CARDIOPULMONARY TOXICITY INDUCED BY AMBIENT PARTICULATE MATTER
(BI CITY CONCENTRATED AMBIENT PARTICLE STUDY)

FINAL SCIENTIFIC REPORT

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ABSTRACT

Alterations in heart rate variability (HRV) have been reported in rodents exposed to concentrated ambient particles (CAPs) from different regions of the United States. The goal of this study was to compare alterations in cardiac function induced by CAPs in two distinct regional atmospheres. AirCARE 1, a mobile laboratory with an EPA/Harvard fine particle (particulate matter <2.5μm; $PM_{2.5}$) concentrator was located in urban Detroit, MI, where the PM mixture is heavily influenced by motor vehicles, and in Steubenville, OH, where PM is derived primarily from long-range transport and transformation of power plant emissions, as well as from local industrial operations. Each city was studied during both winter and summer months, for a total of four sampling periods. Spontaneously hypertensive rats instrumented for electrocardiogram (ECG) telemetry were exposed to CAPs 8 h/day for 13 consecutive days during each sampling period. Heart rate (HR), and indices of HRV (standard deviation of the average normal-to-normal intervals [SDNN]; square root of the mean squared difference of successive normal-to-normal intervals [rMSSD]), were calculated for 30-minute intervals during exposures. A large suite of PM components, including nitrate, sulfate, elemental and organic carbon, and trace elements, were monitored in CAPs and ambient air. In addition, a unique sampler, the Semi-Continuous Elements in Air Sampler (SEAS) was employed to obtain every-30-minute measurements of trace elements. Positive matrix factorization (PMF) methods were applied to estimate source contributions to $PM_{2.5}$. Mixed modeling techniques were employed to determine associations between pollutants/CAPs components and HR and HRV metrics. Mean CAPs concentrations in Detroit were 518 and 357 $\mu g/m^3$ (summer and winter, respectively) and 487 and 252 $\mu g/m^3$ in Steubenville.

In Detroit, significant reductions in SDNN were observed in the summer in association with cement/lime, iron/steel, and gasoline/diesel factors, while associations with the sludge incineration factor and components were less consistent. In winter, increases in HR were associated with a refinery factor and its components. CAPs-associated HR decreases in winter were linked to sludge incineration, cement/lime, and coal/secondary factors and the majority of their associated components. Specific relationships for increased rMSSD in winter were difficult to determine due to lack of consistency between factors and associated constituents.

In Steubenville, we observed significant changes in HR (both increases and decreases), SDNN, and rMSSD in the summer, but not in the winter. We examined associations between individual source factors/PM components and HRV metrics segregated by predominant wind direction (NE or SW). Changes in HR (both increases and decreases) were linked with metal processing, waste incineration, and iron/steel factors along with most of their associated elemental constituents. Reductions in SDNN were associated with metal processing, waste incineration, and mobile source factors and the majority of elements loading onto these factors. There were no consistent associations between changes in rMSSD and source factors/components. Despite the large number of coal-fired power plants in the region, and therefore the large contribution of secondary sulfate to overall PM mass, we did not observe any associations with the coal/secondary factor or with the majority of its associated components.

There were several inconsistencies in our results which make definitive conclusions difficult. For example, we observed opposing signs of effect estimates with some components depending on season, and with others depending on wind direction. In addition, our extensive dataset clearly would be subject to issues of multiple comparisons, and the "true" significant

results are unknown. Overall, however, our results suggest that acute changes in cardiac function were most strongly associated with local industrial sources. Results for coal-fired power plant-derived PM were inconsistent and largely nonsignificant, whereas traffic and metal-related industries appeared to have stronger impacts. Importantly, our findings highlight the value of combining both factor analytical and component-specific analyses when interpreting results; relying on only one approach may not provide a complete picture of exposure-health relationships.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

The Bi City Concentrated Ambient Particle Study (Bi City CAPS) was designed to investigate the sources and components of fine particulate matter ($PM_{2.5}$) responsible for adverse health effects, with an emphasis on coal-fired power plant-derived PM. The project was a multi-site field study to investigate the toxicity of secondary $PM_{2.5}$ derived from coal-fired power plants and other sources, including mobile sources. A mobile ambient particle concentrator coupled with a toxicological laboratory was employed to assess the health effects of CAPs in regions dominated by different PM sources. The project included two study locations, each evaluated during both winter and summer seasons to exploit different meteorological regimes. The first study location was located near the Ambassador Bridge in Detroit, MI, and is heavily influenced by both idling diesel truck traffic and gasoline-fueled commuter traffic. The second site is located in Steubenville, OH, an area dominated by both regional power plant-derived PM as well as local industrial sources. The selection of sites and seasons was based on achieving the highest degree of variability in PM composition and contribution from different sources.

Spontaneously hypertensive (SH) and normal (Wistar-Kyoto; WKY) rats were exposed to CAPs from these locations for 13 consecutive days and assessed for a wide suite of cardiopulmonary endpoints. The rats were implanted with telemeters and evaluated for pulmonary, systemic, and cardiovascular effects, including changes in heart rate (HR) and heart rate variability (HRV), cellularity and biochemical content of bronchoalveolar lavage fluid, lung histology, and serum analysis. At the same time, comprehensive exposure characterization was carried out to enable linking of adverse health impacts with PM composition. Integrated (8-hour) samples were analyzed for major PM components and trace elements, while a Semi-Continuous Elements in Aerosol (SEAS) sampler was used to collect every-30-minute samples for trace elemental analysis. This novel sampler allowed us to match 30-minute HR and HRV data with corresponding exposure data. Using the SEAS data, receptor modeling was carried out to enable attribution of toxicological effects to specific PM source factors. Non-HRV endpoints, which were collected at the end of each sampling season, were not linked to PM components/source factors as only four data points were available.

Detroit studies were carried out in July 2005 and February 2006. Mean CAPs concentrations were 518 and 357 $\mu g/m^3$ in summer and winter, respectively. In the summer, CAPs mass was dominated by sulfate and organic carbon (OC); in contrast, in the winter, CAP was dominated by nitrate and ammonium, and OC and sulfate were lower. Winter elemental concentrations were generally lower than in summer, with the exception of a few earth (Mg, Al, K) and trace (V, Mo) elements. Positive matrix factorization (PMF) was applied to the 268 30-minute SEAS samples. Combined with wind directionality analysis and correlation analysis with gaseous pollutants and elemental carbon, six factors were identified: secondary aerosol, gasoline- and diesel-powered vehicles, sludge incineration, refining, cement/lime production, and iron and steel manufacturing. In winter, the same methods were applied to the dataset, and six factors were resolved: coal/secondary sulfate (this factor would also contain primary sulfate from local sources), gasoline- and diesel-powered vehicles & iron/steel manufacturing, sludge incineration, refining, cement/lime production, and metal processing & iron/steel manufacturing. Due to low ambient $PM_{2.5}$ mass concentrations and less temporal variability of elemental concentrations in winter, more mixtures of source factors were observed than in summer.

In Detroit, significant reductions in SDNN were observed in the summer in association with cement/lime, iron/steel, and gasoline/diesel factors, while associations with the sludge incineration factor and components were less consistent. In winter, increases in HR were associated with a refinery factor and its components. CAPs-associated HR decreases in winter were linked to sludge incineration, cement/lime, and coal/secondary factors and the majority of their associated components. Specific relationships for increased rMSSD in winter were difficult to determine due to lack of consistency between factors and associated constituents. Overall, the results pointed to a strong influence from local industrial emissions as well as mobile sources.

Steubenville studies were conducted in August 2006 and February 2007. Mean CAPs concentrations were 487 and 252 $\mu g/m^3$ in summer and winter, respectively. As in Detroit, CAPs concentrations during the summer study were dominated by sulfate and organic carbon, but these components were also highest in the winter. Winter elemental concentrations were generally equivalent to summer concentrations, but a few elements were higher (>1.4 x) than in the summer. Six major factors contributed to the observed ambient $PM_{2.5}$ mass during the summer exposure study: coal combustion/secondary, mobile sources, metal coating/processing, iron and steel manufacturing, Pb factor, and waste incineration. In the winter, five factors were resolved, including coal combustion/secondary, gasoline- and diesel-powered vehicles, iron/steel manufacturing, metal coating/processing, and coke oven.

In Steubenville, significant changes in HR (both increases and decreases) were linked with metal processing, waste incineration, and iron/steel factors along with most of their associated elemental constituents. Reductions in SDNN were associated with metal processing, waste incineration, and mobile source factors and the majority of elements loading onto these factors. There were no consistent associations between changes in rMSSD and source factors/components. We did not observe any associations with the coal/secondary factor or with most of its associated components. No significant changes in HR or HRV parameters were observed in the winter As with Detroit, the Steubenville results indicate that changes in cardiac function were most strongly associated with local industrial sources as well as mobile sources.

Associations between source factors and their associated components at the two locations were broadly consistent. In Detroit, we observed associations between cardiac changes and cement/lime, iron/steel, sludge incineration, refinery, coal/secondary, and gasoline/diesel factors. In Steubenville, factors of metal processing, waste incineration, iron/steel, and mobile sources were most strongly associated with HR and HRV changes. In both cities, results indicate that changes in cardiac function were most strongly associated with local industrial sources with high emissions of metals. Results for coal-fired power plant-derived PM were inconsistent and largely nonsignificant, whereas traffic and metal-related industries appeared to have a stronger impact. However, inconsistencies in our results make definitive conclusions difficult. Importantly, the findings also highlight the value of combining both factor analytical and component-specific results when interpreting results; relying on only one of the two approaches may not provide a complete picture of exposure:health relationships.

1.0 INTRODUCTION AND BACKGROUND

Ambient PM is a very complex mixture: it is derived from multiple sources and is comprised not only of primary emissions from stationary and distributed sources, but also of secondary aerosol formed via atmospheric transformation. Because of this, it is a challenge to study in epidemiological and toxicological settings. Most research to date has focused on ambient PM mass and has not involved extensive exposure characterization; therefore, little is known regarding the effects of specific PM sources and components on human health. Because $PM_{2.5}$ is currently regulated on a mass basis, assuming all particles are toxicologically identical, it is clear that in order to determine the most effective way in which to regulate PM and ensure that reductions in PM do in fact improve human health, additional data are required. Such data would be obtained from conducting toxicological investigations focused on PM sources and components, and would be carried out at atmospherically relevant concentrations.

The first toxicological investigations of PM were prompted by epidemiological investigations such as the Harvard Six Cities Study (Dockery et al., 2003) which reported associations between fine particle mass ($PM_{2.5}$) and mortality and morbidity. Initial efforts to identify the constituents responsible for the effects observed focused on nonspecific particle effects and involved exposures to artificially-generated, largely monodisperse particles with inherently low toxicity, such as carbon black and polystyrene. Investigation of this hypothesis in both animals and humans has shown that, except at very high exposure concentrations, these particles have little effect. Later, as it became evident that sulfate and nitrate, both produced as secondary aerosols in the atmosphere through oxidation of combustion-derived NO_x and SO_2, comprise a large portion of the ambient fine particle mass, these were investigated for their potential role in the induction of health effects. Again, however, controlled exposures in humans (Leduc et al., 1995; Utell and Frampton, 1995) and animals (Heyder et al., 1999; Griese et al., 1999; Busch et al., 1986) have in general not provided convincing support for this hypothesis, although high levels of sulfate have been associated with increased airway resistance in guinea pigs (e.g., Chen et al., 1987). The inhalation toxicology community continues to pursue hypotheses related to specific PM components such as transition metals and organic constituents. However, although exposures to specific constituents are useful in investigating mechanistic hypotheses because of the ability to recreate identical exposure conditions, they do not accurately reflect ambient exposures because they lack other PM components and pollutants that may act synergistically to change the physicochemical and toxicological properties of the particles.

In the mid-1990s, the development of virtual impactor particle concentrators and centrifugal particle concentrators allowed inhalation exposures to ambient particles at concentrations 10 to 100-fold higher than found in ambient air. These concentrated ambient particles (CAPs) represent the naturally occurring PM mixture, and thus represent the most realistic PM exposure possible (Sioutas et al., 1995; Green and Armstrong, 2003; Demokritou et al., 2003; Savage et al., 2003). Airborne gases are not concentrated, allowing the researcher to test the effects of real-time exposures of animals to a particle mixture that maintains the physicochemical properties of ambient PM. Using these real-world particle mixtures, biological responses are seen at lower concentrations (e.g., $< 300\ \mu g/m^3$), than instillation and re-aerosolization studies.

To date, many CAPs studies in both humans and animals have not included extensive PM characterization, thus limiting their ability to provide insight into the PM sources and components responsible for health effects. However, these studies are of very high value in demonstrating health effects to ambient PM, and in investigating mechanisms by which health effects may occur. For example, CAPs (200-400μg/m^3) - induced pulmonary inflammation, epithelial cell remodeling, and systemic activation of inflammatory pathways has been documented in allergic rodents (Harkema et al., 2002; Carter et al., 2003; Wagner et al., 2003). Similar concentrations of CAPs also exacerbate inflammatory responses in bronchitic rats (Clarke et al., 1999; Kodavanti et al., 2000). A range of pulmonary responses has been documented in CAPs-exposed animals from little to no effect, to profound and unexpected results (Harkema et al., 2002; Smith et al., 2003; Gordon et al., 1998). A summary of CAPs exposure studies suggests these differences in experimental results are due to strain and health status of the animal, the length and frequency of exposure, and the dependence of geography, weather, and season on the particle mixtures that have been tested across the country (Green and Armstrong, 2003; Kodavanti et al., 1998). Pulmonary effects in humans exposed to CAPs in Chapel Hill, NC have included increased neutrophils in bronchoalveolar lavage fluid and increased blood fibrinogen (Ghio et al., 2000).

In the cardiovascular effects arena, exposure to CAPs has been shown to induce changes in blood cytology (Gordon et al., 1998), and changes in cardiac function (Gordon et al., 2000) in rats exposed to CAPs in Tuxedo, NY. In dogs exposed to Boston CAPs, both pulmonary and cardiac effects have been noted (Godleski et al., 2000). Using CAPs from Taipei, Taiwan, Cheng et al. (2003) demonstrated a reduction in heart rate and blood pressure in rats. In humans, Gong et al. (2003) showed changes in blood mediators of inflammation, in heart rate variability, and blood pressure in healthy and asthmatic adults exposed to CAPs in Los Angeles.

Some recent CAPs studies have attempted to evaluate the impact of specific PM components on health responses. Urch et al. (2004) exposed 24 healthy adults to CAPs in Toronto, Canada and measured brachial artery diameter (BAD) before and after exposure. A significant reduction in BAD (indicative of vasoconstriction) was associated with organic carbon and elemental carbon concentrations, and no association was observed for sulfate or any trace metals.

A number of studies have been conducted by the Harvard group using Boston CAPs, and have investigated the contribution of different CAPs components to health responses. Batalha et al. (2002) exposed chronic bronchitic rats to CAPs in Boston, MA, and found that vasoconstriction of small pulmonary arteries was associated with CAPs silicon and organic carbon concentrations. Clarke et al. (2000) conducted factor analysis on CAPs used for dog exposures, and showed associations between some pulmonary parameters and an aluminum/silicon factor, a vanadium/nickel factor, a bromine/lead factor, and a sulfur factor. This association with silicon was also found by Wellenius et al. (2003) using a canine model of coronary artery occlusion exposed to Boston CAPs. Significant ST-segment elevation was observed, which was correlated with the silicon concentration of the particles and other crustal elements possibly associated with street dust. Saldiva et al. (2003) exposed chronic bronchitic rats to CAPs and found associations between pulmonary inflammation and with vanadium and bromine concentrations. Finally, using a novel *in vivo* chemiluminescence technique, Gurgueira et al. (2002) reported oxidative stress associated with CAPs Fe, Mn, Cu, and Zn content (lung) and Fe, Al, Si, and Ti content (heart).

It can therefore be inferred, since CAPs consistently induce biological effects and yet sulfate, nitrate, and nonspecific particles do not, that other components of PM, or synergistic effects between PM components, are responsible for the induction of adverse effects. Unfortunately, efforts to fractionate ambient PM to further investigate compositional issues have met with limited success. Water-soluble components can be isolated, but to date only intratracheal instillation techniques have been used for such exposures; moreover, separation of organic material is not possible due to concerns about loss of chemical and physical sample integrity. In addition, exposure to PM collected using high-volume samplers, or extracts of such PM, is not reflective of ambient exposures because of distortion of physiochemical and toxicological properties during sample collection and storage. Particle size, chemical composition, and oxidative properties can change from the time of collection to the time of exposure. Exposure to specific combustion-derived sources, eg. diesel exhaust, can help to identify potential toxicants; however, because these emissions change significantly through atmospheric reaction, the representativeness of such exposures to real life conditions can be questioned. One study, Toxicological Evaluation of Realistic Emissions of Source Aerosols (TERESA) has incorporated atmospheric aging into the study design to evaluate the health impacts of coal-fired power plant emissions and mobile source emissions in a more realistic manner (e.g., Ruiz et al., 2007a, b; Kang et al., 2010) Similarly, exposures to combustion-related sources such as residual oil fly ash (ROFA) are unrealistic because of the unrepresentative size of this material, enrichment with transition metals, and the fact that most of the exposures to date have been conducted at very high concentrations.

We can therefore conclude that to elucidate the causative *sources* and *components* of PM-induced health effects, new strategies are required. First, studies that address the source question must consider the formation of secondary aerosols. Populations are not exposed to primary emissions, and in order to generate realistic exposures we must consider actual ambient particles once atmospheric transformation has occurred. Second, comparison of ambient PM in regions dominated by different sources should be conducted in order to maximize compositional variability and thus allow teasing out of specific source effects. Finally, to address the *components* issue, extensive exposure characterization must be conducted to allow correlation with toxicological endpoints and enable determination of the PM components that contribute to the health effects observed. Bi City CAPS, the subject study, meets all of the above criteria, and has generated important data with which to inform the regulatory process regarding $PM_{2.5}$.

2.0 OBJECTIVES

The primary objective of Bi City CAPS as stated in the original study plan was to evaluate the potential for adverse cardiopulmonary effects from ambient exposure to realistic (environmentally relevant) coal-fired power plant and traffic-related PM. Because we examined and considered other potential pollution sources, the objective was slightly modified to "evaluate the potential for adverse cardiopulmonary effects from ambient exposure to realistic (environmentally relevant) PM derived from multiple sources, including coal-fired power plants and traffic."

The formal statistical hypotheses (null hypotheses) that formed the basis of the project and were tested were that:

- Cardiopulmonary toxicity will not be observed in healthy or susceptible rats exposed to CAPs at any of the project locations.
- No differences in biological effects will be observed in response to CAPs in regions dominated by different PM sources.
- No differences in biological effects will be observed in response to CAPs with differing composition.

3.0 EXPERIMENTAL METHODS

3.1 Exposure Sites

Southwest Detroit, Michigan: The Detroit study was conducted next to Maybury Elementary School (4410 Porter St., Detroit, MI) in a residential neighborhood in southwest Detroit, where previous research has been conducted to assess the impact of ambient PM and its components on human populations (Morishita et al., 2009; Baxter et al., 2008; Lewis et al., 2005). This location was selected for study due to its strong influence from both diesel-emission and gasoline-emissions. The site is located within one block of Interstate 75, the primary southbound route from Detroit to Ohio, and one-half mile from the Ambassador Bridge. This important international border crossing connects Detroit to Windsor, Canada and is the busiest border crossing between the two countries. The Michigan Department of Transportation estimates daily traffic volume near this site to be as high as 100,000 vehicles/day. Figure 3.1a is a map of the Detroit area and shows the location of the inhalation exposure studies. The map also indicates some of the major $PM_{2.5}$ point sources in Wayne and Monroe Counties (EPA 2002). Seven counties in southeast Michigan, including Wayne County where the study was performed, have been designated as $PM_{2.5}$ non-attainment areas by the USEPA for violations of the National Ambient Air Quality Standard.

Steubenville, Ohio: The Steubenville study was conducted on the campus of the Franciscan University (40.379 N, 80.620 W; 306 m above mean sea level) overlooking the Ohio River. This location was selected because of its strong influence from both coal-fired power plant and local industrial emissions. There are five large coal-fired utility boilers within a 50-km radius of the site and seventeen within 100 km (Keeler et al., 2006). Figure 3.1b presents overview and detail maps of the Steubenville area showing the location of the inhalation exposure studies. The overview map also indicates $PM_{2.5}$ point sources in the area that are among the top 25 emitters in Pennsylvania, Ohio, and West Virginia (EPA 2002).

(a) (b)

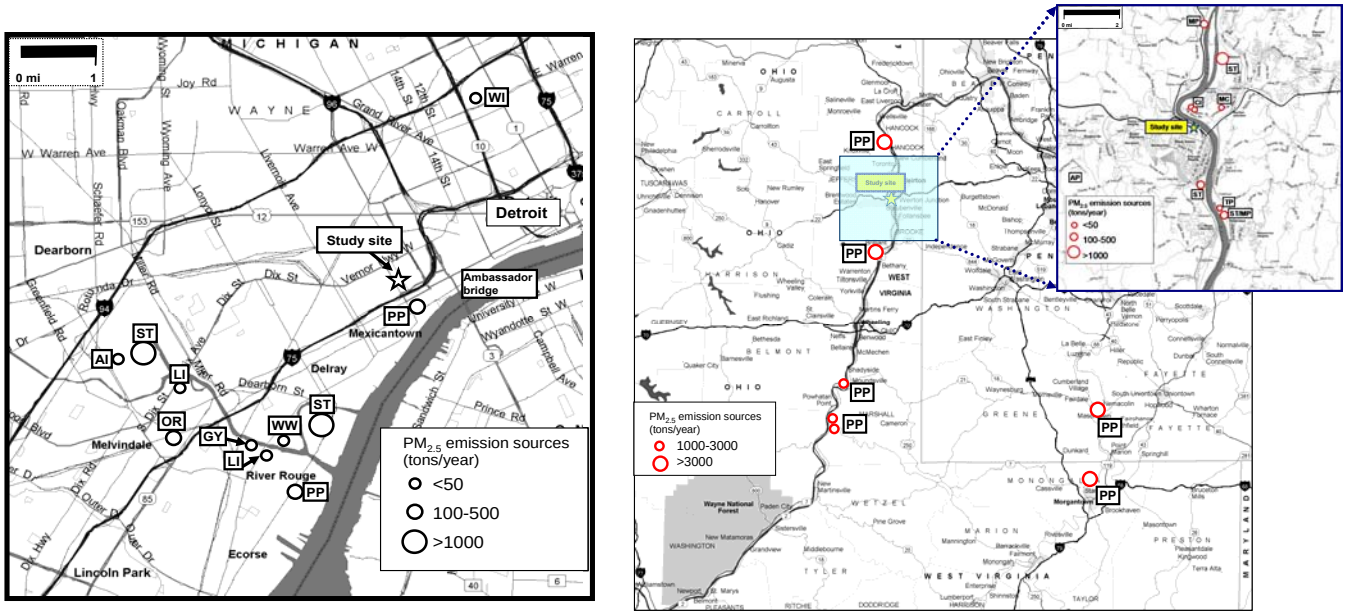


Figure 3.1
(a) A map of southwest Detroit showing the location of the sampling site and major industrial sources for $PM_{2.5}$ in Wayne and Monroe Counties, Michigan (USEPA-Air Data NET, 2002 (AI: auto industries, CE: cement industries, GY: gypsum industries, LI: lime industries, OR: oil refineries, PP: coal-fired power plants, ST: iron/steel industries, WI: waste incinerator, WW: waste water treatment and sludge incinerator).
(b) A map of the Steubenville area showing the location of the sampling site and major emission sources for $PM_{2.5}$ (USEPA-Air Data NET, 2002 (AP: air port, CI: metal can industries, PP: coal-fired power plants, ST: iron/steel industries, MC: metal coating, MP: metal processing, TP: tar production).

3.2 Ambient Aerosol and Gaseous Pollutant Measurements

All equipment and supplies used in trace measurement sampling were rigorously acid-cleaned in a 5-step, 11-day procedure (Landis and Keeler, 1997). Sample filters were placed in 50-mm acid-cleaned petri dishes and sealed with Teflon tape and triple-bagged in the field. The exception to this is the acid aerosol filters, which were handled in acid-free environments and ultrapure Milli-Q (MQ) water cleaned petri dishes. Particle-free gloves were worn when handling the samples in the field. All sample processing and analyses were carried out in the Class 100 clean laboratory at the University of Michigan Air Quality Laboratory (UMAQL).

Size-segregated aerosol sampling was performed with integrating samplers including micro-orifice impactors (MOIs), as well as $PM_{2.5}$ cyclone samplers. These samples were collected for the exposure periods on each day and for the overnight samples to determine chemical and physical characterization of the urban PM (Table 3.1). The volume of air drawn through each particulate sampling train was determined using a calibrated dry test meter (DTM) (Schlumberger, Owenton, KY). The DTMs were calibrated against a spirometer, a primary calibration standard, before and after being deployed in the field. In addition, a calibrated rotameter was used to check the flow rate at the beginning and end of the sampling period.

Table 3.1: Ambient PM Measurements and Analyses

Measurement	PM property	Sampling Media	Sample Duration (hr)	Analytical Method
TEOM	Mass	-	Continuous	-
APS 3020	Size (0.5-20 μm)	-	Continuous	-
SMPS 3936	Size (0.02-1 μm)	-	Continuous	-
Filter ($PM_{2.5}$)	Trace elements	Teflon	8	ICP-MS
ADS	Acid gases & aerosols and major ions	Teflon /denuders	8	IC/pH
Filter	Elemental & organic carbon	Quartz	8	TOA

$PM_{2.5}$: Fine particle mass was sampled onto 47-mm Teflon (PTFE) membrane filters (Gelman Sciences, Ann Arbor MI). Vacuum pumps were used to draw air through a Teflon-coated cyclone inlet (URG Inc.) at a flow rate of 16.7 LPM. In addition, levels of $PM_{2.5}$ were monitored continuously with a Rupprecht and Patashnick Series 1400a Tapered Element Oscillating Microbalance (Rupprecht and Patashnick Inc., Albany, NY) equipped with a sharp-cut cyclone inlet (BGI Inc., Waltham, MA) in near real-time (30 minute intervals). The TEOM filter was heated to 40°C to minimize interference from particle-bound water and semi-volatile components. Rupprecht and Patashnick reported the estimated precision as ±1.5 μg/m³ for 1-hour averages. Filter based measurements of $PM_{2.5}$ using URG cyclone inlets were compared to the $PM_{2.5}$ measured by TEOM.

