." Environmental Health Perspectives **117**(6): 898-903.
- Zhang, J. M. and J. An (2007). "Cytokines, inflammation, and pain." International anesthesiology clinics **45**(2): 27-37.
- Zhang, Y., J. J. Schauer, et al. (2008). "Source apportionment of in vitro reactive oxygen species bioassay activity from atmospheric particulate matter." Environmental science & technology **42**(19): 7502-7509.
- Zhao, X., Z. Wan, et al. (2002). "Genotoxic activity of extractable organic matter from urban airborne particles in Shanghai, China." Mutation research **514**(1-2): 177-192.
- Zhou, J., K. Ito, et al. (2011). "Time-series analysis of mortality effects of fine particulate matter components in Detroit and Seattle." Environmental Health Perspectives **119**(4): 461-466.
- Zuk, M., L. Rojas, et al. (2007). "The impact of improved wood-burning stoves on fine particulate matter concentrations in rural Mexican homes." Journal of exposure science & environmental epidemiology **17**(3): 224-232.
- Zuo, J., J. F. Huang, et al. (2002). "A study of mass transport of linear polystyrene in polyethyl methacrylate gel by dynamic light scattering." Journal of Applied Polymer Science **86**(8): 2062-2066.