Annular Denuder/Filter Pack Systems (ADS): Annular denuder/filter packs were employed to collect the acidic gaseous species including nitrous acid (HONO), nitric acid (HNO_3), and sulfur dioxide (SO_2), and ammonia (NH_3) and inorganic fine particulate ions (SO_4^{2-}, NO_3^-, NH_4^+, H^+). A detailed description of the annular denuder sampling system has previously been reported (Koutrakis et al., 1988; Keeler et al., 1991).

Scanning Mobility Particle Sizer (SMPS): **A** Scanning Mobility Particle Sizer (TSI, 3936) system was used to measure 5-minute average concentrations of sub-micrometer particles ranging from 10 to 800 nm in diameter. It consisted of an Electrostatic Classifier to determine particle size and a Condensation Particle Counter (CPC) to determine particle concentrations. Operation of the APS and SMPS together allowed measurements of the size distribution from about 10 nm to 20 μm.

Gaseous Pollutants: Ambient ozone (O_3) was measured using a continuous UV photometric analyzer (TECO 49). The analyzer was calibrated against an O_3 calibrator (TECO 5009). Samples were collected at a flow rate of 2.0 LPM. Voltage corresponding to the concentration was transmitted from the analyzer to a data logger. The average O_3 concentration was recorded every 30 min. The limits of detection (LOD) of and precision of the instrument were listed as 1 part per billion (ppb) and ±1 ppb, respectively. Continuous ambient carbon monoxide (CO) measurements were made using a non-dispersive infrared analyzer (TECO 48S). The instrument specifications list the LOD and precision of the instrument as 0.04 ppm and ±0.1 ppm, respectively.

Ambient nitrogen oxide (NOx) concentrations were measured using a commercial chemiluminescence detector (TECO 42S). The instrument has a single photomultiplier tube that automatically cycles between the NO and NOx modes. Signals from the photomultiplier tube are conditioned and then send to the microprocessor where a mathematical algorithm is utilized to calculate three independent outputs - NO, NO_2 and NOx. The LOD of instrument and precision were listed as 0.4 ppb and ±0.4 ppb, respectively. Finally, ambient SO_2 concentrations were measured using a pulsed fluorescence technique (TECO 43S). The instrument specifications list the instrument LOD as 0.6 ppm and precision as ±1 ppb.

3.3 Measurements of Concentrated Ambient Particles (CAPs)

CAPs were only collected during animal inhalation exposure periods. As previously described (Sioutas et al., 1995), the Harvard/EPA Ambient Fine Particle Concentrator consists of a series of three virtual impactors to concentrate ambient fine particles. The output flow from the third stage of the concentrator is ~50 LPM with 15 LPM used for characterization measurements (Table 3.2) and the remaining 35 LPM of flow administered to the animal exposure chambers.

Table 3.2: CAPs Measurements and Analyses

Measurement	PM Property	Sampling Media	Sample Duration (hr)	Analytical Method
TEOM	Mass	-	Continuous	-
MOI (stage 2)	Size (6 stages) /trace metals	Teflon	8	Gravimetric ICP-MS
Filter (stage 3)	Trace elements	Teflon	8	Gravimetric ICP-MS
Filter (chamber)	Acid aerosols and ions	Teflon/ denuders	8	Gravimetric IC
Filter (chamber)	Elemental & organic carbon	Quartz	8	TOA

Continuous measurement of CAPs was performed via sampling ports on the exposure chamber. A TEOM 1400a was placed in-line to continuously measure the concentrated fine mass concentration during the 8-hour exposure periods. The TEOM sampled CAPs at a flow rate of 3 LPM and the TEOM filter was also heated to 40°C to match the ambient TEOM measurements. The mass of CAPs was also determined by placing 47-mm Teflon (PTFE) filters (Gelman Science) in Teflon/Teflon-coated aluminum filter packs (URG) attached to the back of the animal exposure chamber, which is downstream of the concentrator, at flow rates of 3 LPM. Sodium carbonate-coated backup filters were placed behind the Teflon filters to correct for volatilization losses of nitrate from the Teflon filter during sampling. Pre-baked Quartz filters (Gelman Science) were placed in URG filter packs mounted on the exposure chamber and sampled at flow rates of 3 LPM. A six-stage MOI was placed after the concentrator's second stage to determine the size distribution.

3.4 Semi-Continuous Elements in Aerosol Sampler (SEAS)

The details of the SEAS have been previously described in detail (Kidwell and Ondov, 2001; 2004). Briefly, the SEAS consists of a state-of-the-art dynamic aerosol concentrator in which particles are grown by condensation of water vapor to allow separation from the air stream. $PM_{2.5}$ is sampled at 90 L min^{-1} and delivered to a sample collector for off-line analysis. In 30 minute sampling intervals, enough slurry (20-30 ml) is collected to permit up to 40 elements to be analyzed using high-resolution ICP-MS, and perform the analysis in triplicate. Minimal sample volumes are needed using micro-liter introduction systems used at the ICP-MS facility at the University of Michigan.

3.5 Analytical Methods

All filters collected for ambient PM and CAPs characterization were prepared and analyzed at the UMAQL. Sample handling, processing, and analysis took place in a Class 100 ultra-clean laboratory designed for ultra-trace element analysis with an emphasis on low-level environmental determinations. A brief overview of the analytical methods used at the UMAQL is provided below.

Gravimetry: All gravimetric determinations of Teflon filters were made using a microbalance (MT-5 Mettler Toledo, Columbus OH) in a temperature/humidity-controlled environment (filter equilibrium within ±5% for relative humidity between 30-40% and within ±2°C for temperature between 20°C-23°C) as described in Federal Reference Method (EPA Code of Federal Regulations, 1999). Measurements including field blanks, filter-lot blanks, replicate analyses, and externally certified standard weights were incorporated into all gravimetric analyses for quality assurance (QA) and quality control (QC) purposes. 10% of the pre- and post weighted filters were re-weighed for the replicate analysis and all the values were within ±15 μg.

Carbonaceous Material: All the quartz filters were pre-baked at 550°C for two hours and stored in a -40°C freezer before sampling. PM samples collected on quartz filters were maintained at -40°C after sampling and were analyzed for temperature-resolved carbon fractions (organic carbon (OC1), OC2, OC3, OC4, pyrolized carbon (PC), EC) by a thermal-optical analyzer using NIOSH Method 5040 (Sunset Labs, Forest Grove, OR). The estimated LODs for organic and elemental carbon were 0.66 $\mu g/cm^2$ and 0.03 $\mu g/cm^2$, respectively. Precision of the organic and elemental carbon data was 5.1% and 17.3%, respectively, based on replicate analyses.

Inorganic Ions: Annular denuder/filter pack samples were analyzed for gaseous species HNO_3, HONO, SO_2 and NH_3 as well as fine particles SO_4^{2-}, NO_3^- and NH_4^+ by ion chromatography (Model DX-600, DIONEX, Sunnyvale, CA). Detailed description of the annular denuder sampling system and the analytical method have been provided previously (Koutrakis 1988; Keeler et al., 1991). After sampling, filter packs were disassembled in an ammonia-free hood and the Teflon filters were placed in a 6-ml solution of 10^{-4} perchloric acid. The sodium carbonate-coated backup filters were placed in 5 mL of ultra pure MQ water (Millipore, Bedford, MA). Denuders were extracted with 10 mL of ultra pure MQ water. Extracts were analyzed for anions and cations by ion chromatography. This analysis method incorporates routine daily QA/QC measures such as field blanks, MQ blanks, replicate analyses and external standards (Simulated

Rainwater #2, ICA and ICB) (High-Purity Standards, Charleston, SC). Accuracy for sulfate and nitrate were determined to be 1.2 % and 6.2%, respectively. Precision of the anion analysis was 9.0% for nitrate and 2.6% for sulfate, which were calculated based on replicate analyses.

Trace Elements: After completion of gravimetric analysis, Teflon sample filters were placed in 15mL centrifuge tubes and were wetted with 150 µl of ethanol before extraction in 10 mL of 10% HNO_3 that was made on the same day it was to be used and dispensed using a repippetor calibrated for 10 mL. The extraction solution was then sonicated for 48 hours in an ultrasonic bath, and then passively acid-digested for two weeks. Extracts were then analyzed via inductively coupled plasma-mass spectrometry (ICP-MS) (ELEMENT2, Thermo Finnigan, San Jose, CA) for a suite of elements including Rb, Sr, Y, Mo, Cd, Sb, Ba, Cs, La, Ce, Sm, Pb, Na, Mg, Al, P, S, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As and Se. The ELEMENT2 is a double focusing magnetic sector field ICP-MS. Calibration curves were created using multi-element standards (SPEX CertiPrep, Metuchen, NJ) in 10% nitric acid solution and the matrix was matched with the samples. This analysis method incorporates daily QA/QC measures such as field blanks, acid blanks, laboratory blanks, and replicate analyses. The National Institute of Standards and Technology (NIST, Gaithersburg, MD) SRM 1643d was used as a quality control standard to check the calibration of the instrument. If the measured values were not within ±20% of the expected values, the calibration procedure was repeated.

3.6 Air Quality Data Analysis

Data describing the parameters of particle number, mass, and species are expressed as the mean group value ± the standard error of the mean.

Receptor Models: Source apportionment analysis was conducted on the exposure CAPs in order to enable linking of specific PM sources to cardiovascular endpoints. In this study, positive matrix factorization (PMF) – a multivariate receptor modeling approach – was employed. In particular, USEPA PMF 3.0 was applied to the four independent particle composition data sets (Detroit and Steubenville, two seasons each) to calculate the source compositions and source contributions to each sample based on the measured data. The sum of the analytical and sampling uncertainty and method detection limit (MDL) were used to calculate the uncertainty (U) assigned to each measured concentration data point as follows:

$$U = \sqrt{(\sqrt{(SC)^2 + (AM)^2} \times (concentration))^2 + (MDL)^2}$$

(where SC = the uncertainty of sample collection and AM = the uncertainty of analytical measurement). Values below the MDL were replaced by half of the MDL for the measured data. Based on EPA's PMF guidelines (2008) and Paaterro and Hopke (2003), |signal-to-noise ratios were used to determine species categorization. If the signal-to-noise ratio was less than 0.2, it was excluded from the analysis. If the signal-to-noise ratio was greater than 0.2 but less than 2, it was categorized as "weak" and down-weighted. The optimal solution was determined by multiple model runs to examine the effect on the numbers of factors assigned and the different FPEAK values on the range of results that were both physically reasonable and where the

objective function Q value did not change substantially. The FPEAK value was set at zero, where the value of robust Q reaches a global minimum.

Trajectory Analysis: Air mass transport to the study site was modeled using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) Model Version 4.8 (Draxler and Hess, 1997). This model was used to understand the transport of particles that impacted communities by calculating air mass backward trajectories using the National Weather Service's Eta Data Assimilation System for 2005-2006. All data were obtained from the National Oceanic and Atmospheric Administration's Air Resources Laboratory.

3.7 AirCARE1 Exposure Facility

AirCARE1, constructed collaboratively by Michigan State University and the University of Michigan, is a 53-ft mobile laboratory containing whole-body inhalation exposure chambers, a biomedical lab, an inhalation exposure lab, and an atmospheric monitoring lab (Harkema et al., 2004). The inhalation toxicology lab inside AirCARE1 is outfitted with an EPA/Harvard $PM_{2.5}$ Concentrator and two Hinner full-body animal exposure chambers (HC-I 00, Lab Products, Maywood, NJ). By drawing in 5000L/min of ambient air through a $PM_{2.5}$ size-selective inlet and then through a series of virtual impactors, fine particles are concentrated 20 to 30 fold, depending on particle size distribution and meteorological conditions. The details of the concentrator performance and the exposure systems in AirCARE 1 have been described in detail elsewhere (Sioutas, 1997; Sioutas et al., 1995). The CAPs are drawn into the exposure chamber at 50L/min for inhalation by the CAPs rats. Alongside the CAPs exposure chamber is an identical Hinner chamber pulling in HEPA-filtered air with matching flow rate which houses the control group, or "AIR" rats. Inside each chamber, custom-built cage separates all 16 rats from one another. Air pressure and pressure were balanced in both exposure chambers by external pumps (Gast Manufacturing, Benton Harbor, MI).

3.8 Animal Exposures

At each site and each season, 16 spontaneously hypertensive (SH) rats and 16 Wistar Kyoto rats (Charles River Laboratories, Portage, MI), 13-14 weeks of age, were used. Animals were maintained and used in accordance with the National Institutes of Health guidelines for the care and use of animals in research, and all protocols were approved by the All University Committee on Animal Use and Care at Michigan State University. Rats were initially housed for 10-12 days in animal facilities at Michigan State University for Detroit expsoures studies, or at the National Institute for Occupational Safety and Health (NIOSH) in Morgantown , WV for Steubenville exposure studies. Five days prior to exposures rats were moved to AirCARE1, where they were housed 2 per polycarbonate cage on corn cob bedding with *ad libitum* access to food and water. Exposures were carried out in two stainless steel Hinners-type whole body inhalation chambers; one received CAPs while the other received HEPA-filtered clean air at the same flow rate as the experimental group. Eight SH and 8 WKY rats were exposed in each chamber from 7:00am – 3:00pm for thirteen consecutive days. After exposures, animals were removed from chambers and returned to their cages. Housing and experimental conditions were maintained at 21 – 24 C and 50 – 60% relative humidity. Light cycles were set on a 12-hr light/dark cycle beginning at 6 am.

3.9 ECG Monitoring and Telemetry

Two to three weeks before exposures, SH rats were surgically implanted with bipotential PhysioTel transmitters (# TA11CTA-F40; Data Sciences International, St. Paul, MN) that emit radio signals of electrocardiograms, heart rate and temperature. Transmitters were placed intraperitoneally with leads terminating in a Lead II configuration. Telemetry receivers (RLA300; DSI) were modified and affixed inside individual cages in exposure chambers that were customized for telemetry studies. Due to space limitations of radiotelemetry, only eight rats could be instrumented in each chamber. We chose to employ all 8 positions for SH rats to collect HR and ECG waveforms. Data streams of 30 second duration were collected every 5 minutes during exposures. Eight WKY rats in each exposure chamber were used to compare exposure-related changes in biochemical and histological parameters between strains.

3.10 Collection and Analysis of Biological Samples

Immediately after exposures, rats were transported to laboratories at Michigan State University (Detroit exposures) or NIOSH (Steubenville exposures). Eighteen hours after exposures rats were anesthetized (i.p. pentobarbital) and blood collected from the abdominal aorta prior to sacrifice by exsanguination. The trachea was cannulated and heart/lung removed *en bloc*. The right lung lobes were removed for future analysis if necessary. Bronchoalveolar lavage fluid (BALF) was collected from the left lobe (2 x 5ml saline), total cell numbers were determined using a hemocytometer, and cell free BAL fluid was generated after centrifugation. The left lung was then fixed for at least 24h in 10% neutral-buffered formalin.

Bronchoalveolar Lavage Fluid (BALF): Total cells and differentials (eosinophils, neutrophil, macrophage /monocytes and lymphocytes) were determined on a cytospin slide stained with Diff-Quik. In cell-free BALF, protein content was determined by the bicinchoninic acid assay method using a commercial kit (Pierce).

***Serum Analysis*:** Serum concentrations of interleukins IL-1, -6,-10, and of TNFα and IFNγ were determined using Cytometric Bead Assay methods (BD Bioscience) using a laser flow cytometer (FACScalibur). An ELISA approach was used to estimate serum levels of C-reactive protein (Alpha Diagnostics, San Antonio, TX).

Preparation of Lung Tissues for Airway Histopathology and Morphometry: Specific approaches for preparing site-specific analyses in the lung are detailed elsewhere (Harkema and Hotchkiss 1991; Harkema and Hotchkiss 1992). Two transverse sections (5 μm) were taken at the level of the 5^{th} and 11^{th} airway generation. Lung tissue sections were histochemically stained with 1) hematoxylin and eosin (H&E) for evaluation of epithelial and tissue 2) Alcian Blue (pH 2.5)/Periodic Acid Schiff's sequence (AB/PAS) to detect acidic and neutral mucosubstances for quantification of intraepithelial mucosubstances (IM).

Morphometric Analyses: Morphometric analyses of IM was conducted as previously described (Harkema et al., 1997; Harkema and Hotchkiss, 1992) using digitized images and NIH Image analysis software. The volume density (Vs) of IM in the airway epithelium was determined by

standard morphometric techniques (Harkema et al., 1987). Amounts of IM per unit area are expressed as the mean volume (nl) of IM / mm^2 of basal lamina.

3.11 ECG Data Processing

Automated ECG analysis was applied to the recorded signals using Physiostat® ECG Analysis Data Analysis Software to generate the raw data for heart rate variability analysis. Automated analysis allows for R-wave detection on a beat-to-beat basis to calculate the interbeat interval (IBI) for the entire recording. The IBI for all normal beats (N-N intervals) were used to calculate time-domain measures of heart rate variability: SDNN (standard deviation of the normal-to-normal intervals) and rMSSD (square root of the mean squared differences of successive normal-to-normal intervals).

Outlying data beyond a reasonable physiological range of IBI (50-400 msec) were initially removed so as to not to distort the normal distribution of IBI when calculating the time-domain parameters. Subsequent data points beyond two standard deviations of the mean were eliminated. Breaks in the data often incurred a shift resulting in an erroneous r-MSSD calculation. Consecutive IBI readings exceeded 30msec were removed to reduce misleading variability in the dataset caused by the breaks. The average number of recordings per 30-minute time period exceeded 700; if the number of recordings for each rat fell below 200 the data were excluded from the dataset.

3.12 Statistical Methods

Data describing the magnitude of pulmonary responses are described as the mean group value, and its dispersion calculated as the standard error of the mean (mean $\pm$ SEM). SigmaStat statistical software (Jandel Scientific) was used to perform two-way analysis of variance (ANOVA) for factors of strain and exposure (Air vs. CAPs). Significant differences between group means were based on comparisons using Student-Newman Keuls *post hoc* test, with the criterion for significance set at $p \leq 0.05$.

For associations of cardiac responses and air pollution exposure, two levels of mixed model analyses were conducted to determine (1) differences in HR and HRV between air- and CAPs-exposed SH rats; and (2) where these differences were significantly different, associations of air pollutant measures with changes in HR and HRV in CAPs-exposed rats.

For the non-continuous toxicological endpoints (BAL fluid analysis, histology, serum analysis), linking of response to CAPs components and source factors was not possible, due to the fact that the endpoints were measured only once (at the end) during each exposure period. With such a small number of data points (n=4), only very simple comparisons could be made between sites and seasons.

Repeated measures Analysis of Variance (ANOVA) was used in a mixed model framework (PROC MIXED, SAS, Carey, NC). The basic structure of a mixed model that tests for marginal differences in AIR and CAPs rats as well as for changes in time profiles of HRV between AIR versus CAPs rats is given below in Equation 3.1 to detect high-resolution changes in HRV. The

"Group" refers to whether rats are CAPs or AIR, and "Time" refers to each 30-minute data point. For each HRV parameter, $\boldsymbol{Y_{ij}}$, the interaction term, $\boldsymbol{\beta_3}$, of "Group * Time" ultimately determines if CAPs-exposed rats are statistically different from the control group ($p<0.05$). Data comprised of both 8-hour means and 30-minute means were analyzed using this approach.

Equation 3.1 Description of the mixed model that was used to compare the difference in HRV measures of AIR and CAPS as averaged over time and to describe any changes in HRV profiles across time.

$$\boldsymbol{Y_{ij} = (\beta_0 + u_i) + \beta_1(Group_i) + \beta_2(Time_{ij}) + \beta_3(Group_i * Time_{ij}) + e_{ij}}$$

Y_{ij} = Response or HRV measures (e.g. Heart Rate, ln(SDNN), ln(r-MSSD)) measured on the ith rat on the jth time point.
$Group_i$=A binary variable indicating the Group that rat i belongs to, (e.g. AIR, CAPs)
$Time_{ij}$=Time corresponding to the jth observation on the ith rat
β_0=Overall intercept
u_i= Random intercept corresponding to the ith rat
β_1 =Overall effect of Group (e.g. CAPs versus AIR)
β_2 =Overall effect of Time (e.g. Day1 versus Day2, averaged HRV across both groups)
β_3 =Coefficient corresponding to Group and Time interaction
e_{ij} =Measurement error corresponding to the jth observation on the i-th rat. u_i and e_{ij} are assumed to be independently distributed random variables with mean zero and variance σ^2_b and σ^2 respectively. Given this assumption, the resultant covariance matrix has a compound symmetry structure.

Trace elements collected from filters (8 hour collection time) and from SEAS slurry samples (30 minute collection time), other PM components, and gases were time-matched with HR and HRV parameters. Equation 3.2 is a mixed model that was used to determine the effect estimate of a given pollutant with each HRV parameter. This equation could be calculated using the raw mass values for each pollutant; however, since the scales for each constituent are on different orders of magnitude, it was more useful to normalize the data, allowing for a comparative analysis of the effect estimates of each pollutant. Therefore, the raw concentration for every component of $PM_{2.5}$ was normalized using its inter-quartile range. The 75^{th} and 25^{th} percentiles were calculated for each pollutant. The interquartile range (IQR) for each component was then internally scaled based on the pollutant's inherent variability rather than its raw concentration. For example, the effect estimate of S (mean=3000 ng/m^3) can be reasonably compared against the effect estimate of La (mean=2 ng/m^3), to determine which pollutant was more strongly associated with HRV changes.

Equation 3.2 Mixed modeling equation used to associate air pollution concentrations with the changes in HRV in CAPs-Exposed Rats.

$$\boldsymbol{Y_{ij} = (\beta_0 + u_i) + \beta_1(Pollutant_j) + e_{ij}}$$

Y_{ij} = Response or HRV measures (Heart Rate, ln(SDNN), ln(r-MSSD)) measured on the ith rat on the jth time point.
$Pollutant_j$=A continuous variable indicating the concentration at the jth observation
β_0=Overall intercept

u_i = Random intercept corresponding to the jth time point
β_1 =overall effect of the Pollutant (e.g. Effect estimate)
e_{ij} =Measurement error corresponding to the jth observation on the i-th rat. u_i and e_{ij} are assumed to be independently distributed random variables with mean zero and variance σ^2_b and σ^2 respectively. Given this assumption, the resultant covariance matrix has a compound symmetry structure.

Another component of mixed modeling is maximum likelihood estimation, used to calculate the upper and lower confidence interval for the effect estimate. The entire range of the confidence interval for the effect estimate must fall above (or below) the origin for determination of the statistical significance of the association ($p<0.05$). Any range that would cross the origin indicates that the effect estimate may be positive or negative, thus a significant directional association cannot be achieved. Furthermore, the narrower the range of the beta estimate, the stronger the statistical strength of the association between the component of $PM_{2.5}$ and HRV.

4.0 DETROIT RESULTS: SUMMER 2005

4.1 CAPs Characterization

The average ambient $PM_{2.5}$ and CAPs concentrations during the 13-day exposure period were 19±12 μg/m^3 and 518±506 μg/m^3, respectively. Daily variations of CAPs mass and major chemical component concentrations during 13-day exposure study are shown in Table 4.1. The concentration of particulate organics was estimated by multiplying the measured concentration of organic carbon by a factor of 1.8 as suggested by Turpin (2001). An estimate of oxygen content of crustal elements was applied and calculated as 2.14Al+2.43Fe+1.54Si, where Si was estimated as potassium (K)/0.15 (Mason 1982). Based on levels of $PM_{2.5}$ mass concentration in both ambient air and in the chamber, concentration enrichment factors (CEFs) – ratios of concentrated particle mass concentration to ambient concentration – were calculated for each inhalation exposure period to evaluate concentrator performance. The overall average CEF of particle mass concentrations during the 13-day exposure study was 25.

Table 4.1 CAPs major components from the 13-day summer exposure study. All concentrations in μg/m^3.

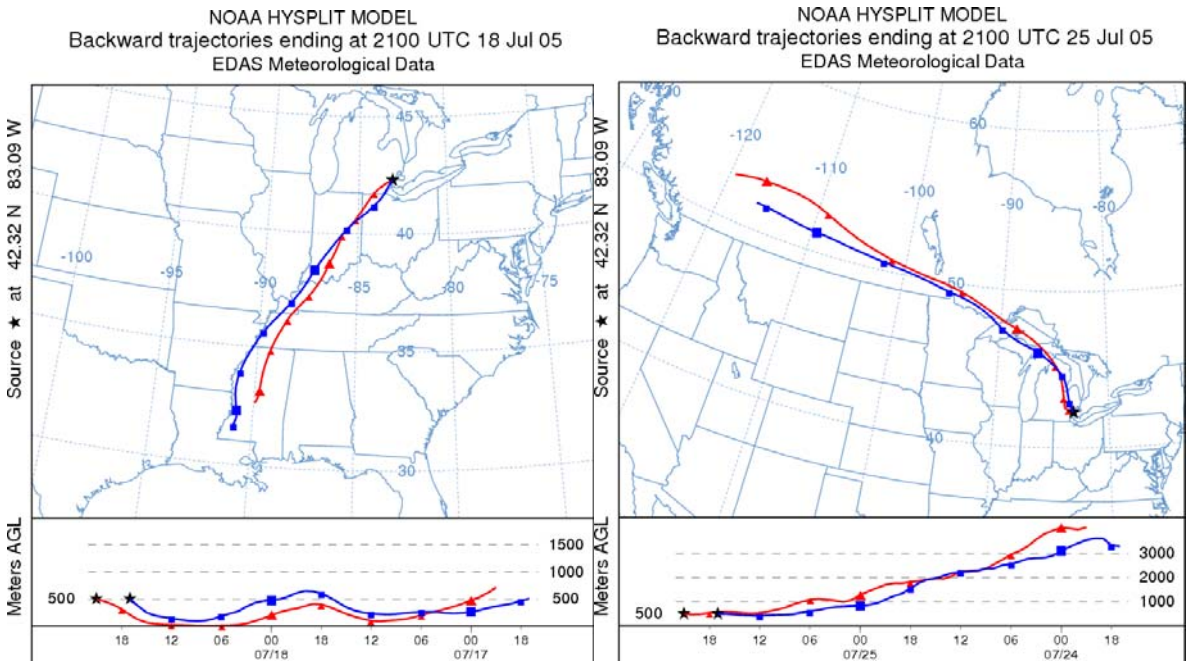
July 2005	CEF [1]	Mass	OM [2]	EC	Sulfate	Nitrate	Ammonium	Urban dust [3]	Unidentified
7/16	15	576	109	3	149	29	55	21	211
7/17	65	1437	269	5	379	152	190	44	399
7/18	41	1638	381	10	648	64	252	45	237
7/19	28	360	236	7	33	15	20	35	13
7/20	13	255	132	16	13	10	10	43	31
7/21	39	594	142	3	121	37	57	23	212
7/22	26	314	121	4	45	8	22	26	88
7/23	16	115	61	3	6	1	7	14	24
7/24	7	81	50	5	6	4	6	13	0
7/25	5	68	31	1	11	1	8	16	0
7/26	31	843	339	6	186	17	92	30	173
7/27	23	213	185	8	11	6	12	19	0
7/28	23	240	6	2	5	12	6	40	169
TWA	**25**	**518**	**159**	**6**	**124**	**27**	**57**	**28**	**120**

1. CEF (concentration enrichment factor)
2. Organic mass (OM) was estimated from organic carbon (OC) x 1.8
3. Urban dust: 1.89*Al+1.4*Ca+1.43*Fe+2.14*Si, where Si is estimated by K/0.15

The Harvard group previously characterized the concentrator and found that the CEFs were likely to be influenced by environmental factors such as levels of ambient PM concentration and size distribution, and meteorological conditions such as humidity. They also observed that the operation of the concentrator, including the minor 3 pressure drop, the minor-to-total-flow ratio and the alignment of the slits of the virtual impactors had a significant impact on the CEFs observed. This investigation supported their findings that the CEFs were influenced by meteorological conditions and the low ambient concentrations. During this study, a relatively clean and dry (for summer months) air mass was driven in via northerly winds for several days.

As expected, the CEFs as well as ambient PM levels were low during this time, and the concentration of the CAPs delivered to the exposure chamber increased by smaller factors. On the other hand, when relatively humid air masses brought increased levels of pollutants via southerly winds, the fine particle concentrator generated concentrated particles in the size range of 0.1 to 2.5 μm effectively and the CEFs were higher than 30.

Relatively high concentrations of ambient $PM_{2.5}$ (> 30 μg/m^3) were generally shown to be associated with transport of air masses from the south and southwest, in addition to various $PM_{2.5}$ inputs from local combustion sources. Transport from these directions may include emissions from coal-fired power plants and various coke processing and steel manufacturing plants located along the Ohio River Valley. For example, the highest CAPs concentration was observed on July 18th during the 13-day exposure period (Table 4.1). The HYSPLIT 48-hr back-trajectory model in Figure 4.1a shows very long-range transport of air mass that passed through Missouri, Kentucky, Indiana and Ohio. During this period in Detroit, the predominant southerly wind associated with a high-pressure system and increased concentration of secondary or aged aerosol in the accumulation mode was observed. This situation usually arises because the monitoring site is influenced strongly by regional transport (secondary PM) in addition to the local sources. More details on chemical composition to support this conclusion are discussed later in this report.



(a) (b)

Figure 4.1. HYSPLIT-generated backward trajectories showing the history of air mass arriving the Detroit site on (a) July 18, 2005 and (b) July 25, 2005 (Data are from NOAA 2005).

In contrast, much lower pollutant concentrations were seen with transport from the north. For example, the lowest CAPs concentration was observed on July 25th. During the exposure period,

the predominant wind direction was from the north, and the HYSPLIT back-trajectory model in Figure 4.1b shows that air masses was transported from Canada. As expected, days with northerly winds were usually associated with low pollutant concentrations due to the fact that northerly air masses are usually cleaner than southerly ones and that only a limited number of stationary sources are north of the sampling site.

As shown in Table 4.1, CAPs mass during the summer study were dominated by organic carbon and sulfate. As discussed in the previous sections, elevated CAPs concentrations during this study were associated with a combination of transported secondary air pollutants and local combustion sources. During the 13-day exposure period, contributions from local combustion sources were indicated by elevated levels of signature elements such as Pb, Zn and Cd for municipal incinerators; La, V, and Ni for oil combustion/refineries; and Fe, Pb and Mn for iron/steel manufacturing (Table 4.2). For example, during the 8-hr exposure period on July 18th, many elements in CAPs including La, Ce, Mo, Se, Ni, Co, Cr, Ti, Ca, K, and S reached the highest levels of the 2005 campaign. These trace elements are often used as tracers or fingerprints for identifying emission sources for atmospheric pollutants, and characterization of ambient $PM_{2.5}$ sources are described in the next section.

Table 4.2 CAPs elemental composition during the summer Detroit exposure study. All concentrations in ng/m^3.

July 2005	7/16	7/17	7/18	7/19	7/20	7/21	7/22	7/23	7/24	7/25	7/26	7/27	7/28
Mg	244	475	1296	894	1758	477	800	431	189	119	883	279	1383
Al	0	246	941	371	1245	212	875	0	449	308	877	278	755
P	308	179	302	604	426	280	109	146	4	143	267	571	385
S	58163	131986	210868	15925	6033	44243	18950	3790	2998	5473	78273	5074	3372
K	1182	2616	1722	1379	1363	952	1055	651	571	1003	1335	980	1356
Ca	753	753	6414	3502	5903	1302	2388	1488	827	443	3124	924	4712
Ti	17.3	28.1	88.5	51.5	67.1	36.7	54.8	20.8	8.3	7.6	37.0	26.9	70.7
V	17.8	16.2	21.3	4.7	7.1	6.6	7.0	4.2	2.9	1.7	13.3	1.9	62.8
Cr	62	93	324	38	65	111	74	33	39	87	129	143	156
Mn	60	94	205	191	411	106	118	39	53	9	190	84	529
Fe	2162	3343	7041	6848	9350	4802	3942	1671	1937	491	3207	2579	8527
Co	0.8	1.8	6.4	2.0	1.9	2.3	1.4	0.6	1.6	0.2	1.5	1.6	3.6
Ni	40.1	64.1	211.0	26.9	18.7	69.8	24.6	12.4	43.2	4.9	26.6	48.6	98.1
Cu	46	114	116	209	105	120	70	39	24	8	118	61	149
Zn	155	319	769	1629	2419	1192	336	48	35	122	966	272	16737
As	17.5	56.0	40.9	17.2	11.5	13.6	6.6	5.5	3.8	1.5	16.9	4.7	11.5
Se	69.8	67.0	164.7	20.5	13.1	25.7	15.2	7.4	4.0	2.6	60.4	4.4	12.5
Rb	2.1	1.6	3.6	1.9	3.9	1.3	1.4	0.7	1.1	0.5	3.6	2.1	4.6
Sr	11.3	58.0	34.3	19.1	38.2	15.5	18.0	8.8	9.3	2.6	17.6	7.2	33.9
Mo	5.5	14.6	62.5	11.2	16.1	18.7	19.0	5.0	4.3	2.6	4.3	16.6	27.3
Cd	2.3	2.3	4.1	5.0	4.0	2.9	1.0	1.0	0.4	0.1	2.4	1.4	7.7
Sb	7.8	39.8	20.9	116.9	74.0	19.4	18.8	5.2	2.9	2.4	9.1	10.7	63.5
Ba	36	191	174	150	183	156	185	90	21	24	84	106	376
La	0.6	1.3	24.4	1.2	22.0	1.1	1.2	0.4	1.3	0.3	6.3	0.3	11.3
Ce	0.8	1.8	16.5	1.9	14.2	1.3	2.0	0.7	1.0	0.3	4.5	0.8	6.4
Sm	0.03	0.04	0.19	0.11	0.18	0.05	0.11	0.03	0.03	0.02	0.13	0.02	0.27
Pb	36	59	355	921	789	133	25	36	11	3	53	17	172

4.2 Characterization of Ambient $PM_{2.5}$ Sources

Figure 4.2 shows temporal variations of ambient $PM_{2.5}$ concentrations measured by TEOM in southwest Detroit during the 13-day period in July 2005. These relatively high concentrations are representative of particulate mass concentrations in Southwest Detroit during summer months (Harkema et al., 2004; Morishita et al., 2006). Seven counties in southeast Michigan, including Wayne County where the study was performed, have been designated as $PM_{2.5}$ non-attainment areas by the USEPA for violations of the National Ambient Air Quality Standard.

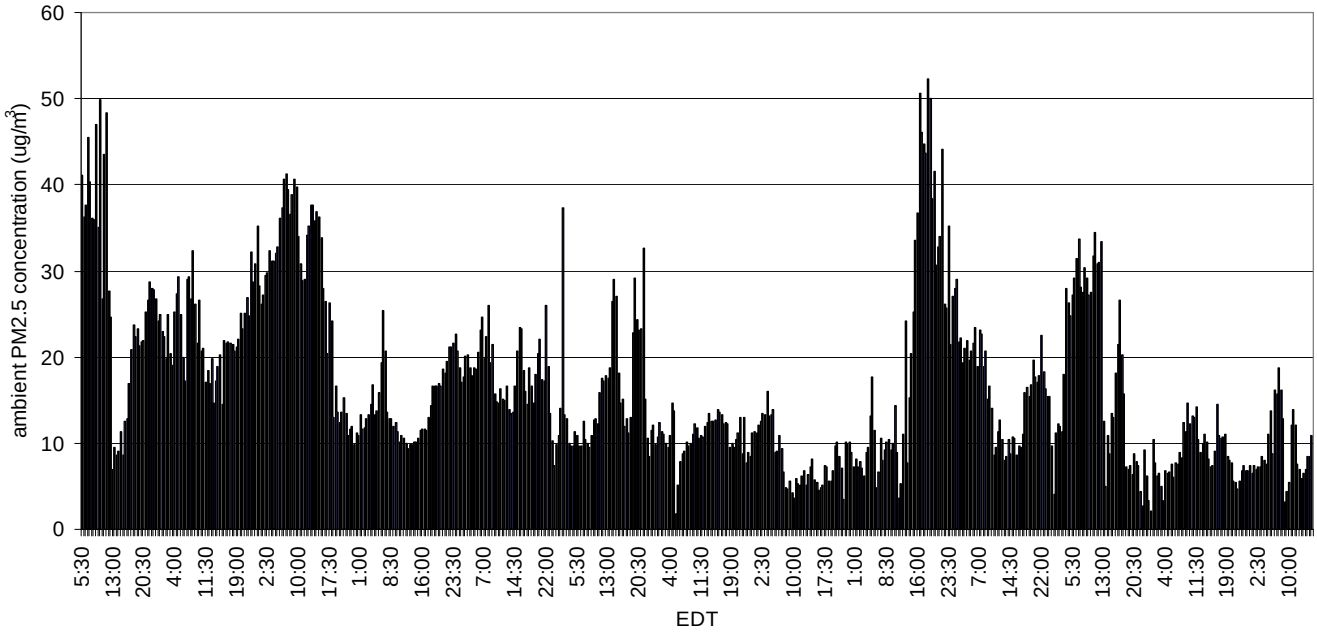


Figure 4.2 Temporal variations of ambient $PM_{2.5}$ concentrations measured by TEOM in Detroit during the 13-day exposure days (7/16/05-7/28/05).

Detailed results of the PMF analysis are described in an in-press manuscript (Morishita et al., 2010a). The aims of this paper were to: (1) present detailed source apportionment results for $PM_{2.5}$ measured in Detroit, (2) compare the PMF results between 8-hour integrated filter samples and semi-continuous elemental samples, and (3) evaluate the PMF modeling results using observed meteorological data and gaseous pollutant concentrations.

In brief, PMF was applied to the 268 half-hour ambient samples collected from SEAS. Combined with wind directionality analysis and correlation analysis with gaseous pollutants and elemental carbon, six factors were identified and the contribution of each element to each of the identified source factors is shown in Figure 4.3. The identified source factors included secondary aerosol (S, Se), gasoline- and diesel-powered vehicles (Fe, Ba, Zn, Al, Mg), sludge incineration (Cd, Sb, As, Cr, Zn, Rb, K, Ni, Cu), refining (La, Ce), cement/lime production (S, Sr, Ca, Mg), and iron and steel manufacturing (Fe, Pb, Zn). Figure 4.4 shows that during the 13-day summer exposure study conducted in July 2005, the contributions from the secondary aerosol factor were

the highest (33%), followed by gasoline and diesel vehicles (31%), cement/lime, and iron/steel manufacturing factors.

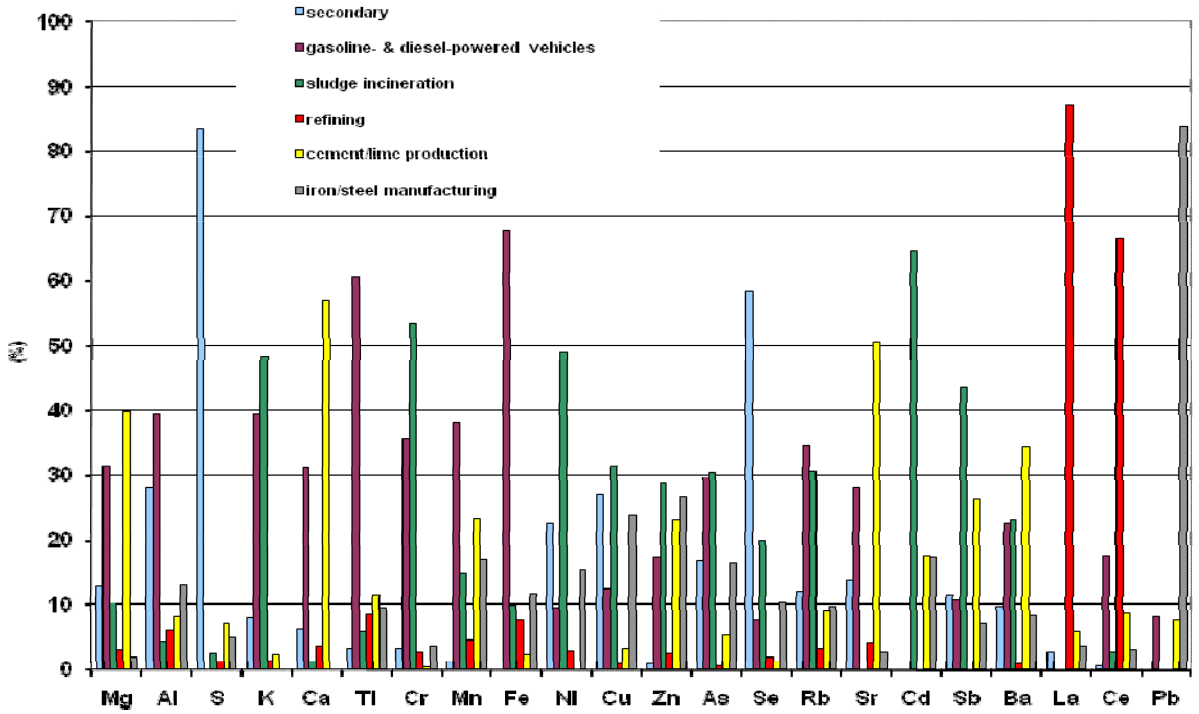


Figure 4.3 Percentage contribution of each element to each identified source factor during summer studies.

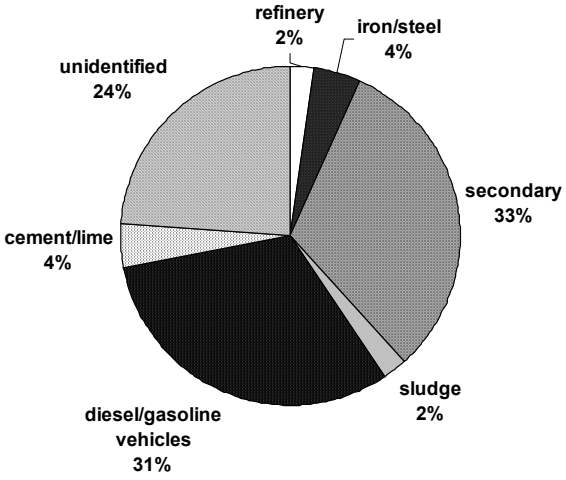


Figure 4.4. Average factor contributions to ambient $PM_{2.5}$ during the 13-day exposure period (7/16/05-7/28/05): Results from 268 30-minute SEAS samples collected in July and August 2005.

Figure 4.5 shows wind rose plots of the time-averaged PMF factor contributions as a function of wind direction for the 30-minute SEAS data as observed at the Detroit sampling site. Different scales were used for the y-axes to cover a large range of $PM_{2.5}$ concentrations. During the 13-day exposure period, the dominant wind direction was west-southwest. While the dominant SW wind direction made source differentiation a challenging task, the 30-minute data were able to show source directionality during the study. For example, the highest refinery contribution was associated with southwesterly winds; this is consistent with the location of the refinery on a heading of 240° from the measurement site. The iron/steel manufacturing factor showed increased contributions when the winds were coming from both west and south at approximately 190°. These directions are consistent with the locations of iron and steel manufacturing facilities - the Rouge Steel complex (265° from the measurement site) and National Steel complex (190°), respectively. The gasoline/diesel vehicle factor showed increased contributions when the wind was coming from the southwest, particularly from the direction of I-75.

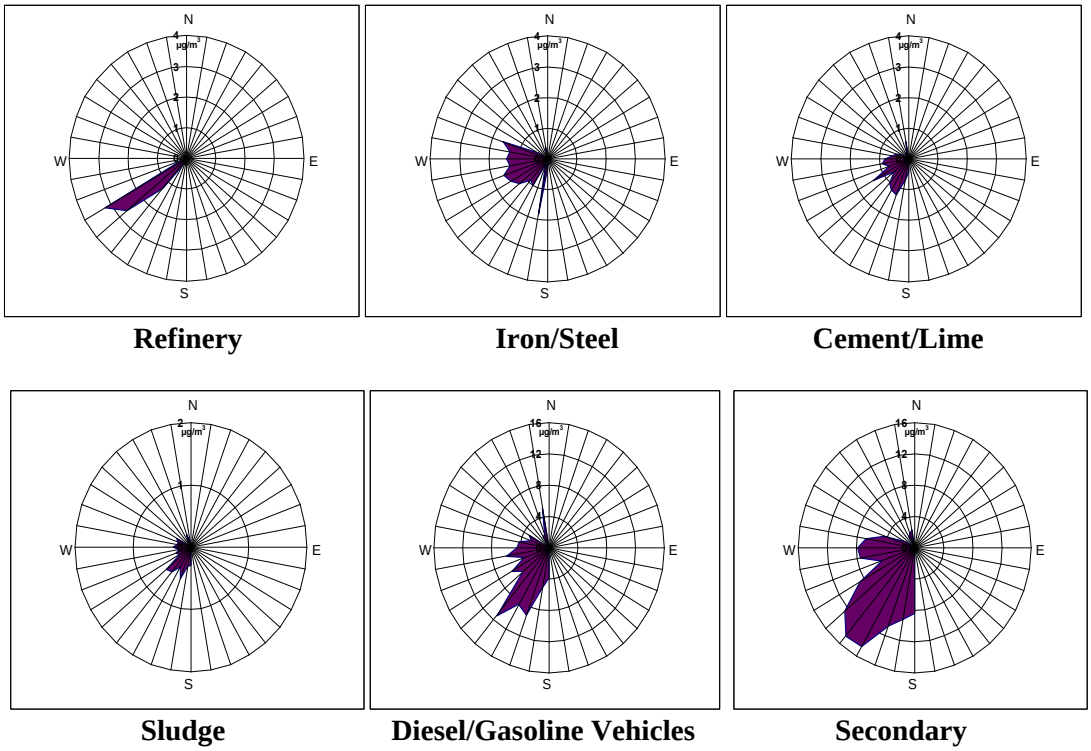


Figure 4.5. Average factor contributions versus wind direction from 268 SEAS samples collected in July and August 2005.

Table 4.3 Primary gaseous pollutants measured during 13 8-hour exposure periods in Detroit.

	SUMMER			
CO	0.5 (1.5)	±	0.2	(ppm)
SO_2	5.2 (36)	±	4.1	(ppb)
NO	5.9 (101)	±	12.8	(ppb)

Parenthetic values are maxima

Average concentrations of the primary gaseous pollutants are shown in Table 4.3. Numerous locally-dependent pollutant concentrations were also observed and Figure 4.6, for example, shows temporal variations of primary gaseous pollutants including CO, NO and SO_2 concentrations during the first week of August. Fluctuations in these pollutants are dependent on traffic volume, episodic activity in industrial effluent, wind directions and microclimate environments. In particular, NO and CO spiked during morning rush hour, supporting the predicted impact of the diesel/gasoline vehicle source factor during those hours. Later in the day on August 3rd, additional large peaks were seen suggesting strong impacts from local fossil fuel combustion sources emitting SO_2.

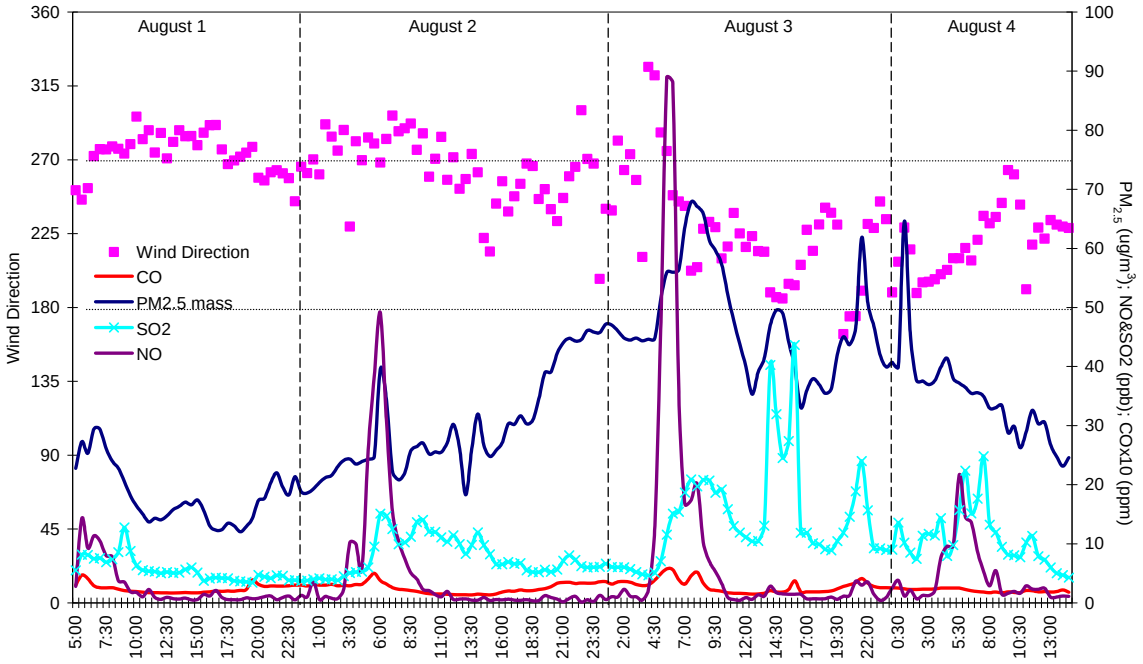


Figure 4.6 Temporal variations of ambient $PM_{2.5}$ and primary gaseous pollutants in Detroit between August 1st and August 4th, 2005.

4.3 Bronchoalveolar Lavage Fluid, Tissue, and Serum Analysis

Exposure to CAPs caused significant decreases in all BALF cell types in WKY rats. By comparison, no CAP-associated changes in cellularity were detected in SH rats (Fig 4.7). WKY rats exposed to air had greater airway total cells and macrophages in BALF compared to air-exposed SH rats.

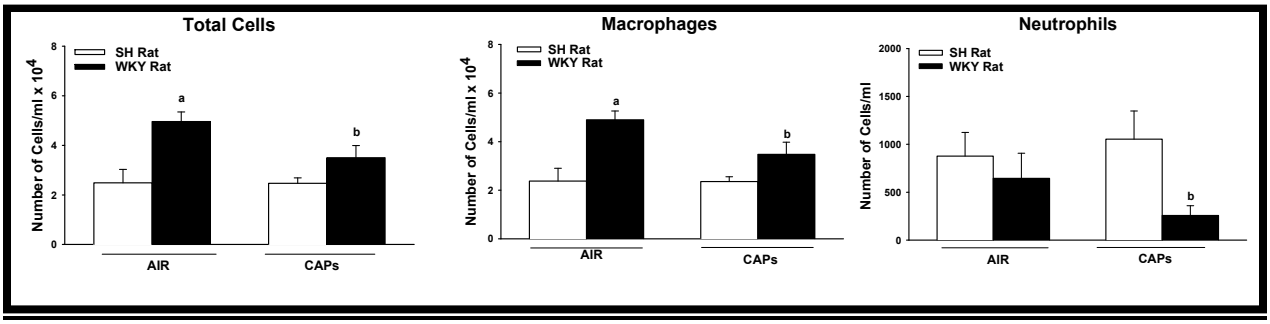


Figure 4.7 Effect of CAPs exposure on bronchoalveolar lavage cellularity, Detroit, Summer 2005.

Exposure to CAPs caused significant increases in intraepithelial mucosubstances (IM) in both proximal and dista axial airways of WKY rats (Fig 4.8). However, no changes in IM were induced by CAPs inhalation in SH rats. WKY rats exposed to air had modest amounts of IM in proximal airways that were 3-fold greater than found in SH rats. It should be noted that the IM detected in airways of both strains was minimal, consisting of 4-5 cells per field.

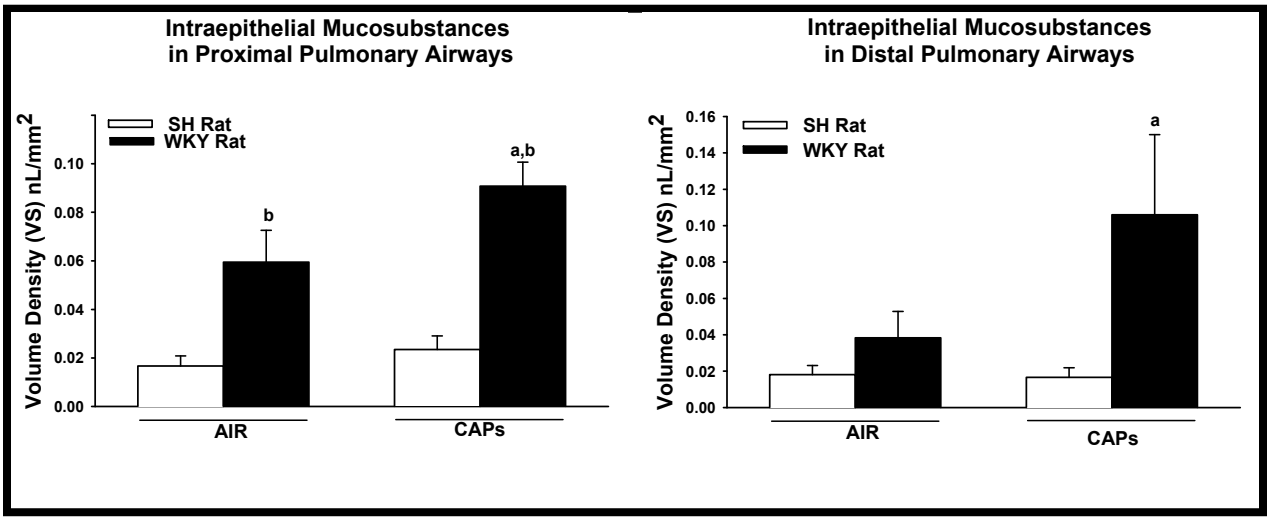


Figure 4.8 Effect of CAPs exposure on intraepithelial mucosubstances in proximal and distal pulmonary airways, Detroit, Summer 2005.

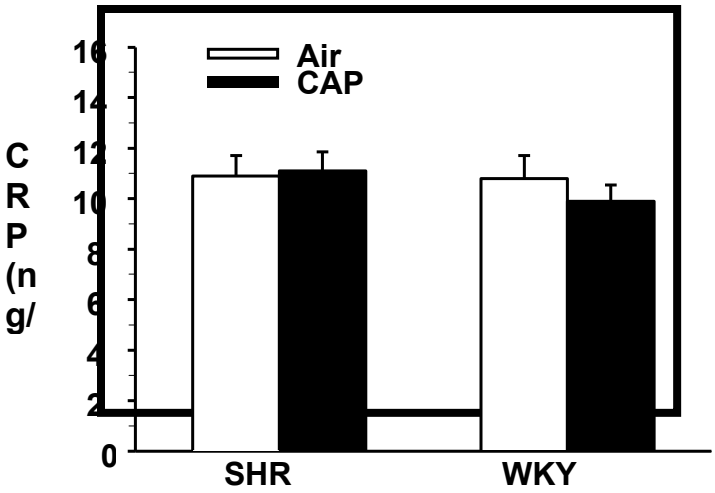


Figure 4.9 Effect of CAPs exposure on serum C-reactive protein, Detroit, Summer 2005.

CAPs exposure had no effect on serum CRP levels in either strain, and no strain differences were detected between SH and WKY rats (Fig. 4.9). Serum levels of TNFα, IFNγ, IL-10 and IL-6, where detectable, were not significantly different across strains or exposures (data not shown).

4.4 Heart Rate and Heart Rate Variability Responses

The results described here are presented in a submitted manuscript (Rohr et al., 2010a). Mixed model analyses using daily (8-hour) means of HR and HRV parameters from air- and CAPs-exposed rats failed to detect differences in responses (Table 4.4). The term of interest is the interaction term ("exposure x time"). However, changes over time occurred for all cardiovascular endpoints ("time" variable), which we believe is due to changes over the course of the 8-hour exposure, especially immediately at the start of exposures, or over the 13 day exposure period.

In contrast, mixed model analysis using 30-minute data allowed us to detect differences in SDNN between air and CAPs rats (Table 4.5). Furthermore, marginally significant differences in HR between exposure groups were observed (p = 0.0576).

Table 4.4. Mixed modeling results using daily means (8-hour) for heart rate, SDNN and rMSSD in SH rats exposed to air or CAPs for 13 consecutive days, Detroit, Summer 2005.

Parameter		*df* N	*df* D	F *Value*	P *Value*
Heart Rate	Exposure	1	12	0.18	0.6782
	Time	11	131	30.97	<0.0001
	Expos x Time	11	131	0.69	0.7465
ln (SDNN)	Exposure	1	12	2.24	0.1603
	Time	11	131	4.67	<0.0001
	Expos x Time	11	131	0.47	0.9212
ln (rMSSD)	Exposure	1	12	1.65	0.2232
	Time	11	131	5.24	<0.0001
	Expos x Time	11	131	0.58	0.8412

Table 4.5. Mixed modeling results using 30-minute means for heart rate, SDNN and rMSSD in SH rats exposed to air or CAPs for 13 consecutive days, Detroit, Summer 2005.

Parameter		*df* N	*df* D	F *Value*	P *Value*
Heart Rate	Exposure	1	12	0.21	0.6546
	Time	191	1872	11.19	<0.0001
	Expos x Time	191	1872	1.18	**0.0576**
ln (SDNN)	Exposure	1	12	1.7	0.2171
	Time	191	1872	2.87	<0.0001
	Expos x Time	191	1872	1.33	**0.0029***
ln (rMSSD)	Exposure	1	12	1.57	0.2345
	Time	191	1872	2.54	<0.0001
	Expos x Time	191	1872	0.89	0.8458

We applied mixed model approaches to determine if individual pollutants were associated with acute changes in cardiac endpoints; specifically, we used semi-continuous elemental measurements from the slurry sampler and 30-minute means of cardiac parameters. Although differences in HR between air- and CAPs-exposed rats were only marginally significant, we did evaluate associations with components and source factors. Increased HR was associated with a number of components, including (in order of largest to smallest effect estimate) Ni, Al, CAPs mass, As, Se, Fe, S, Mg, Rb, Ti, Sr, Ca, P, Cd, V, Ba, Cu, Sb, and Cr (Figure 4.10). By comparison, reductions in SDNN (also in decreasing order of effect estimate) were associated with EC, Fe, Sr, Mg, As, Ca, Ti, Mn, Se, Ba, Sb, Pb, Ce, and Zn (Figure 4.11).

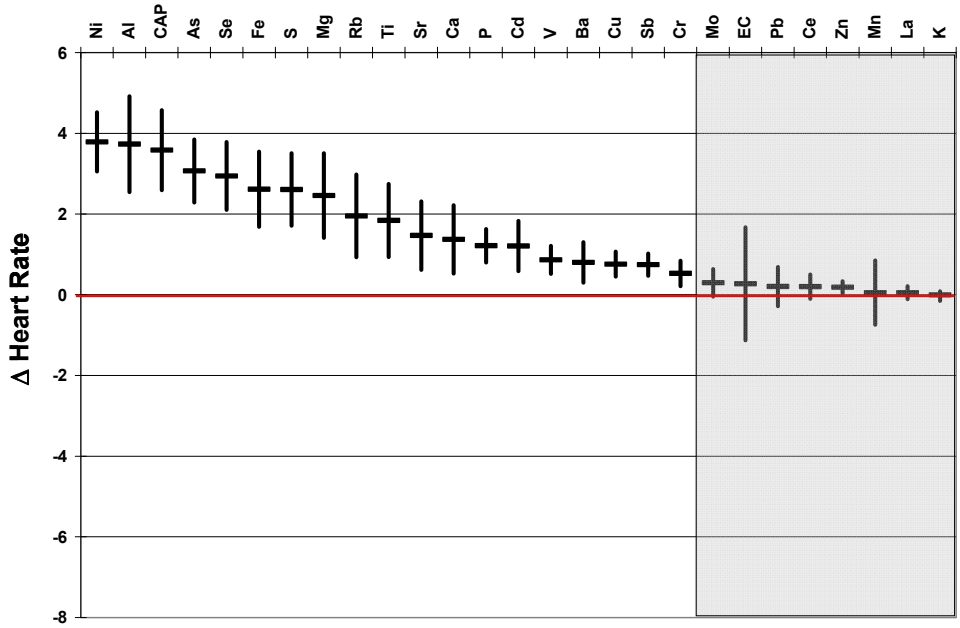


Figure 4.10 Relationship between PM components and heart rate, Detroit, Summer 2005.

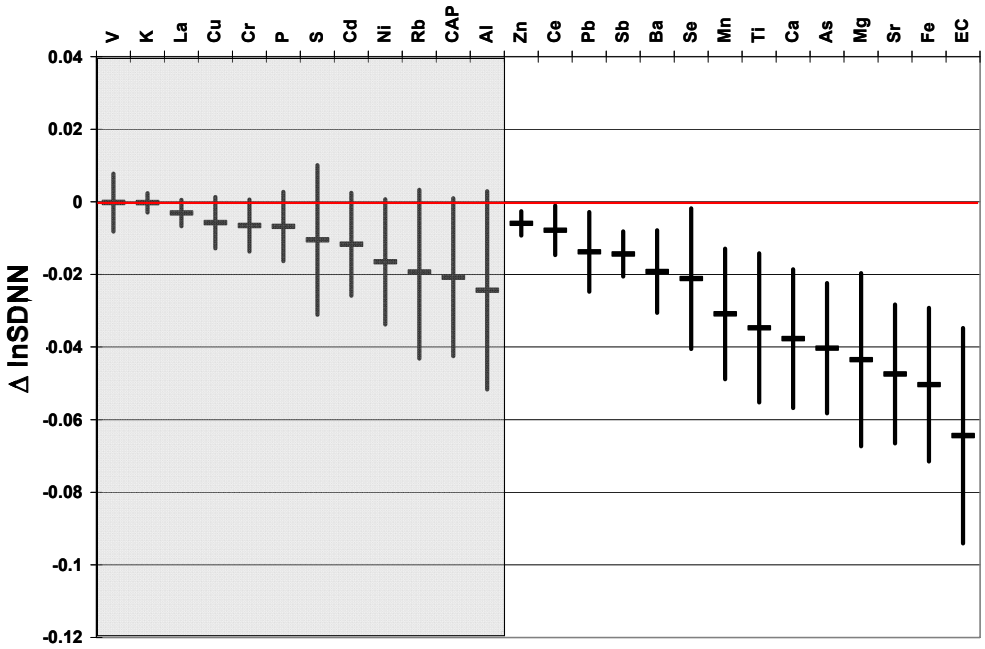


Figure 4.11 Relationship between PM components and SDNN, Detroit, Summer 2005.

As described earlier, we determined six primary source factors in Detroit during the summer study. These source factors included secondary aerosol, cement/lime, sludge incineration, refinery, motor/diesel (traffic), and iron/steel manufacturing. In the current study, secondary aerosol, sludge incineration, and traffic source factors were associated with increased HR in CAPs-exposed SH rats (Figure 4.12).Decreased SDNN was associated with factors of motor/diesel, cement/lime, sludge incineration and iron/steel manufacturing (Figure 4.13).

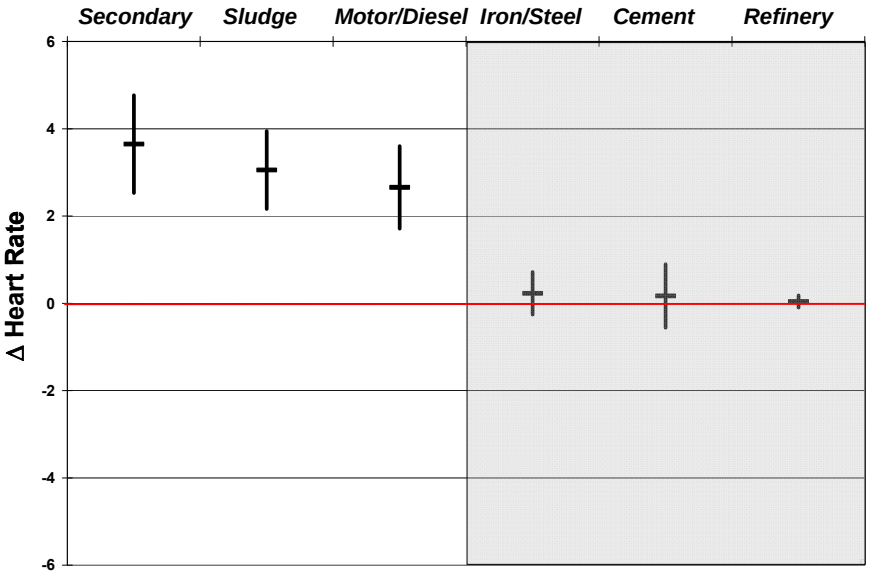


Figure 4.12 Relationship between $PM_{2.5}$ source factors and heart rate, Detroit, Summer 2005.

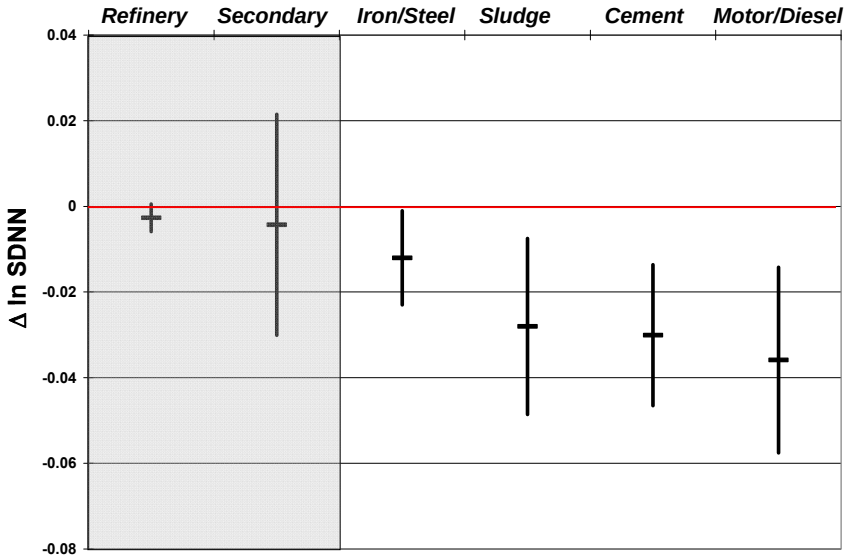


Figure 4.13 Relationship between $PM_{2.5}$ source factors and SDNN, Detroit, Summer 2005.

5.0 DETROIT RESULTS: WINTER 2006

5.1 CAPs Characterization

The average ambient $PM_{2.5}$ and CAPs concentrations during the 13-day exposure period were 14±9 μg/m^3 and 357±218 μg/m^3, respectively. Daily variations in CAPs mass and major chemical component concentrations during 13-day exposure study are shown in Table 5.1. The overall average CEF during the 13-day exposure study was 29. In contrast to the summer exposure study, CAPs were dominated by nitrate and ammonium, and OM and sulfate were lower during the winter 13-day exposure period. Winter elemental concentrations (Table 5.2) were generally lower than in summer, with the exception of a few earth (Mg, Al, K) and trace (V, Mo) elements.

Table 5.1 CAPs major components from the 13-day winter exposure study in Detroit. All concentrations in μg/m^3.

February 2006	CEF[1]	Mass	OM[2]	EC	Sulfate	Nitrate	Ammonium	Urban dust[3]	Unidentified
2/11	46	564	64	5	94	229	145	22	3
2/12	41	126	39	6	30	16	16	18	2
2/13	41	262	34	2	51	62	37	40	36
2/14	29	565	57	6	69	215	148	42	27
2/15	20	234	48	8	19	52	24	36	47
2/16	29	691	98	3	153	224	151	25	37
2/17	36	58	16	5	9	2	5	16	5
2/18	26	116	7	1	15	8	10	24	51
2/19	20	202	23	4	27	36	22	36	54
2/20	22	323	45	6	34	80	38	30	91
2/21	20	395	41	5	33	96	53	29	137
2/22	21	703	174	17	56	170	151	48	87
2/23	23	400	80	7	22	88	43	34	127
TWA	**29**	**357**	**56**	**6**	**47**	**98**	**65**	**31**	**54**

1. CEF (concentration enrichment factor)
2. Organic mass (OM) was estimated from organic carbon (OC) x 1.8
3. Urban dust: 1.89*Al+1.4*Ca+1.43*Fe+2.14*Si, where Si is estimated by K/0.15

Table 5.2 CAPs elemental composition during the winter Detroit exposure study. All concentrations in ng/m^3.

February 2006	2/11	2/12	2/13	2/14	2/15	2/16	2/17	2/18	2/19	2/20	2/21	2/22	2/23
Mg	258	289	775	841	718	271	492	2232	2603	1980	941	1059	1152
Al	289	449	1278	1451	719	416	352	969	851	1028	1135	1119	934
P	197	208	376	277	0	278	87	136	149	190	169	435	33
S	36095	12605	20916	28590	7083	49434	3488	6258	10790	13115	13114	22644	9714
K	1284	931	1568	1934	1403	1294	722	1320	1949	1555	1346	2647	1190
Ca	737	738	5767	3536	5946	1260	2360	1527	827	414	2950	890	3796
Ti	13.6	17.5	23.4	48.7	36.2	24.8	11.7	10.3	26.0	41.8	34.8	45.2	45.5
V	11.3	3.2	4.7	8.0	6.8	240.6	2.0	2.3	4.7	6.4	6.4	11.6	5.2
Cr	153	38	39	38	30	22	18	8	62	3	35	34	79
Mn	68	29	198	203	127	138	36	21	184	165	118	214	195
Fe	1644	2083	5010	4773	4699	2502	940	888	3659	3420	2588	4952	6731
Co	0.9	0.8	0.8	0.9	0.8	1.0	0.4	0.2	0.5	0.7	0.8	1.2	1.0
Ni	13.7	22.9	21.6	5.1	17.6	54.5	3.5	0.7	8.3	5.3	12.7	13.3	24.0
Cu	46	46	63	94	60	68	26	12	20	50	64	79	79
Zn	443	323	2098	1519	752	838	106	135	1105	863	670	1497	512
As	16.4	12.3	9.6	12.9	8.6	12.3	7.8	4.3	8.8	10.0	11.8	24.0	9.2
Se	44.4	9.9	6.4	28.9	19.6	32.8	2.0	1.3	5.5	8.0	15.3	86.0	9.8
Rb	2.4	1.3	3.7	4.2	3.1	2.5	0.9	1.5	3.3	3.3	3.1	9.1	2.5
Sr	5.4	8.7	14.8	20.4	24.7	10.8	9.7	17.0	25.7	37.5	25.0	34.0	35.3
Mo	4.4	2.8	5.3	6.7	6.4	396.2	2.3	0.9	1.9	6.2	14.8	9.5	8.0
Cd	1.7	1.4	1.7	3.5	2.4	5.0	1.1	0.8	2.5	2.4	11.7	6.5	1.9
Sb	37.9	14.0	6.1	8.9	11.4	14.8	11.0	2.8	3.7	7.4	13.6	20.4	9.8
Ba	35	187	156	151	184	150	182	92	21	22	79	102	303
La	1.3	0.6	7.8	38.1	5.5	4.4	0.3	0.2	17.3	20.0	9.7	2.4	8.7
Ce	0.7	0.6	4.8	22.3	3.6	1.9	0.6	0.5	9.4	9.9	5.3	1.9	4.8
Sm	0.02	0.02	0.05	0.09	0.10	0.18	0.03	0.04	0.09	0.11	0.09	0.12	0.11
Pb	42	19	44	68	62	72	32	17	48	72	90	111	98

Although generally lower ambient $PM_{2.5}$ concentrations were observed during the winter study, the sampling site was often impacted by short-term increases in ambient $PM_{2.5}$. For example, the highest CAPs concentration was observed on February 22, 2006, and meteorological data showed that the increased pollution event was associated with south and SES winds (Figure 5.1). Figure 5.2 shows temporal variations of primary gaseous pollutants including CO, NO and SO_2 concentrations and ultrafine particle number concentrations during the 8-hr exposure period on February 22. In the early morning, simultaneous increases in NO, CO and number concentrations of ultrafine particles are assumed to be the result of motor vehicle emissions during the morning rush hour. The figure also shows that in the late morning, primary gaseous pollutants including NO, CO, SO_2, and ultrafine particle concentrations all increased simultaneously. Furthermore, during the 8-hr exposure period on February 22, many elements in CAPs including Pb, Rb, Se, As, Co, Mn, and K reached the highest levels of the winter study campaign. As described, the largest industrial $PM_{2.5}$ emission sources in Wayne County are located south of the sampling site, and winds from the south significantly increase the likelihood of pollutant transport, which is evident by the elevated SO_2, NO and CO as well as ultrafine particle concentrations measured at the site. Combined results from trace elements and meteorological data showed strong impacts of local combustion sources during these hours.

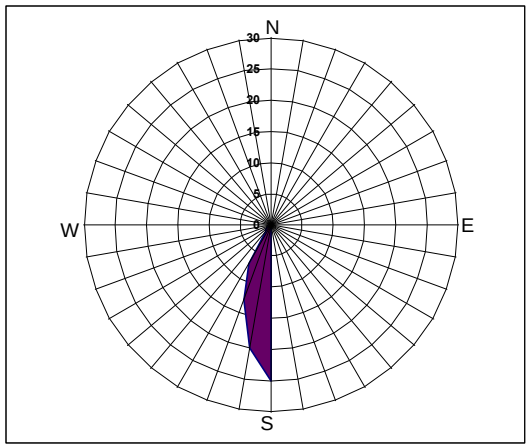


Figure 5.1 Time-averaged ambient $PM_{2.5}$ concentration as a function of wind direction.

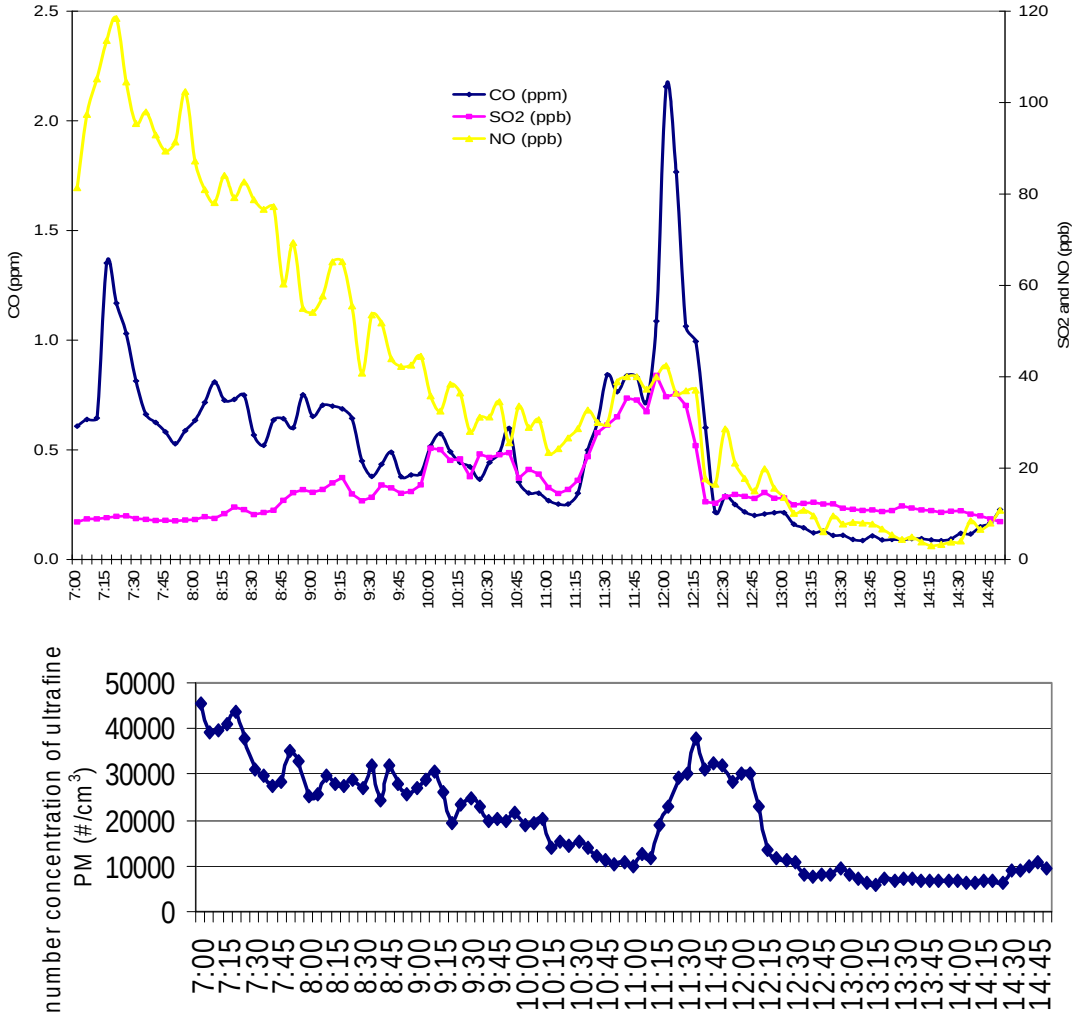


Figure 5.2 Temporal variations of 5-min average concentrations of CO, NO and SO_2 and $PM_{0.1}$ number concentrations during the 8-hr exposure period on February 22, 2006.

5.2 Characterization of Ambient $PM_{2.5}$ Sources

Figure 5.3 shows temporal variations of ambient $PM_{2.5}$ concentrations measured by TEOM in southwest Detroit during the 13-day periods in February 2006. Less temporal variability was observed than during the summer exposure period. Average concentrations of the primary gaseous pollutants are shown in Table 5.3.

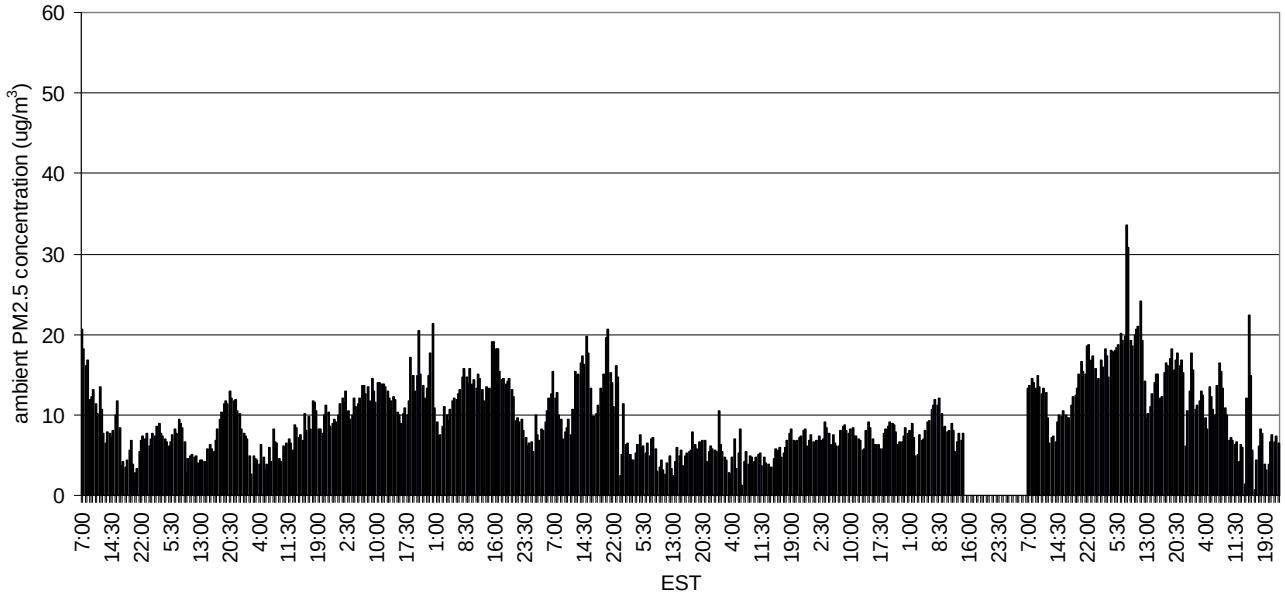


Figure 5.3 Temporal variations of ambient $PM_{2.5}$ concentrations measured by TEOM in Detroit during the 13-day exposure period.

Table 5.3 Primary gaseous pollutants measured during 13 8-hour exposure periods in Detroit.

	WINTER			
CO	0.3 (1.1)	±	0.2	(ppm)
SO_2	6.0 (35)	±	4.5	(ppb)
NO	16.5 (105)	±	18.8	(ppb)

Parenthetic values are maxima

In winter, the same PMF methods were applied to the dataset, and six factors were resolved: coal/secondary sulfate, gasoline- and diesel-powered vehicles & iron/steel manufacturing, sludge incineration, refining, cement/lime production, and metal processing & iron/steel manufacturing. It should be noted that because different datasets were input into PMF for the two seasons in Detroit, the identified source factors were different, and, furthermore, even the source factors with common names (e.g., sludge incineration, refining, and cement/lime production) differed in terms of the elemental factor loadings. The contribution of each element to the winter factors is

depicted in Figure 5.4. Due to low ambient $PM_{2.5}$ mass concentrations and less temporal variability of elemental concentrations in winter, more mixtures of source factors were observed than in summer. There were some similarities in factor composition between seasons (e.g., gasoline and diesel-powered vehicles, refinery, and cement/lime production), but also some differences (e.g., sludge incineration, iron/steel manufacturing). Sewage sludge in Detroit is a by-product of treatment of both impervious-surface runoff and some industrial discharge to the sewers. Thus, variability in industrial inputs may have contributed to the observed seasonal differences in the sludge incineration factor.

Figure 5.5 shows that during the winter exposure study in Detroit, the contributions from the secondary aerosol factor were the highest (33%), followed by gasoline and diesel vehicles (24%), cement/lime, and metal processing/iron & steel manufacturing factors. Figure 5.6 shows wind rose plots of the time-averaged PMF factor contributions as a function of wind direction for the 30-minute SEAS data as observed at the Detroit sampling site. It should be noted that the secondary aerosol factor would also contain some contribution of primary sulfate emitted from local sources, versus long-range transported secondary sulfate.

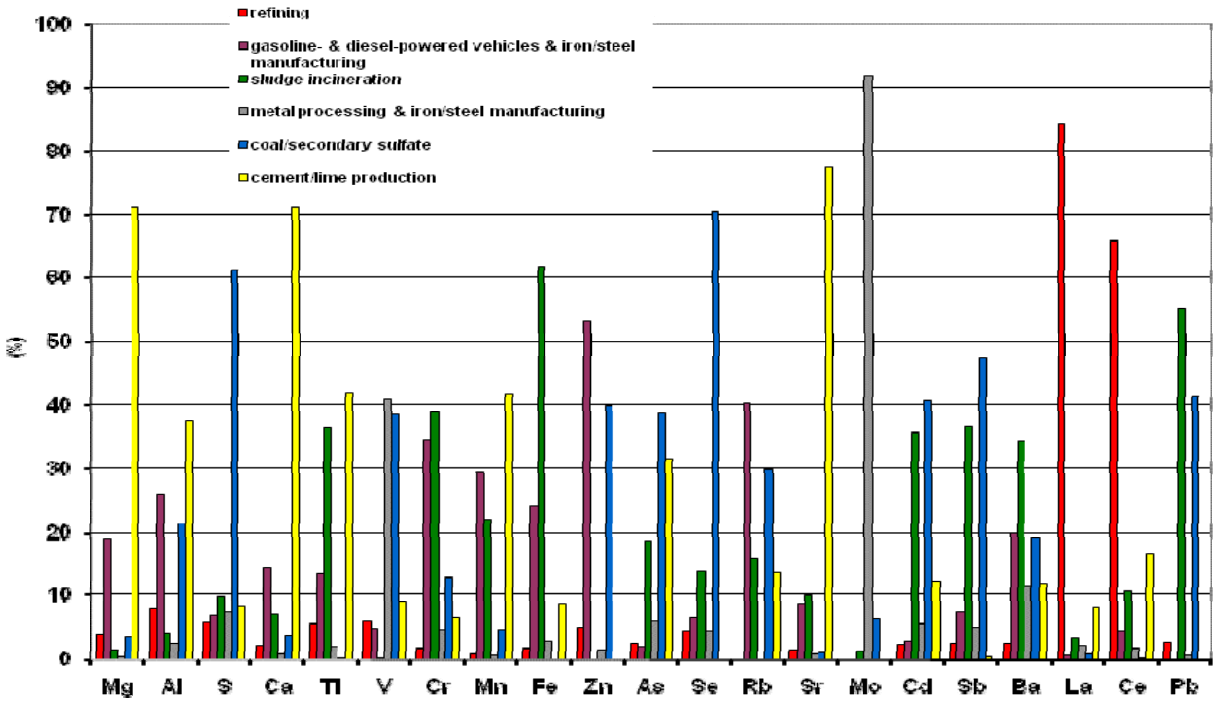


Figure 5.4 Percentage contribution of each element to each identified source factor during winter studies in Detroit.

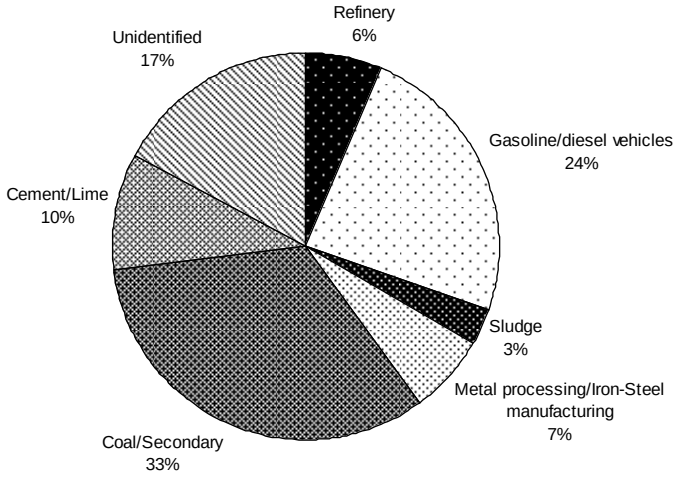


Figure 5.5. Average factor contributions to ambient $PM_{2.5}$ during the winter exposure period in Detroit: Results from 236 30-minute SEAS samples collected in February 2006.

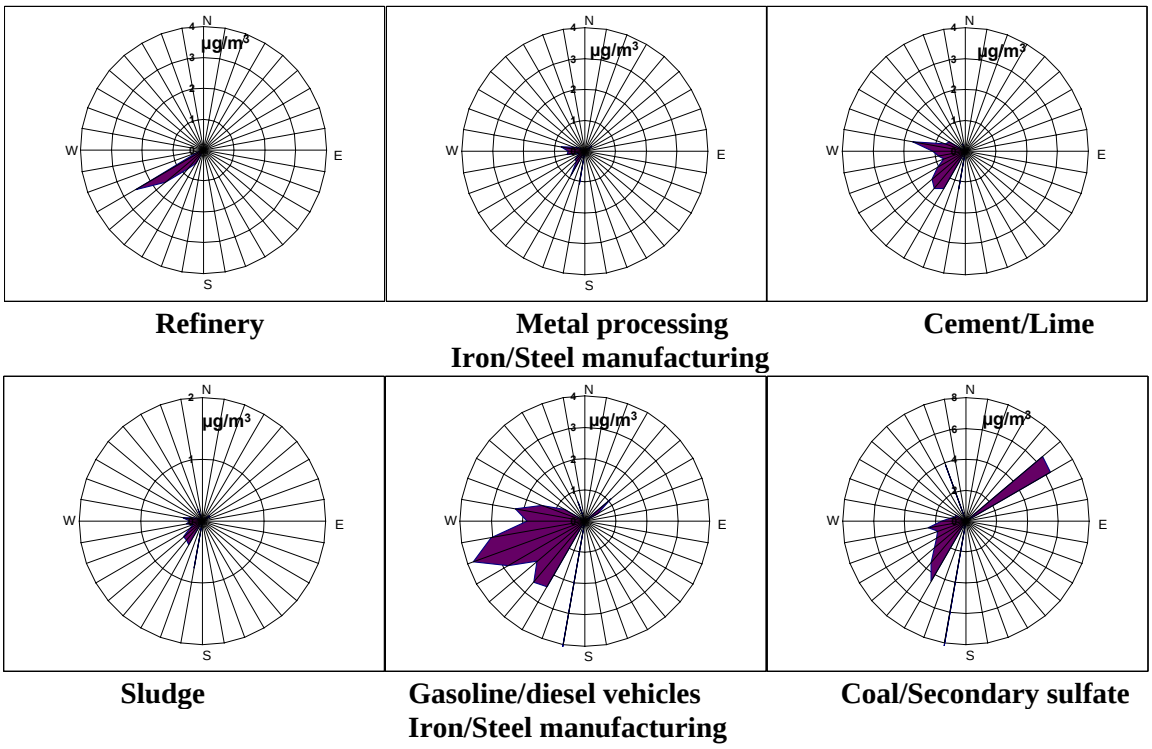


Figure 5.6 Average factor contributions versus wind direction from 236 SEAS samples collected in February 2006 in Detroit.

5.3 Bronchoalveolar Lavage Fluid, Tissue, and Serum Analysis

Exposure to CAPs had no effect on BALF cellularity in either WKY or SH rats (Fig. 5.7). WKY rats exposed to air had fewer airway neutrophils in BAL fluid compared to air-exposed SH rats; however, these account for less than 2% of the total cells, and are likely not biologically significant differences.

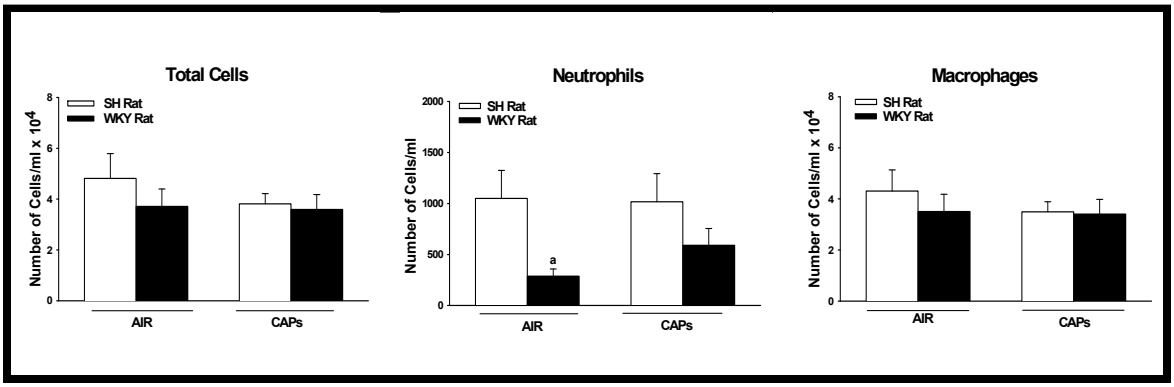


Figure 5.7 Effect of CAPs exposure on bronchoalveolar lavage cellularity, Detroit, Winter 2006.

No changes in intraepithelial mucosubstances (IM) were induced by CAPs inhalation in SH rats, and apparent decreases of IM in WKY rats after CAPs exposure were not significant. WKY rats had significantly greater amounts of IM in proximal airways compared to SH rats regardless of exposure regimen (Fig. 5.8). Mucus cells associated with these levels of IM are approximately 3-5 cells per field.

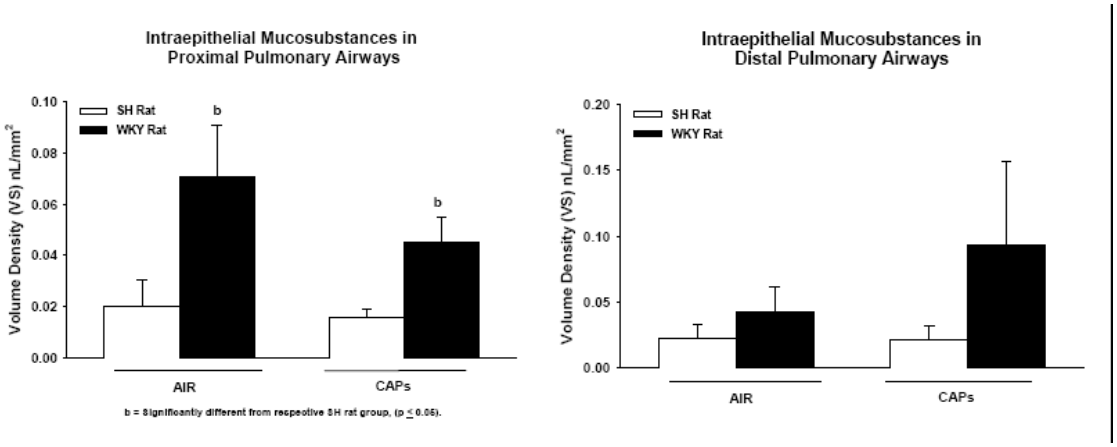


Figure 5.8 Effect of CAPs exposure on intraepithelial mucosubstances in proximal and distal pulmonary airways, Detroit, Winter 2006.

CAPs exposure had no effect on serum C-reactive protein (CRP) levels in either strain (Fig. 5.9). No strain differences in CRP were detected. Lastly, serum levels of TNF-α, IFN-γ, IL-10 and IL-6, where detectable, were not significantly different across strains or exposures (data not shown).

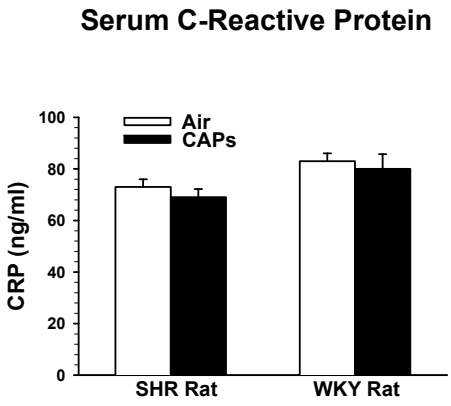


Figure 5.9 Effect of CAPs exposure on serum C-reactive protein, Detroit, Winter 2006.

5.4 Heart Rate and Heart Rate Variability Responses

The results described here are presented in a submitted manuscript (Rohr et al., 2010a). We initially used daily (8-hour) means of HR and HRV parameters from air- and CAPs- exposed rats in our mixed model analyses to determine potential differences between groups (Table 5.4). Only HR was found to be different between groups. As we had previously found during the summer exposures, all cardiovascular endpoints showed a significant temporal pattern.

Table 5.4 Mixed modeling results using daily means (8-hour) for heart rate, SDNN and rMSSD in SH rats exposed to air or CAPs, Detroit, Winter 2006.

Parameter		*df* N	*df* D	F *Value*	P *Value*
Heart Rate	Exposure	1	12	0.18	0.9017
	Time	11	102	30.97	<0.0001
	Expos x Time	11	102	0.69	**0.0194**
ln (SDNN)	Exposure	1	12	2.24	0.5089
	Time	11	102	4.67	<0.0001
	Expos x Time	11	102	0.47	0.5488
ln (rMSSD)	Exposure	1	12	1.65	0.6835
	Time	11	102	5.24	<0.0015
	Expos x Time	11	102	0.58	0.6125

Extending the mixed model analyses by using the 30-minute dataset, we detected significant differences between air- and CAPs-exposed rats for both HR and rMSSD (Table 5.5).

Table 5.5 Mixed modeling results using 30-minute means for heart rate, SDNN and rMSSD in SH rats exposed to air or CAPs, Detroit, Winter 2006.

Parameter		*df* N	*df* D	F *Value*	P *Value*
Heart Rate	Exposure	1	12	0.11	0.7416
	Time	204	1802	7.14	<0.0001
	Expos x Time	204	1802	1.68	**<0.0001**
ln (SDNN)	Exposure	1	12	0.05	0.8294
	Time	204	1802	2.60	<0.0001
	Expos x Time	204	1802	1.07	0.2478
ln (rMSSD)	Exposure	1	12	0.33	0.5748
	Time	204	1802	2.72	<0.0001
	Expos x Time	204	1802	1.21	**0.0278**

Only the 30-minute data were used in mixed model analyses to determine the strength of association of trace elements with cardiovascular responses, in this case HR and rMSSD (Figs 5.10, 5.11). Increased HR was associated with only La, while reductions in HR were associated with Ba, As, Pb, EC, Cd, Zn, S, Sr, Mn, Ca, Ti, Fe, Rb, Cr, Mg, Se, Sb, K, Cu (in decreasing magnitude of effect estimates). Increased rMSSD was associated with Ba, EC, Zn, As, and Rb (Figure 5.12).

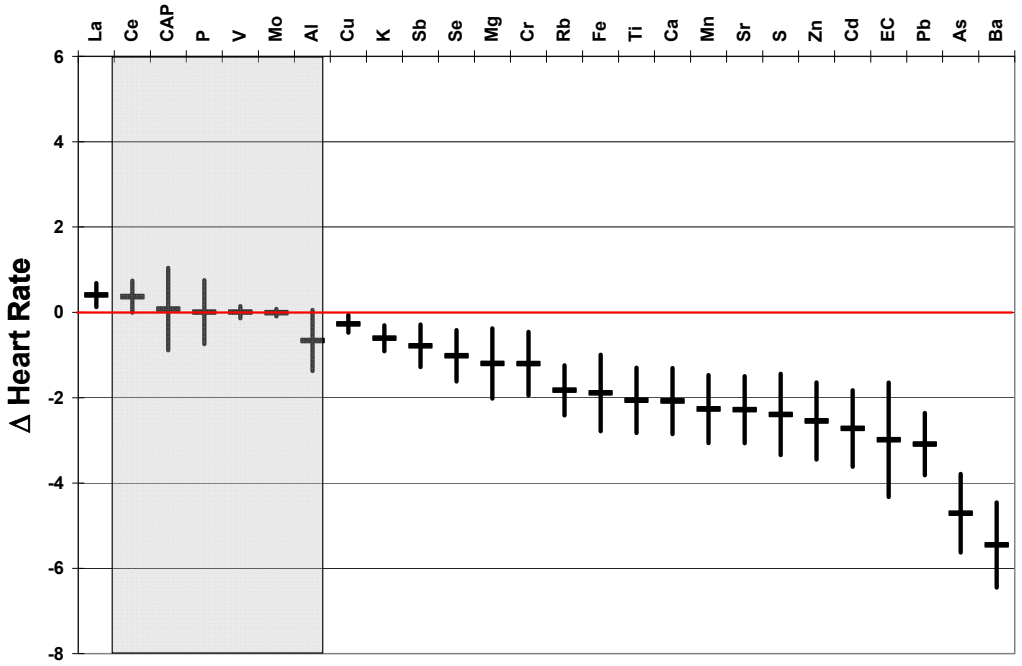


Figure 5.10 Relationship between PM components and heart rate, Detroit, Winter 2006.

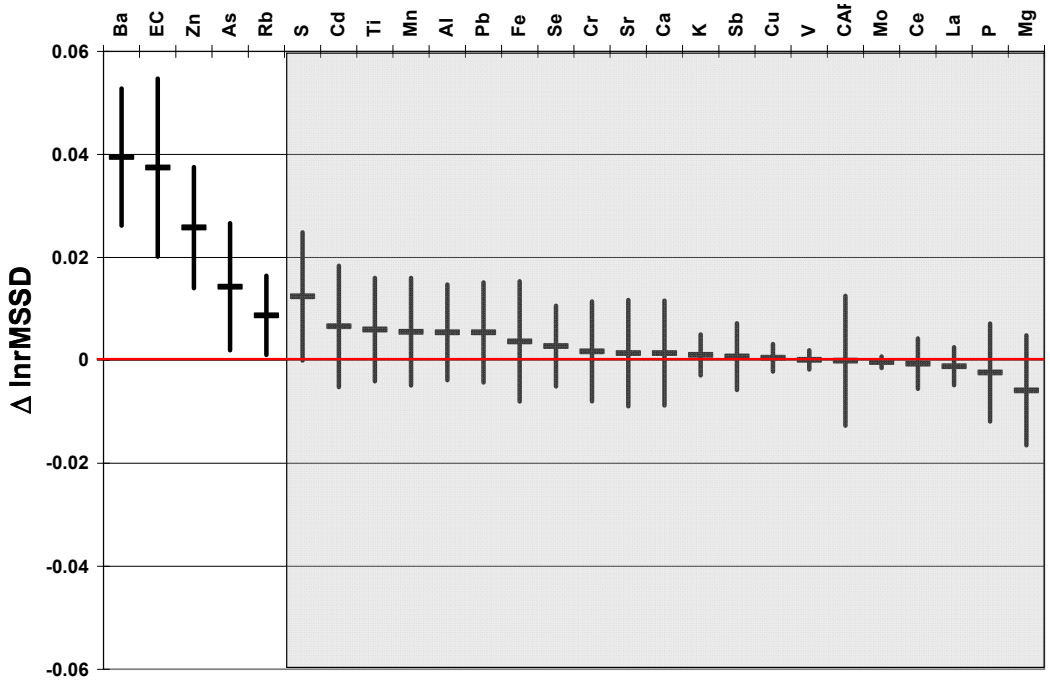


Figure 5.11 Relationship between PM components and rMSSD, Detroit, Winter 2006.

During the winter we found bidirectional effects of PM source factors on HR. The refinery factor was related to increases in HR, whereas secondary, sludge incineration, and cement/lime factors were associated with decreased HR in CAPs-exposed SH rats (Figure 5.12). Increased HRV as indicated by rMSSD was associated with mobile source and coal/secondary factors (Fig 5.13).

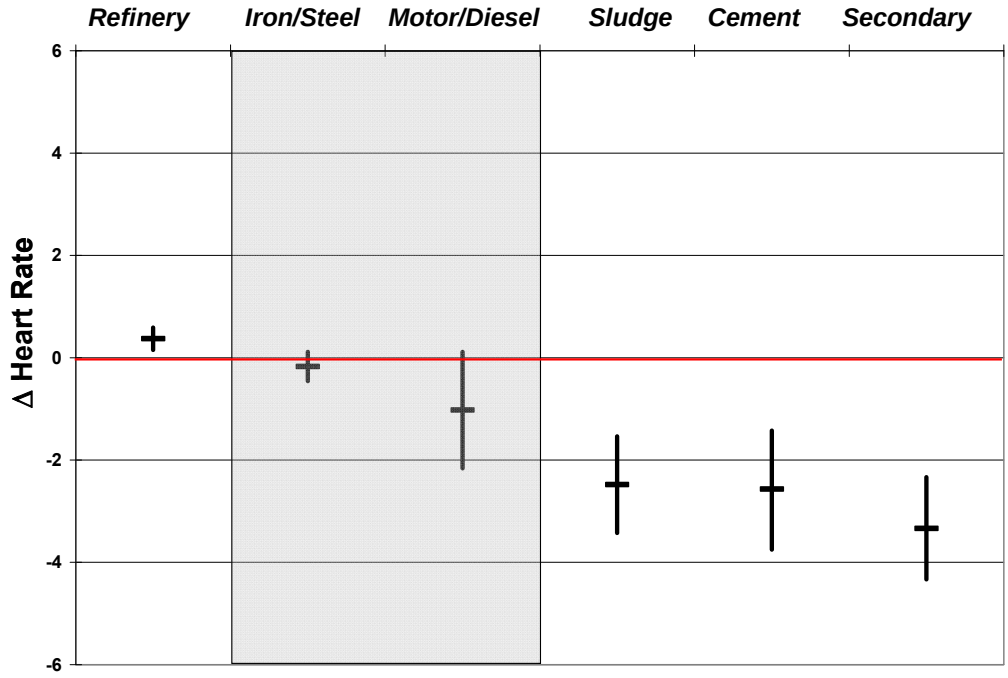


Figure 5.12 Relationship between $PM_{2.5}$ source factors and heart rate, Detroit, Winter 2006.

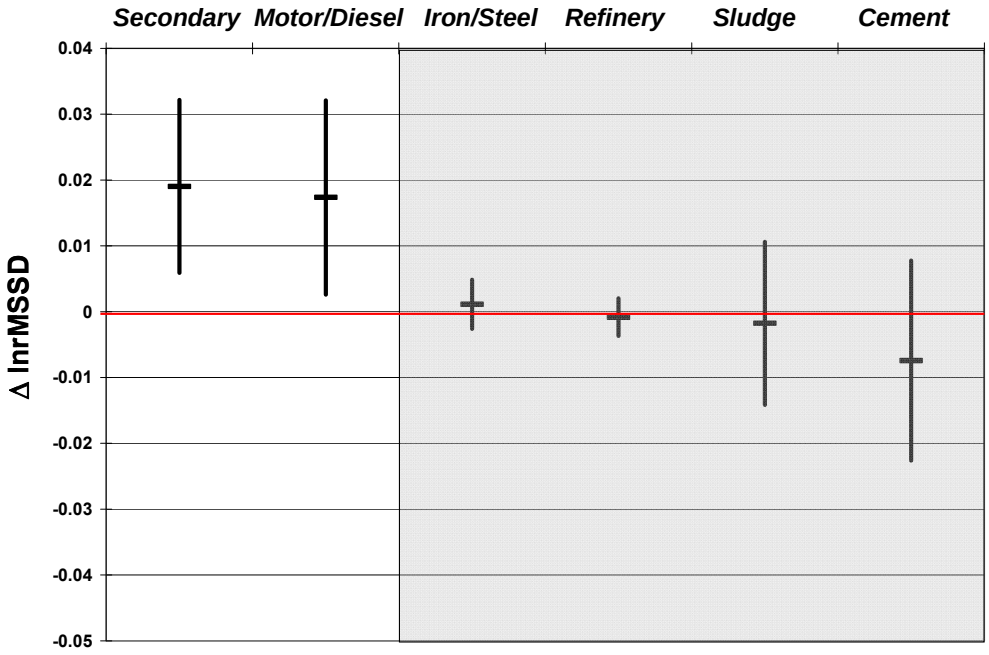


Figure 5.13 Relationship between $PM_{2.5}$ source factors and rMSSD, Detroit, Winter 2006.

6.0 STEUBENVILLE RESULTS: SUMMER 2006

6.1 CAPs Characterization

The average ambient $PM_{2.5}$ and CAPs concentrations during the 13-day exposure period were 25±11 μg/m^3 and 487±321 μg/m^3, respectively. Daily variations of CAPs mass and major chemical component concentrations during the 13-day exposure study are shown in Table 6.1. The overall average CEF of particle mass concentrations during the 13-day exposure study was 19.

Table 6.1 CAPs major components from the 13-day summer exposure study in Steubenville. All concentrations in μg/m^3.

August 2006	CEF[1]	Mass	OM[2]	EC	Sulfate	Nitrate	Ammonium	Urban dust[3]	Unidentified
8/2	32	1155	272	10	321	8	206	20	338
8/3	31	794	192	7	175	5	54	14	362
8/4	32	382	155	5	51	5	25	11	141
8/5	19	294	146	8	17	3	15	12	105
8/6	16	559	487	3	94	1	36	11	0
8/7	28	1011	266	10	234	6	67	13	429
8/8	20	295	80	7	40	3	23	8	141
8/9	10	243	93	19	36	6	18	31	72
8/10	16	571	128	7	146	3	45	11	242
8/11	13	267	57	7	90	5	32	19	76
8/12	9	95	34	1	6	1	5	10	49
8/13	12	259	80	15	89	5	13	19	56
8/14	10	400	135	16	136	6	42	26	65
TWA	**19**	**487**	**163**	**9**	**110**	**4**	**45**	**16**	**139**

1. CEF (concentration enrichment factor)
2. Organic mass (OM) was estimated from organic carbon (OC) x 1.8
3. Urban dust: 1.89*Al+1.4*Ca+1.43*Fe+2.14*Si, where Si is estimated by K/0.15

Table 6.2 CAPs elemental composition during the summer Steubenville exposure study. All concentrations in ng/m^3.

August 2006	8/2	8/3	8/4	8/5	8/6	8/7	8/8	8/9	8/10	8/11	8/12	8/13	8/14
Mg	544	550	210	242	247	283	268	764	259	674	321	495	718
Al	1391	957	233	442	225	418	216	1033	245	762	215	462	521
P	430	212	207	181	157	76	128	246	113	113	194	127	320
S	78917	42539	13087	5961	24528	57929	10273	12772	42159	11885	2562	7621	41286
K	778	565	533	601	618	589	265	1046	529	418	469	827	1014
Ca	1457	1225	1354	699	136	992	1243	5485	839	5505	1173	2977	2905
Ti	33.8	27.7	61.1	148.6	122.0	22.0	87.5	275.2	20.9	83.3	18.4	148.6	31.3
V	22.6	18.9	8.5	25.6	28.7	7.0	13.9	38.4	6.1	11.2	1.6	29.2	15.0
Cr	24	21	9	45	19	22	49	68	41	31	25	43	25
Mn	71	45	56	83	79	135	148	225	67	155	46	85	307
Fe	2840	1648	1009	1170	972	1354	1464	4378	1473	2485	922	1318	4730
Co	1.0	1.8	0.2	0.5	0.7	0.5	1.5	1.3	0.4	0.6	0.5	0.8	0.8
Ni	13.5	8.1	3.8	10.7	5.2	3.2	56.7	21.3	5.7	11.4	7.1	14.9	11.8
Cu	29	11	21	57	20	10	11	46	29	25	56	25	26
Zn	200	94	97	1031	152	53	365	1635	587	474	635	1770	4821
As	16.1	9.3	9.9	10.1	9.8	13.6	7.9	11.8	16.2	8.9	2.8	19.6	13.4
Se	88.6	63.4	46.9	17.4	180.0	70.9	21.1	38.0	45.1	13.8	8.4	22.4	42.0
Rb	2.7	2.2	1.1	1.6	1.3	1.2	1.2	2.5	1.1	1.4	1.0	1.5	4.3
Sr	12.5	11.1	5.6	6.0	9.1	7.5	5.3	14.0	6.6	13.2	4.8	12.7	9.3
Mo	2.8	2.1	0.9	5.7	14.6	2.4	6.4	39.1	5.5	9.1	11.9	38.7	4.7
Cd	20.7	7.4	5.7	34.7	7.6	4.9	3.4	15.1	8.0	11.4	7.9	35.2	16.4
Sb	16.8	7.2	4.2	10.5	9.7	9.3	5.4	12.4	17.6	9.3	6.0	12.9	20.0
Ba	45	35	33	44	36	29	24	75	38	53	37	45	40
La	2.4	1.3	0.4	1.0	0.6	0.6	1.1	1.2	1.1	1.3	0.5	1.0	0.7
Ce	3.2	2.1	0.8	1.2	0.8	1.2	1.3	2.4	1.6	2.3	0.9	1.6	1.4
Sm	0.24	0.21	0.07	0.07	0.08	0.11	0.07	0.20	0.07	0.22	0.08	0.11	0.12
Pb	123	58	59	250	84	56	74	208	80	101	65	311	208

Similar to what was observed during the Detroit summer study, CAPs concentrations during the Steubenville summer study were dominated by organic carbon and sulfate. Relatively high concentrations of ambient $PM_{2.5}$ (> 30 μg/m^3) were frequently observed at this site and were shown to be associated with transport of air masses from the area where coal-fired power plants and other industrial facilities are concentrated. As discussed, the Ohio River Valley is characterized by numerous coal-fired power plants and other industrial facilities, and these sources are known to be significant contributors to ambient air pollution in the Eastern US (Kim et al., 2007; Pekney et al., 2006). In particular, the Steubenville (OH) – Weirton (WV) area contains several large point sources, including coal-fired power plants, steel mills, and coke facilities. Table 6.2 shows the elemental composition of CAPs during the summer study in Steubenville. Compared to the summer exposure study in Detroit, Ti, V, Se and Cd concentrations were higher during the Steubenville study.

6.2 Characterization of Ambient $PM_{2.5}$ Sources

Figure 6.1 shows temporal variations of ambient $PM_{2.5}$ concentrations measured by TEOM at the Steubenville site during the 13-day period in August 2006. Detailed results of the PMF analysis

from the Steubenville study are described in a manuscript that was submitted for publication (Morishita et al., 2010b). The aims of this paper were: (1) to present chemical composition of ambient $PM_{2.5}$ and its potential emission sources in Steubenville, (2) to evaluate the PMF modeling results using observed meteorological data, and (3) to illustrate temporal variations of the source contribution and observed HRV during the inhalation exposure period. As a brief summary, results from PMF indicated that six major factors contributed to the observed ambient $PM_{2.5}$ mass during the summer exposure study (Figure 6.2); elemental contributions to each of these factors are shown in Figure 6.3. Primary sources included (1) coal combustion/secondary, (2) mobile sources, (3) metal coating/processing, (4) iron and steel manufacturing, (5) Pb factor, and (6) waste incineration.

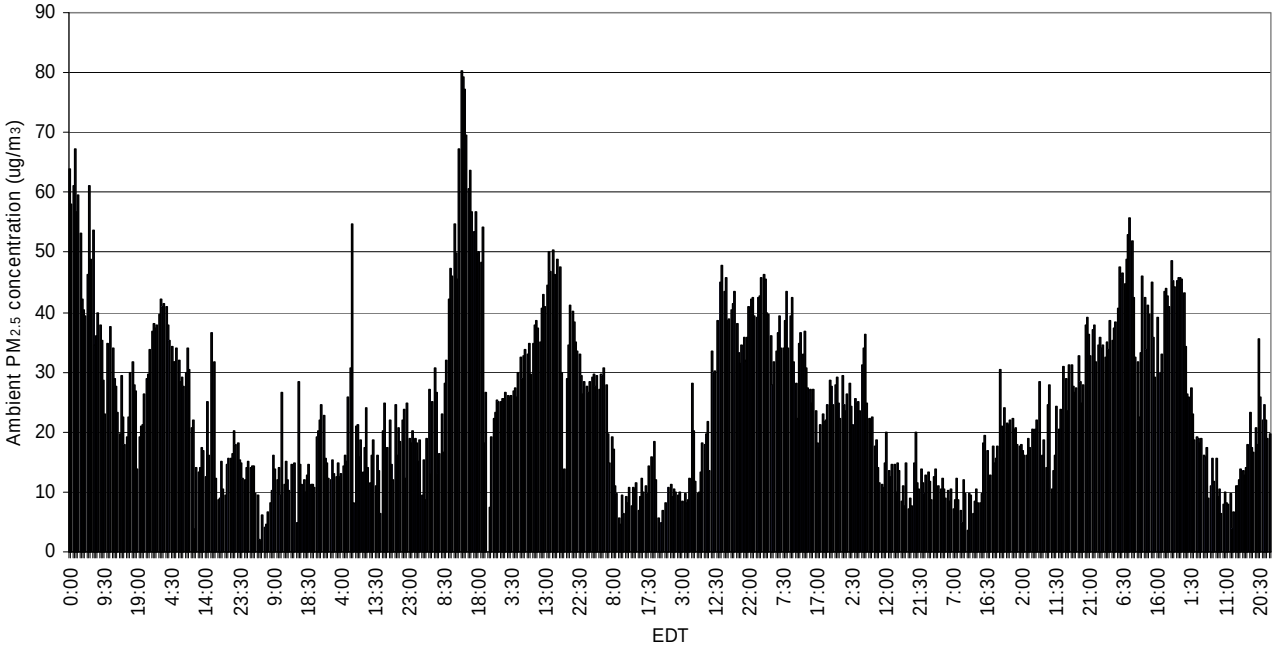


Figure 6.1 Temporal variations of ambient $PM_{2.5}$ concentrations measured by TEOM in Steubenville during the 13-day exposure days (8/2/06-8/16/06).

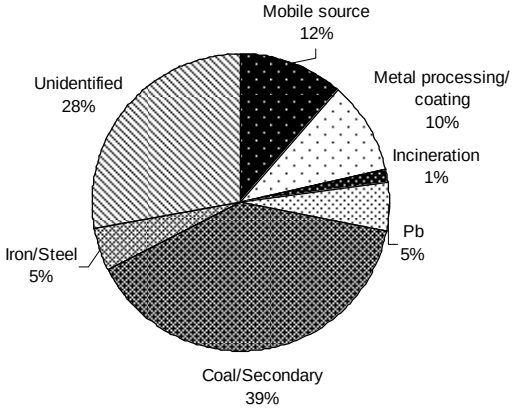


Figure 6.2 Average factor contributions to ambient $PM_{2.5}$ during the 13-day exposure period: Results from 221 30-minute SEAS samples collected in August 2006.

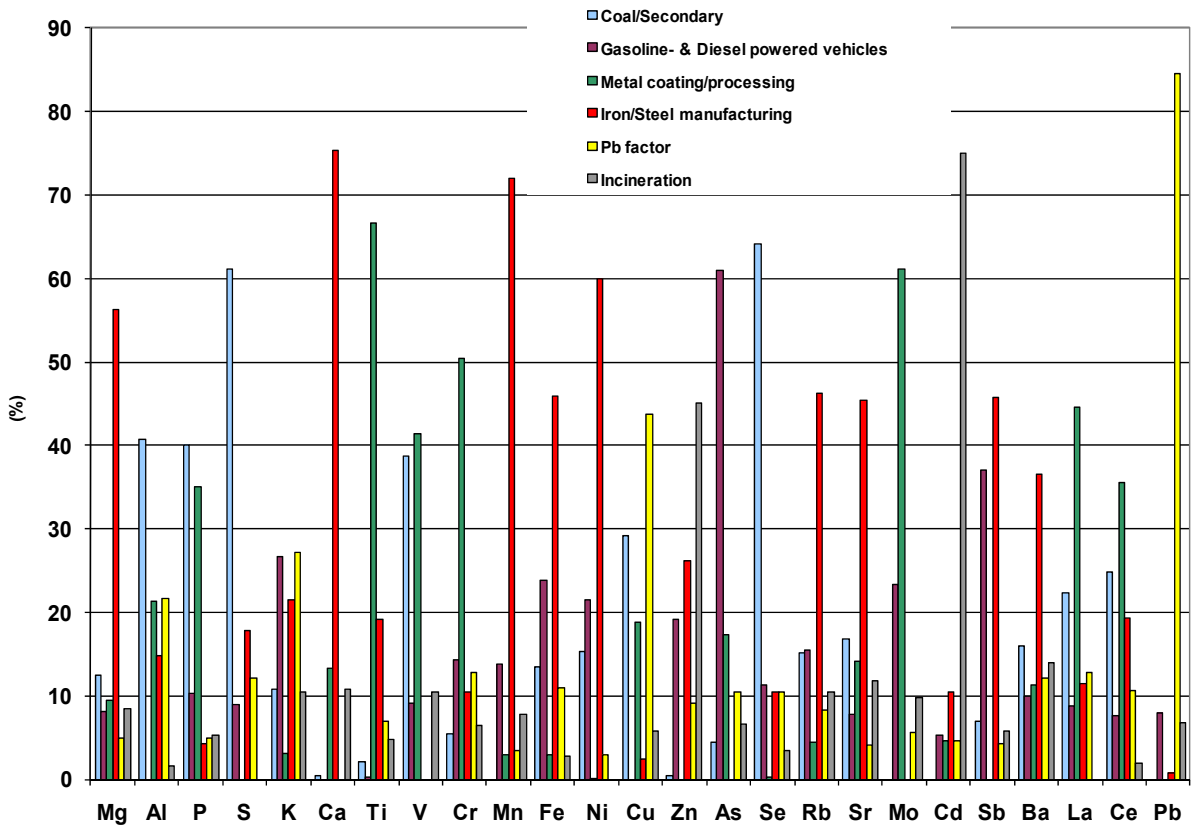


Figure 6.3 Percentage contribution of each element to each identified source factor during summer studies.

Figure 6.2 shows that during the 13-day summer exposure study conducted in August 2006, the contributions from the secondary aerosol factor were the highest (39%), followed by mobile source (12%), metal processing/coating (10%), and iron/steel manufacturing (5%).

Table 6.3 Primary gaseous pollutants measured during 13 8-hour exposure periods in Steubenville.

	SUMMER			
CO	0.3 (1.0)	±	0.1	(ppm)
SO_2	10.2 (104)	±	14.6	(ppb)
NO	4.6 (40)	±	4.6	(ppb)

Parenthetic values are maxima

Figure 6.4 shows wind rose plots of time-averaged PMF factor contributions as a function of wind direction for the 30-minute SEAS data from the Steubenville sampling site. During the 13-day exposure period, the dominant wind directions were northeasterly and southwesterly. Average concentrations of the primary gaseous pollutants are shown in Table 6.3. Compared to

the Detroit summer study, the average SO_2 concentration in Steubenville was twice as high, and the maximum concentration was over 100 ppb. These high concentrations were generally observed with the SWS wind direction. Furthermore, as shown in Figure 6.5, the highest ambient $PM_{2.5}$, S and Se concentrations were associated with southwesterly winds; this is consistent with the locations of multiple large coal-fired power plants in the Ohio River Valley on a heading of 180-200° from the measurement site. A recent study in Steubenville also reported that many pollution episodes exhibited strong covariation between $PM_{2.5}$ and primary gaseous pollutants including CO, NOx and/or SO_2, although these episodes were frequently observed in cooler months (Connell et al., 2005). According to the USEPA Facility Emission Report, four coal combustion utilities located in a southerly direction from Steubenville were the largest sources of $PM_{2.5}$ in the region, emitting over 2000 tons of $PM_{2.5}$ per year (EPA 2002; Figure 1b). The sampling site was often impacted by coal combustion during the exposure period; an example is presented in Figure 6.6, which shows 30-minute temporal variations of Se and S concentrations during the exposure period on August 6. In the early morning, the dominant wind direction was south-southeasterly (150-180°) and gradually shifted to the south-southwest (190-210°) by early afternoon. Per the figure, as the wind direction changed, the levels of S and Se concentrations increased simultaneously, and the average ratios of S to Se between 10:00 and 15:30 was 551 (with a minimum of 290). Previous studies have reported S/Se ratios for coal-fired power plant stack emissions of <200 (Olmez et al., 1988) and <100 (England et al., 2007; Lee 2001) for eastern U.S. coal (with electrostatic precipitation emission controls). It was also reported that in the ambient environment, S/Se ratios drop to less than 1000 in areas of strong SO_2 and Se sources. Two-day backward trajectories calculated via HYSPLIT were used to investigate the upwind history of air masses that impacted the sampling site. The most probable path of air masses reaching the site in the late morning and mid-afternoon on August 6th is shown in Figure 6.7a.

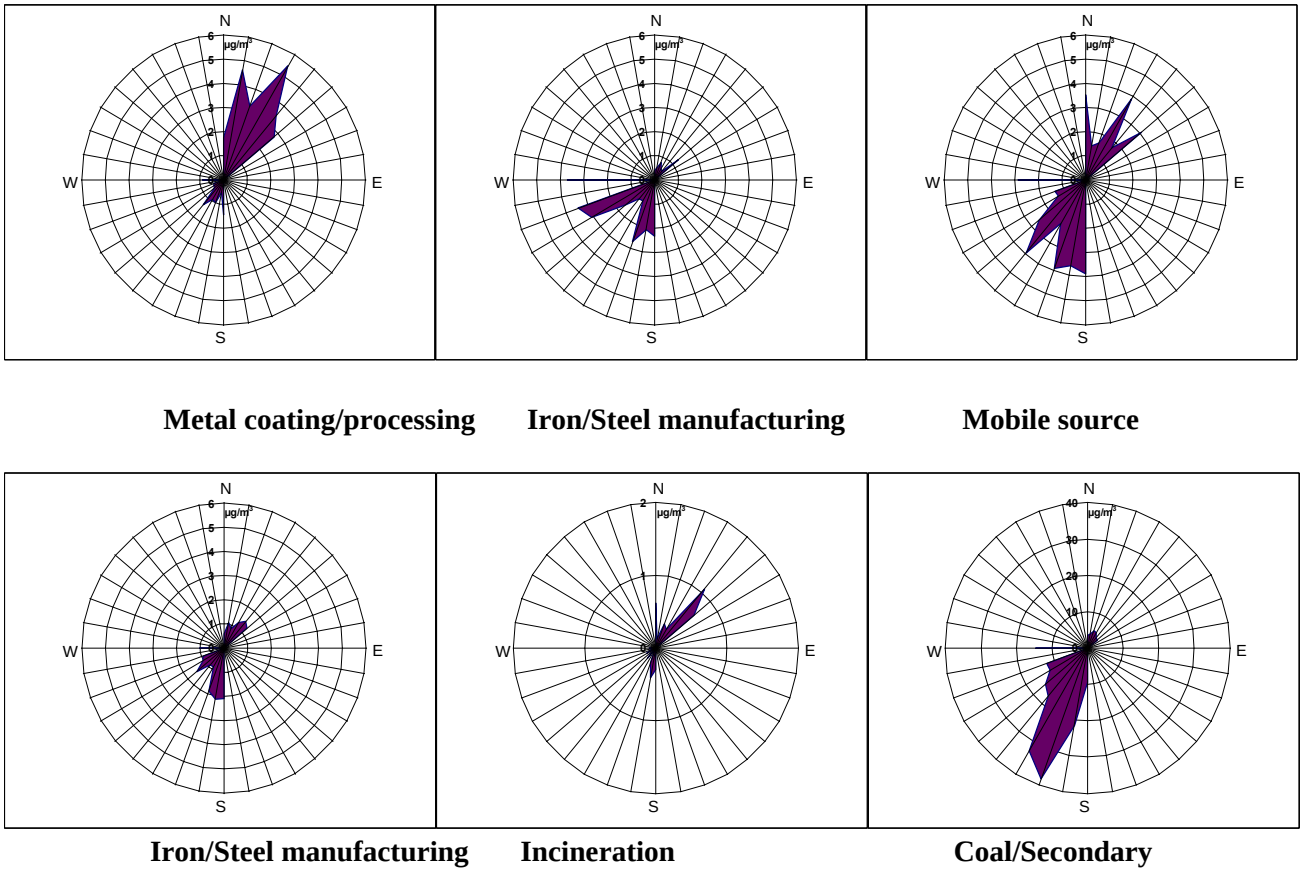


Figure 6.4. Average factor contributions versus wind direction from 221 SEAS samples collected in August 2006.

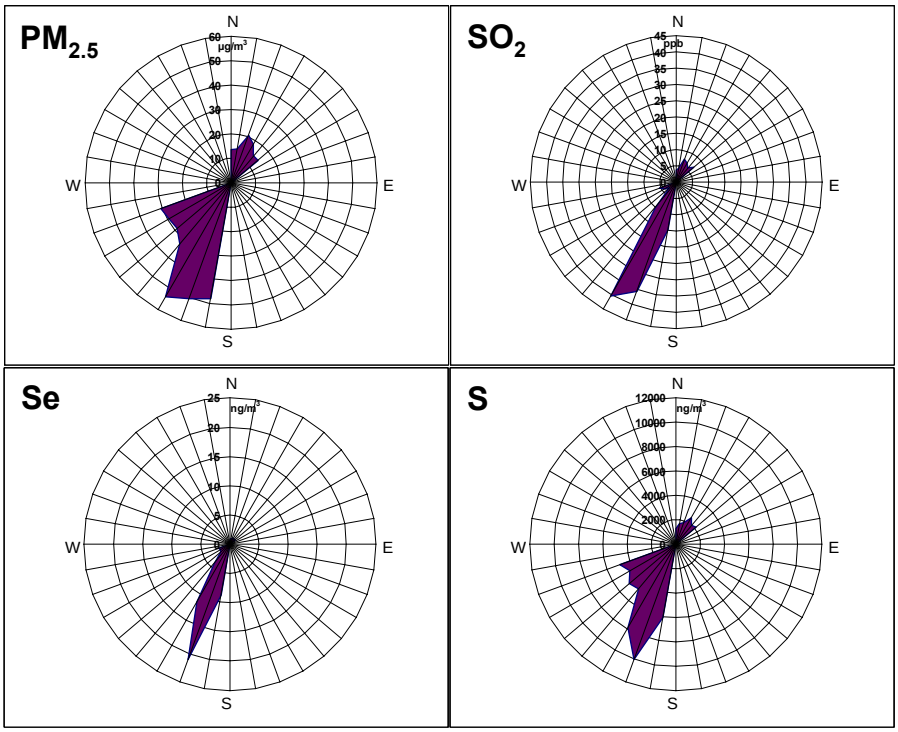


Figure 6.5 Average $PM_{2.5}$, SO_2, Se and S concentrations as a function of wind direction from 221 data points collected in August 2006.

The upwind path of the air masses through regions with a high density of coal-fired plants further supports the PMF modeled impact from these plants to the study site using the SEAS samples. This factor is dominated by secondary sulfate particles attributable to regional SO_2 sources, with coal burning the largest of the SO_2 sources in the Midwest, and coal-fired power plants the largest of the coal users in the region. In contrast, lower pollutant concentrations were generally seen with long-range transport of air masses from the north. For example, the lowest CAPs concentration was observed on August 12th. During the exposure period, the predominant wind direction was from the north, and the HYSPLIT back-trajectory model in Figure 6.7b shows that the air mass was transported from Canada.

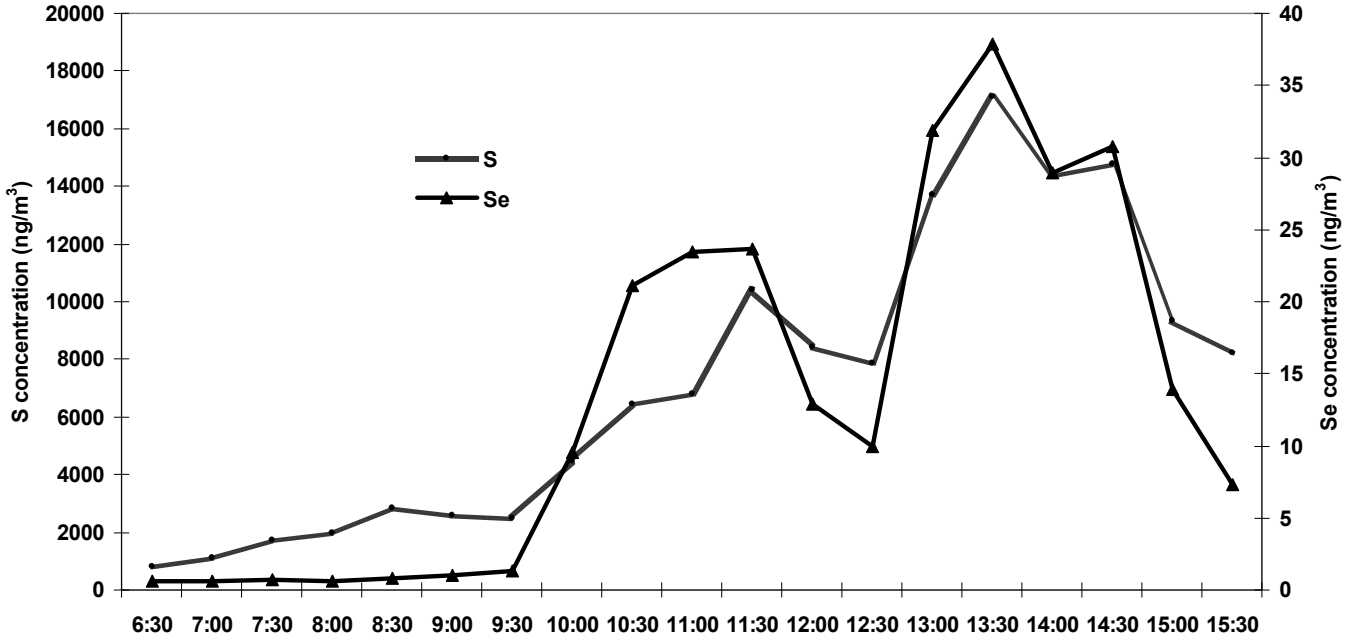


Figure 6.6 Temporal variations in Se and S concentrations from SEAS samples collected on August 6, 2006.

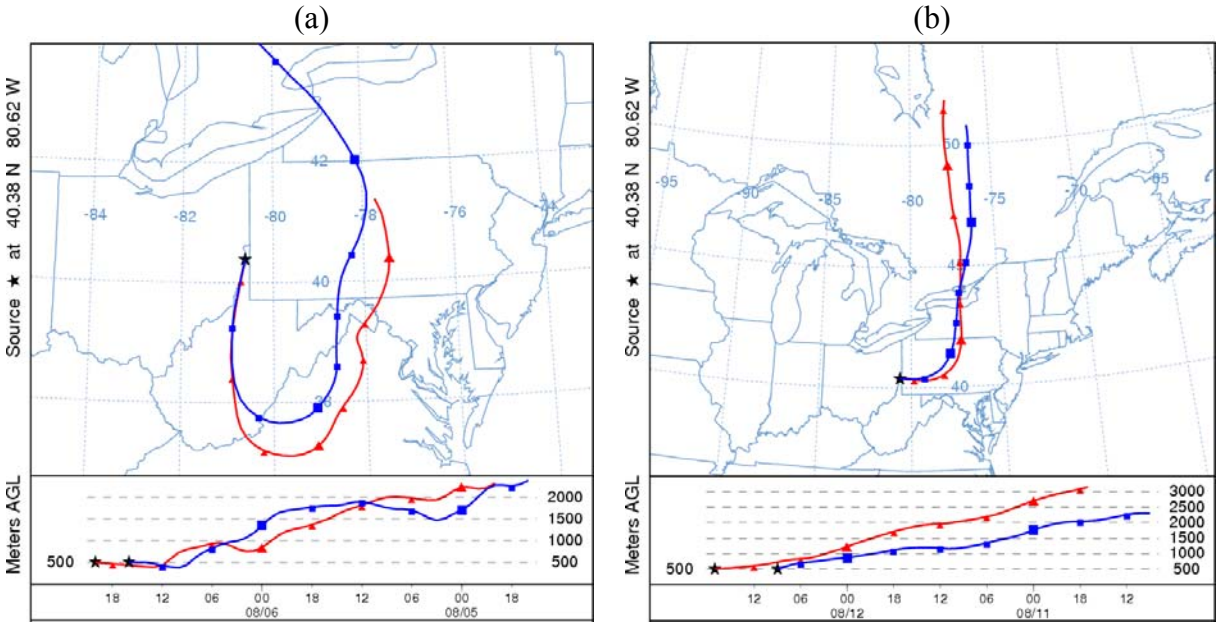


Figure 6.7 HYSPLIT-generated backward trajectories showing the history of air mass arriving at the Steubenville site on (a) August 6th 2006 and (b) August 12, 2006 (Data are from NOAA 2006).

6.3 Bronchoalveolar Lavage Fluid, Tissue, and Serum Analysis

CAPs exposure caused increased BAL cellularity in WKY, but not SH rats (Fig. 6.8). Increased BAL cells in WKY rats were predominately macrophages, with a minor contribution from neutrophils.

Inhalation of CAPs had no effect on stored mucus (intraepithelial mucosubstances, IM) (Fig. 6.8). WKY rats had more intraepithelial mucosubstances in proximal pulmonary airways compared to SH rats regardless of air or CAPS exposure. The IM detected in WKY rats was minimal, and accounted for 1-2 mucous cells per airway section. In distal airways, IM was undetectable in either strain or exposure group.

No strain or exposure related differences were detected in BALF content of total protein (Fig. 6.11). Air-exposed WKY rats had lower serum C-reactive protein (CRP) than air exposed SH rats, but this relationship was not evident after CAPs exposure. Lastly, serum levels of TNFα, IFNγ, IL-10 and IL-6, where detectable, were not significantly different across strains or exposures (data not shown).

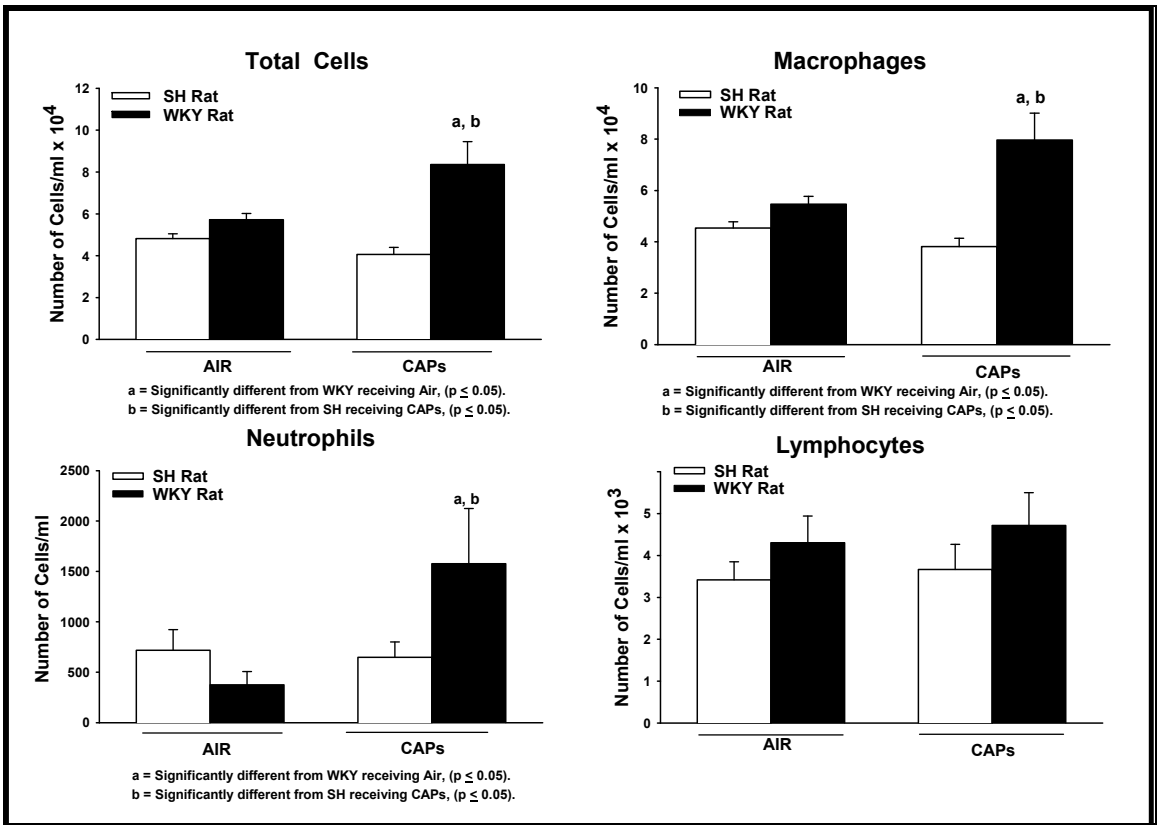


Figure 6.8 Effect of CAPs exposure on bronchoalveolar lavage cellularity, Steubenville, Summer 2006.

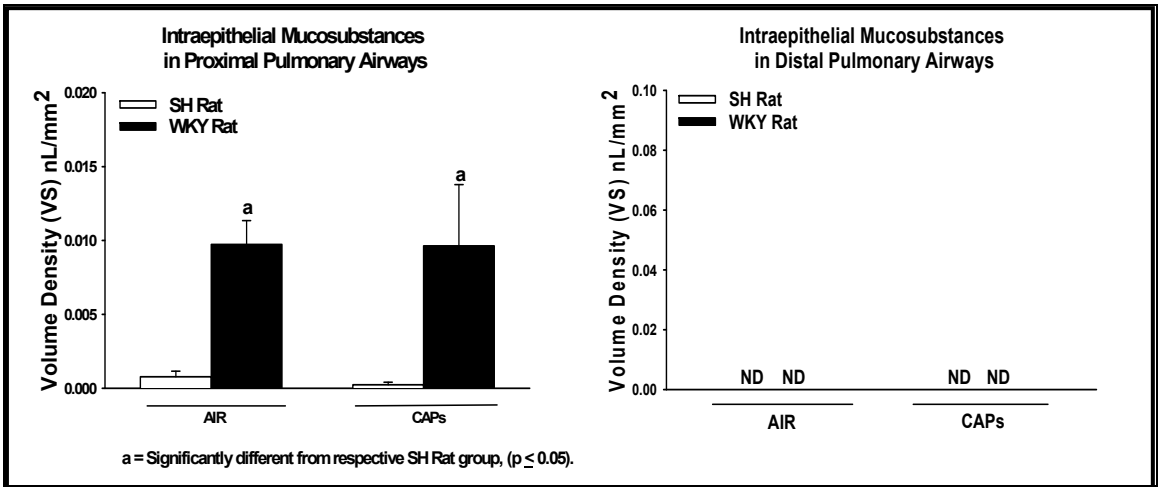


Figure 6.9 Effect of CAPs exposure on intraepithelial mucosubstances in proximal and distal pulmonary airways, Steubenville, Summer 2006.

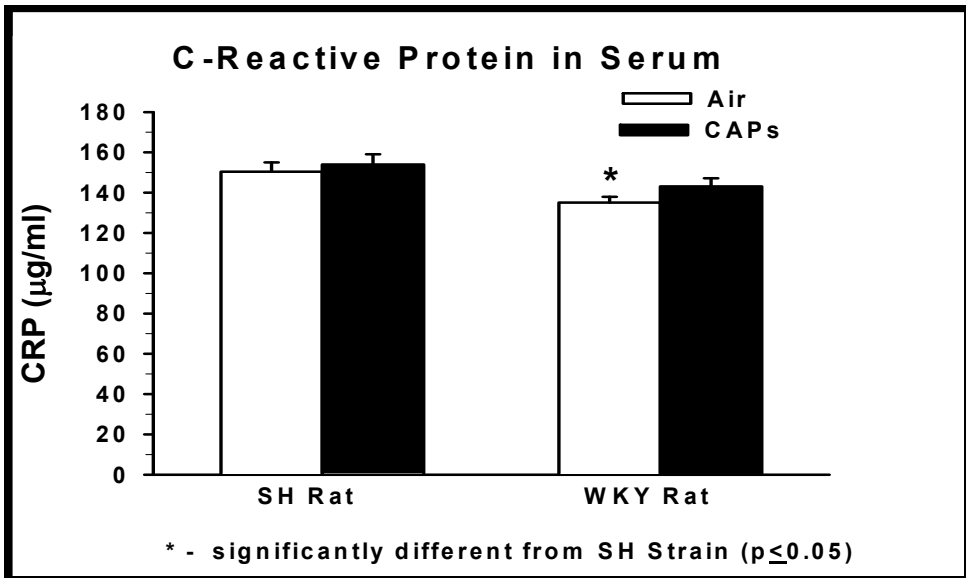


Figure 6.10 Effect of CAPs exposure on serum C-reactive protein, Steubenville, Summer 2006.

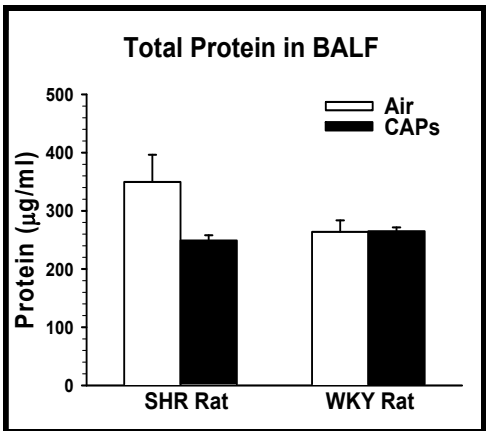


Figure 6.11 Effect of CAPs exposure on total protein in bronchoalveolar lavage fluid, Steubenville, Summer 2006.

6.4 Heart Rate and Heart Rate Variability Responses

During the summer exposure period in Steubenville, over 95% of the exposure period had winds coming from the northeast (NE) or southwest (SW) direction. As such we further divided the analysis of HRV and air pollution into these categories: All Winds, NE Winds and SW Winds. Using the 30-minute resolution data, differences in HR and SDNN between air and CAPs-exposed rats were significant for all wind categories, while only SW winds were associated with changes in rMSSD (Table 6.4).

Table 6.4 Mixed modeling results for wind direction-dependent changes in heart rate, SDNN and rMSSD in SH rats exposed to air or CAPs, Steubenville, Summer 2006**.**

	Heart Rate	***df* N**	***df* D**	**F Value**	***p* Value**
All Wind	Exposure(Exp)	1	14	0.21	0.6574
	Time (tm)	207	2335	6.52	<.0001
	Exp*tm(date)	**207**	**2335**	**1.4**	**0.0003**
NE Winds	Exposure(Exp)	1	14	0.08	0.78
	Time (tm)	115	1270	5.46	<.0001
	Exp*tm(date)	**115**	**1270**	**1.52**	**0.0006**
SW Winds	Exposure(Exp)	1	14	0.13	0.7234
	Time (tm)	91	1051	7.75	<.0001
	Exp*tm(date)	**91**	**1051**	**1.34**	**0.0210**
	ln(SDNN)				
All Wind	Exposure(Exp)	1	14	0.01	0.915
	Time (tm)	207	2335	1.7	<.0001
	Exp*tm(date)	**207**	**2335**	**1.33**	**0.0018**
NE Winds	Exposure(Exp)	1	14	0.05	0.8298
	Time (tm)	115	1270	1.37	0.0081
	Exp*tm(date)	**115**	**1270**	**1.31**	**0.0195**
SW Winds	Exposure(Exp)	1	14	0.25	0.6241
	Time (tm)	91	1051	1.97	<.0001
	Exp*tm(date)	**91**	**1051**	**1.35**	**0.0204**
	ln(r-MSSD)				
All Wind	Exposure(Exp)	1	14	0.68	0.4218
	Time (tm)	207	2335	1.46	<.0001
	Exp*tm(date)	207	2335	1.05	0.3162
NE Winds	Exposure(Exp)	1	14	0.46	0.5093
	Time (tm)	115	1270	1.35	0.01
	Exp*tm(date)	115	1270	0.87	0.8267
SW Winds	Exposure(Exp)	1	14	0.99	0.3371
	Time (tm)	91	1051	1.72	<.0001
	Exp*tm(date)	**91**	**1051**	**1.32**	**0.0272**

As a first step to determine differences in physicochemical differences in $PM_{2.5}$ from each wind direction, and simple comparison of the trace elements are listed in Table 6.5, depicting those predominately from NE or SW directions. For some elements the differences were not great, but for others the concentrations were two-fold greater depending on wind direction. For example from the SW, total PM, Pb, Fe, Zn, S and Se were notably greater than when winds were from the NE; EC was also higher when winds were from this direction. By comparison, Mo, Cr, Cd, Sn, and Ti were roughly two-fold greater when winds came from the NE.

Table 6.5 Relative contribution of trace elements by wind direction. All units ng/m^3.

a) Elemental Concentrations Greater from the Southwest

Variable	NE Flow	Std Dev	**SW Flow**	**SW STDEV**
Se	0.98	0.62	**4.20**	**6.65**
Pb	5.57	6.26	**16.35**	**20.25**

PM	451.16	250.12	**1094.06**	**532.06**
S	1991.23	1040.60	**4781.04**	**2526.96**
Zn	35.41	44.35	**81.70**	**219.37**
K	42.62	45.99	**67.99**	**59.19**
Fe	38.24	49.29	**60.56**	**101.34**
Rb	0.10	0.06	**0.15**	**0.19**
Sb	0.85	0.40	**1.22**	**1.35**
Mn	4.40	3.17	**5.95**	**7.14**
Al	26.79	28.49	**35.66**	**19.33**
Cu	3.23	3.38	**4.06**	**3.30**
EC	5906.72	3364.30	**7107.19**	**2581.04**
Mg	32.15	14.62	**37.74**	**26.35**
Sr	0.59	0.37	**0.67**	**0.45**
Ba	3.62	1.53	**3.78**	**1.69**

b) Elemental Concentrations Greater from the Northeast

Variable	**NE Flow**	**Std Dev**	SW Flow	SW STDEV
Mo	**1.35**	**1.35**	0.58	0.74
Cr	**0.81**	**2.09**	0.35	0.33
Cd	**1.01**	**1.61**	0.49	0.78
Sn	**1.55**	**2.34**	0.75	0.79
Ti	**1.58**	**2.13**	0.84	0.87
P	**13.06**	**11.25**	10.26	9.30
La	**0.03**	**0.02**	0.02	0.01
Ca	**193.78**	**155.66**	164.41	162.28
Ce	**0.04**	**0.03**	0.04	0.02
As	**1.23**	**1.30**	1.16	0.81
V	**1.32**	**1.79**	1.28	1.04

To better determine the association of wind direction and trace elements with cardiovascular responses, we used mixed modeling approaches using all the data, as well as that dichotomized by NE and SW wind directions (Table 6.6 and Figure 6.12). CAPs exposure induced bi-directional changes in HR and HRV that were associated with specific trace elements and wind directions (Table 6.6). La and V were both associated with elevated HR from either the NE or SW sectors, and for combined wind data. Overall, SW winds were associated with increases in HR and linked to Mo, Ce, As, Ti and $PM_{2.5}$ mass. From the NE sector conversely, several elements were linked to reduced HR including S and Se, Pb, Rb, Mn, Mg, Sr, Zn, Fe, Cd and Ca. The only trace element from the SW associated with decreased HR was K.

With regard to exposure-related changes in HRV, we found the strongest associations for trace elements with reductions in SDNN. Sb and As were associated with a decreased SDNN from both the NE and SW sectors, as well as combined wind data (Table 6.6, Fig 6.12). Northeasterly winds brought higher concentrations of V, Ti, Cr, Sn and Cd that were associated with decreased SDNN. Interestingly, although higher concentrations of Zn, Pb, and K were found with SW winds (Table 6.5), their effect on SDNN was seen only with NE winds. Similarly, Mo is greater with NE flow (Table 6.5), but was associated with SW winds and decreased SDNN. As such, the effects of trace element concentration on SDNN may not be driven by the mass of the specific

element alone. Source contributions and the effects of copollutants may be influencing these significant associations.

Table 6.6 Comparison of the associated pollutants with changes in HR and HRV based on prominent wind directions. Elements are listed in order of effect estimates.

Heart Rate	**INCREASE**	**Heart Rate**	**DECREASE**
All Wind	NOx, La, V, Ce, Sb, Mo	All Wind	K, Mg, Pb, Zn
NE	V, La	NE	S, Se, Rb, Pb, Mn, Mg, Zn, Sr, SO_2, Fe, Ca, Cd
SW	Mo, La, Ce, PM, NOx, V, Ti, As, Sb, SO_2	SW	K
ln(SDNN)	**INCREASE**	**ln(SDNN)**	**DECREASE**
All Wind		All Wind	EC, As, PM, Mo, Sn, V, NOx, Sb, Cd, Ti, Fe,
NE		NE	Sb, Pb, Zn, As, Sn, K, V, Cd, Ti, Cr
SW	Al	SW	Mo, As, PM, NOx, Sb, Fe
ln(r-MSSD)	**INCREASE**	**ln(r-MSSD)**	**DECREASE**
All Wind	Not assessed as not sig. in mixed models	All Wind	Not assessed as not sig. in mixed models
NE	Not assessed as not sig. in mixed models	NE	Not assessed as not sig. in mixed models
SW	Al, Mg	SW	Mo, V, SOx

Figure 6.12 -*continued*

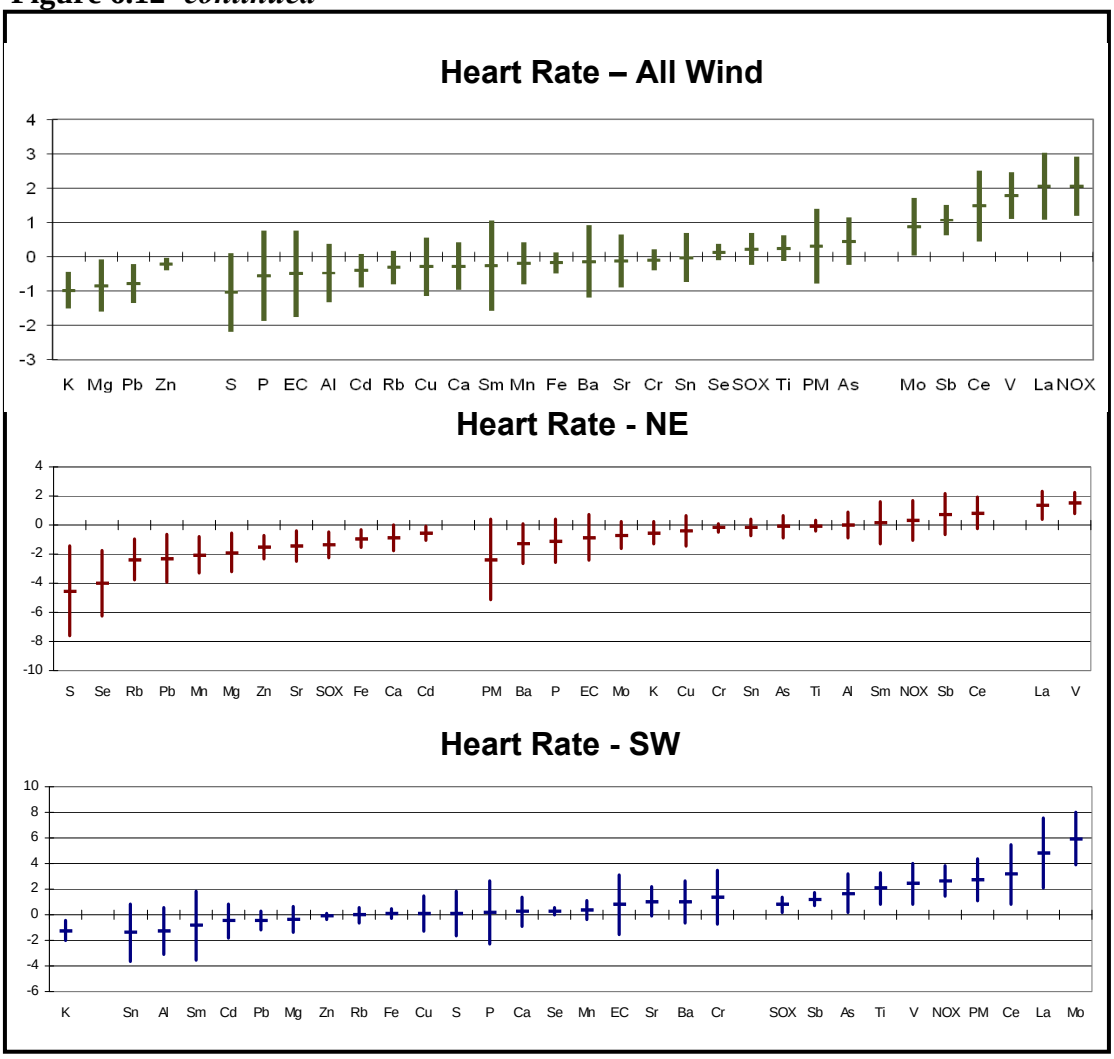
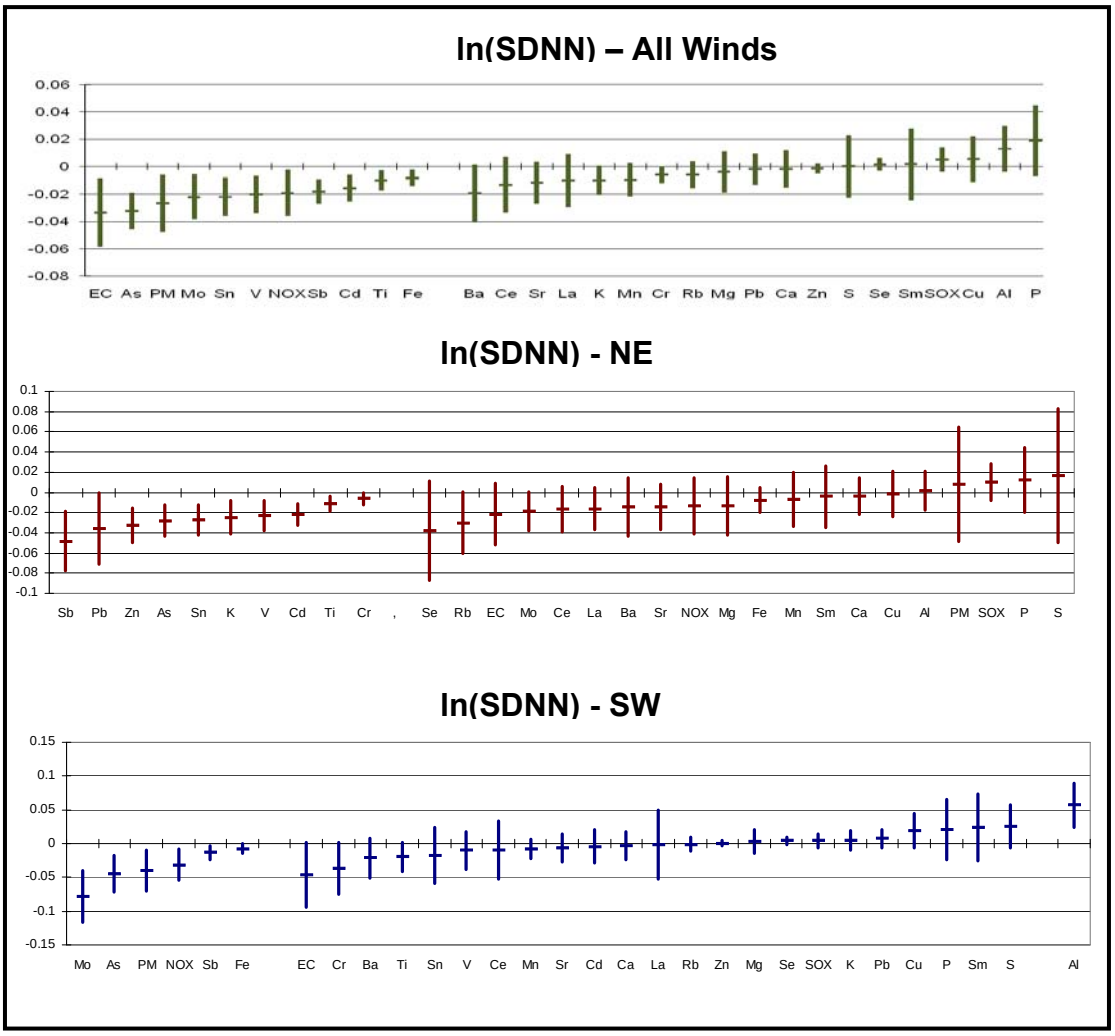


Figure 6.12 -*continued*



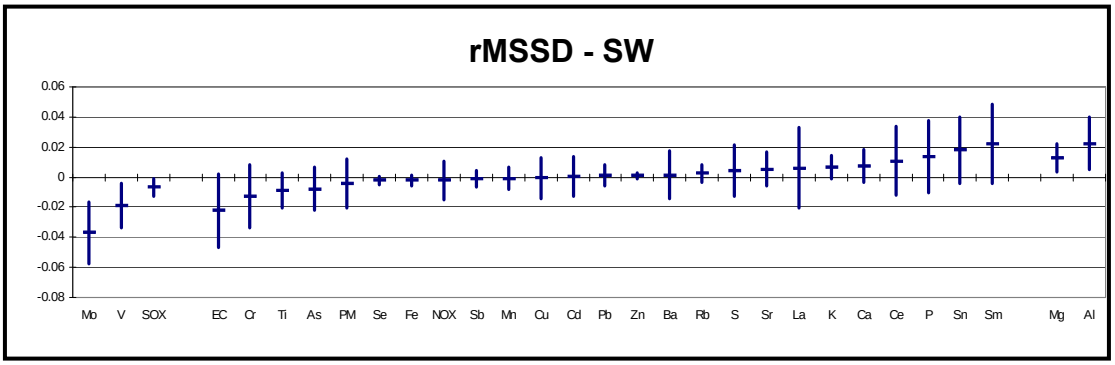


Figure 6.12 Effect estimates for the associations between HR and HRV parameter and individual PM components and gases, Steubenville, Summer 2006: for rMSSD only SW winds are depicted as significant differences between air and CAPs rats were observed.

In the exposure assessment section of this report, we identified six primary source factors at the site during the exposure period. These source factors include coal combustion/secondary, mobile sources, metal coating/processing, iron and steel manufacturing, Pb factor, and waste incineration. In the current study, SW winds and the metal factor were associated with increased HR, whereas factors of incinerator, lead and steel in NE winds were associated with decreased HR (Figure 6.13). This bidirectional pattern of responses generally agrees with the trace elements and wind impacts shown in Table 3. The steel factor also had contributions during SW winds.

During NE winds, decreased SDNN was associated with the incinerator, metal, and mobile source factors, and during SW winds with the metal factor. This result is consistent with the element contributions of Zn, Cd from the NE and Mo from the SW. Changes in rMSSD were not significantly associated with any source factors.

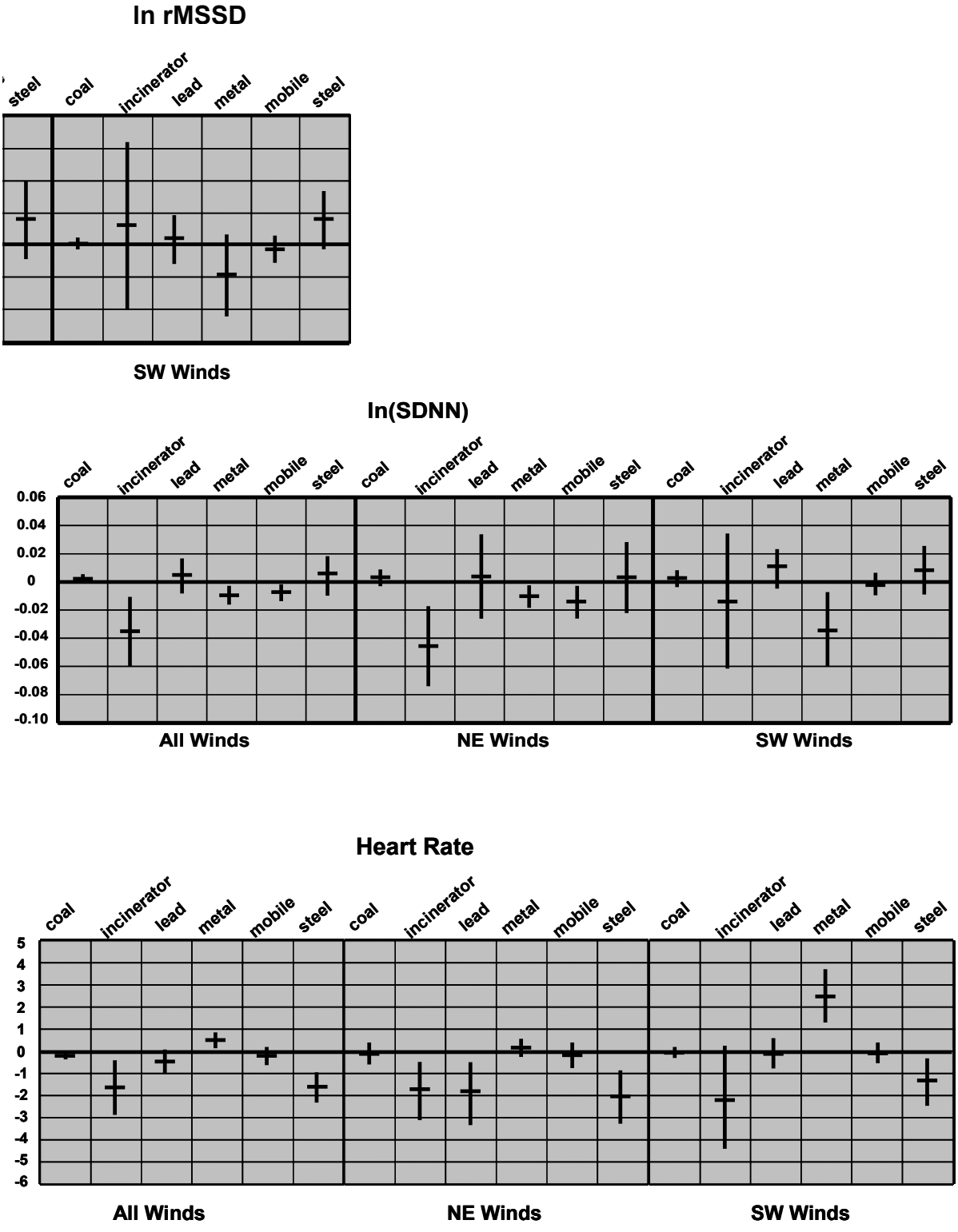


Figure 6.13 Dependence on wind direction for source factor associations with heart rate, SDNN, and rMSSD, Steubenville, Summer 2006; for rMSSD only SW winds are depicted as significant associations between air and CAPs rats were observed.

7.0 STEUBENVILLE RESULTS: WINTER 2007

7.1 CAPs Characterization

The average ambient $PM_{2.5}$ and CAPs concentrations during the 12-day exposure period were 18±10 μg/m^3 and 252±135 μg/m^3, respectively. Due to a severe ice storm, the exposure period on February 14th was canceled. Daily variations of CAPs mass and major chemical component concentrations during the 12-day exposure study are shown in Table 7.1. The overall average CEF of particle mass concentrations during the 12-day exposure study was 18. Similar to the summer CAPs, OM and sulfate were the highest-concentration components during the winter 12-day exposure period. Winter elemental concentrations (Table 7.2) were generally equivalent to summer concentrations, but a few elements including Al, P, Ca, Cu and Cd were all higher (>1.4 x) than in the summer. Compared to the Detroit winter exposure study, the average concentrations of P, Ti, Cd and Pb at the Steubenville site were more than twice as high.

Table 7.1. CAPs major components from the 12-day winter exposure study. All concentrations in μg/m^3.

February 2007	*CEF*[1]	*Mass*	*OM*[2]	*EC*	*Sulfate*	*Nitrate*	*Ammonium*	*Urban dust*[3]	*Unidentified*
2/9	13	150	35	1	14	34	17	23	50
2/10	15	228	40	0	21	62	26	19	79
2/11	12	184	44	3	18	31	17	17	72
2/12	18	253	79	9	30	34	23	18	77
2/13	30	367	86	5	87	57	46	15	85
2/15	14	134	23	2	15	25	10	17	60
2/16	13	179	41	3	18	37	16	15	63
2/17	41	567	169	13	262	24	87	32	12
2/18	3	197	51	2	43	43	34	16	24
2/19	18	251	56	6	47	43	36	23	63
2/20	11	104	23	6	15	4	8	16	47
2/21	24	416	82	5	183	43	96	23	7
TWA	**18**	**252**	**61**	**5**	**63**	**37**	**35**	**20**	**34**

1. CEF (concentration enrichment factor)
2. Organic mass (OM) was estimated from organic carbon (OC) x 1.8
3. Urban dust: 1.89*Al+1.4*Ca+1.43*Fe+2.14*Si, where Si is estimated by K/0.15

Table 7.2. CAPs elemental composition during the winter Steubenville exposure study. All concentrations in ng/m^3.

February 2007	2/9	2/10	2/11	2/12	2/13	2/15	2/16	2/17	2/18	2/19	2/20	2/21
Mg	523	557	266	292	268	307	325	719	282	700	368	523
Al	2020	1697	1077	1260	919	1201	1073	1687	1017	1545	1071	1247
P	715	531	541	510	420	400	468	565	439	462	556	469
S	65433	37727	12392	5689	20516	48487	9944	10950	34242	11165	2587	7120
K	882	732	731	782	692	726	489	1125	666	628	677	993
Ca	2565	2428	2603	2041	1583	2203	2542	5131	1855	5551	2264	3548
Ti	31	28	60	137	103	21	85	234	20	80	20	137
V	18.7	16.8	7.9	23.3	24.1	6.0	13.2	32.7	5.1	10.6	1.6	26.7
Cr	27	25	16	49	20	26	54	65	40	36	30	46
Mn	63	44	56	80	69	118	144	196	59	149	46	81
Fe	2373	1473	951	1078	807	1146	1397	3750	1222	2335	857	1221
Co	1.0	1.8	0.4	0.6	0.7	0.6	1.6	1.2	0.5	0.8	0.6	0.9
Ni	11.2	7.9	5.1	10.0	6.1	4.4	45.1	13.8	4.7	8.8	6.1	10.8
Cu	40	25	35	67	30	24	27	54	39	39	67	38
Zn	204	121	128	973	157	82	383	1417	514	476	615	1644
As	13.0	7.9	8.8	8.9	7.7	11.0	7.1	9.6	12.8	7.8	2.1	17.4
Se	63.9	48.4	37.2	12.4	147.3	51.2	16.0	24.6	28.4	8.7	4.4	14.9
Rb	3.3	2.9	2.0	2.5	2.0	2.0	2.2	3.1	1.9	2.4	2.0	2.4
Sr	14.8	14.1	9.6	9.8	11.6	10.6	9.4	16.2	9.7	16.8	9.0	15.9
Mo	3.2	2.8	1.7	6.1	13.1	2.9	7.0	34.4	5.4	9.4	11.8	36.4
Cd	24.3	13.6	12.4	38.6	13.0	11.2	10.4	19.8	13.5	17.8	14.6	39.3
Sb	12.3	5.6	3.3	8.4	7.9	6.9	4.4	8.4	11.6	6.9	4.3	9.4
Ba	36	30	30	38	32	24	23	56	28	44	30	37
La	1.9	1.2	0.4	0.9	0.6	0.5	1.0	1.0	0.8	1.1	0.5	0.9
Ce	2.7	2.0	0.9	1.2	0.9	1.1	1.4	2.0	1.4	2.1	0.9	1.5
Sm	0.21	0.20	0.09	0.09	0.10	0.11	0.09	0.17	0.08	0.21	0.09	0.11
Pb	147	98	102	264	124	94	118	205	105	132	104	301

7.2 Characterization of Ambient PM Sources

Figure 7.1 shows temporal variations of ambient $PM_{2.5}$ concentrations measure by TEOM in Steubenville during the 13-day exposure periods in February 2007. Compared to the Detroit winter study, more temporal variability and higher ambient $PM_{2.5}$ concentrations were observed. Average concentrations of the primary gaseous pollutants are also shown in Table 7.3.
PMF was applied to 190 ambient $PM_{2.5}$ SEAS samples, and five factors were resolved: coal/secondary sulfate, gasoline- and diesel-powered vehicles, iron/steel manufacturing, metal coating/processing, and coke oven. Figure 7.2 shows that during the 13-day winter exposure study, the contributions from the gasoline & diesel powered-vehicle factor was highest (21%),

followed by iron/steel manufacturing (20%), and coal/secondary (19%). Figure 7.3 shows the average contribution of each element to the identified source factors during the exposure period.

Figure 7.4 shows the wind-rose plots of the time-averaged PMF factor contributions as a function of wind direction for the 30-minute SEAS data as observed at the Steubenville sampling site. During the 12-day exposure period, the dominant wind directions were southwesterly and northwesterly. Similar to what was observed during the summer study, elevated levels of ambient $PM_{2.5}$ and SO_2 were associated with southerly winds.

Table 7.3. Primary gaseous pollutants measured during 12 8-hour exposure periods in Steubenville.

	WINTER			
CO	0.3 (4.7)	±	0.4	(ppm)
SO_2	17 (202)	±	32	(ppb)
NO	10 (73)	±	14	(ppb)

Parenthetic values are maxima

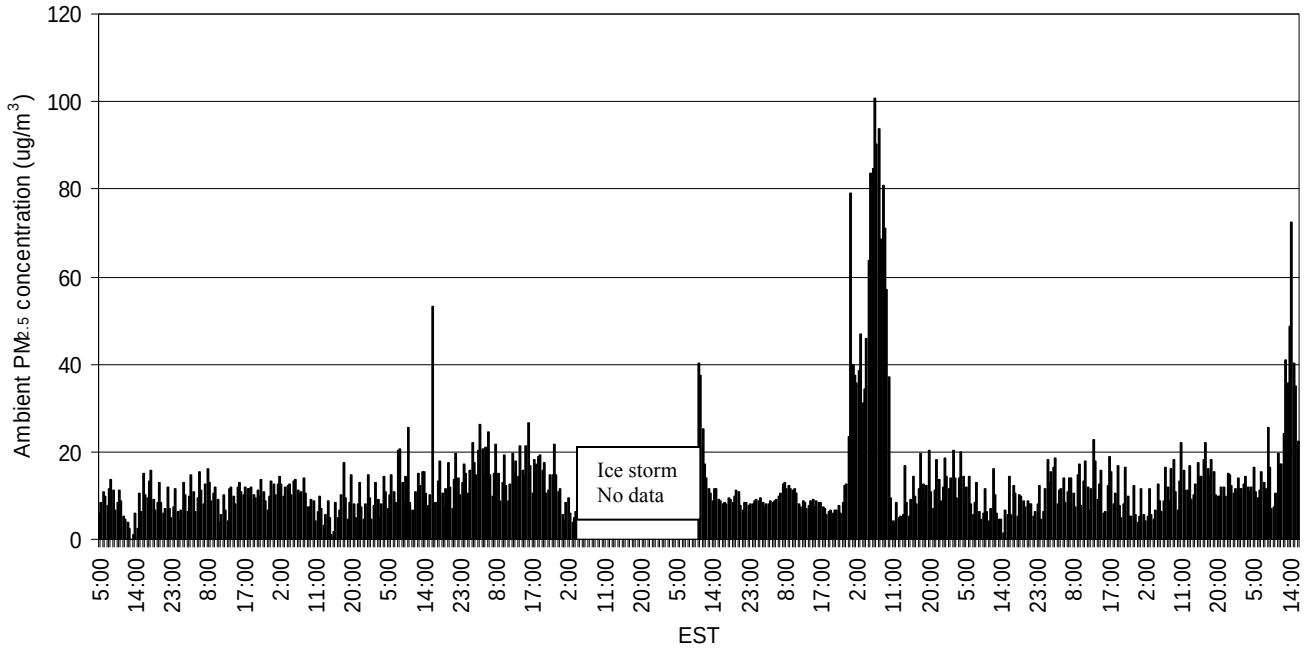


Figure 7.1 Temporal variations of ambient $PM_{2.5}$ concentrations measured by TEOM in Steubenville, Winter 2007.

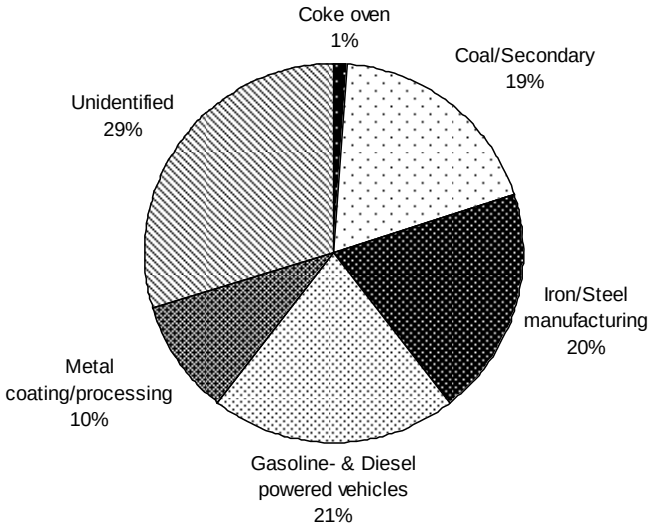


Figure 7.2. Average factor contributions to ambient $PM_{2.5}$ during the 12-day winter exposure period in Steubenville: Results from 190 30-minute SEAS samples collected in February 2007.

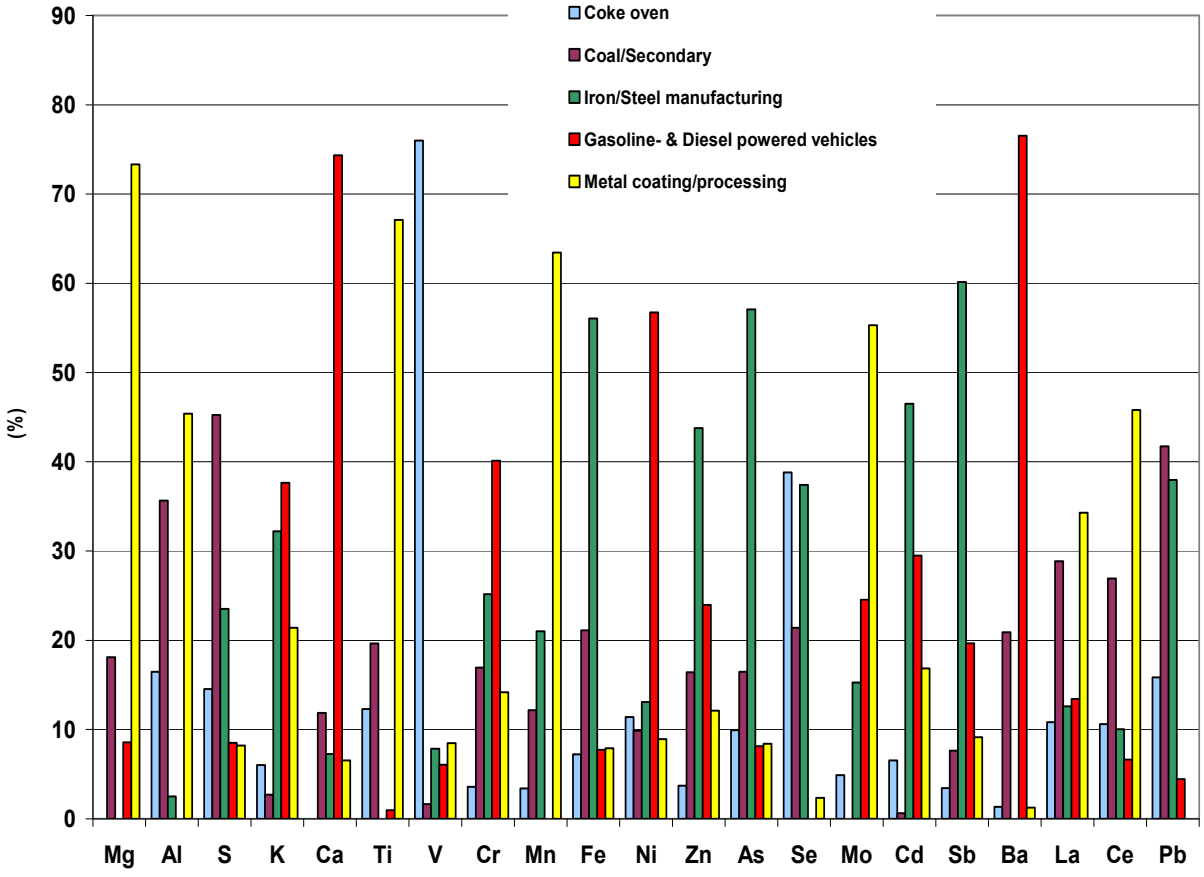


Figure 7.3 Percentage contribution of each element to each identified source factor during Steubenville winter study.

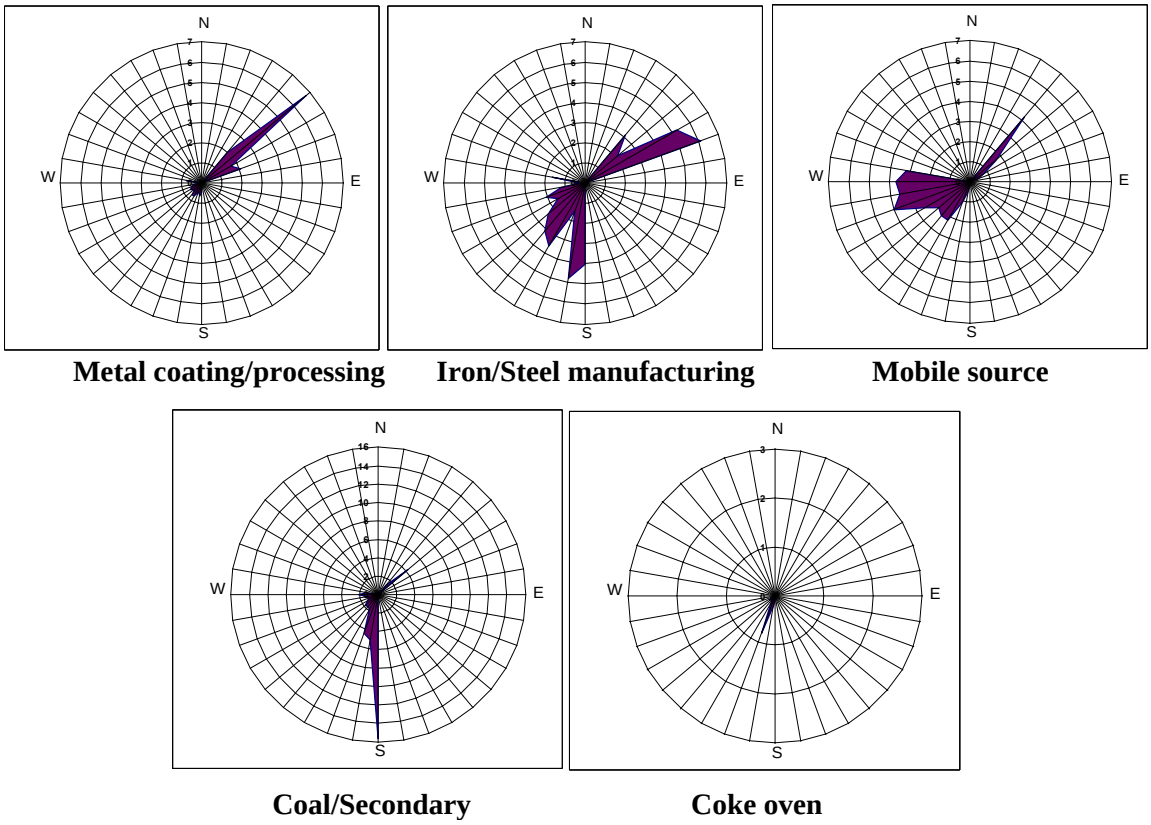
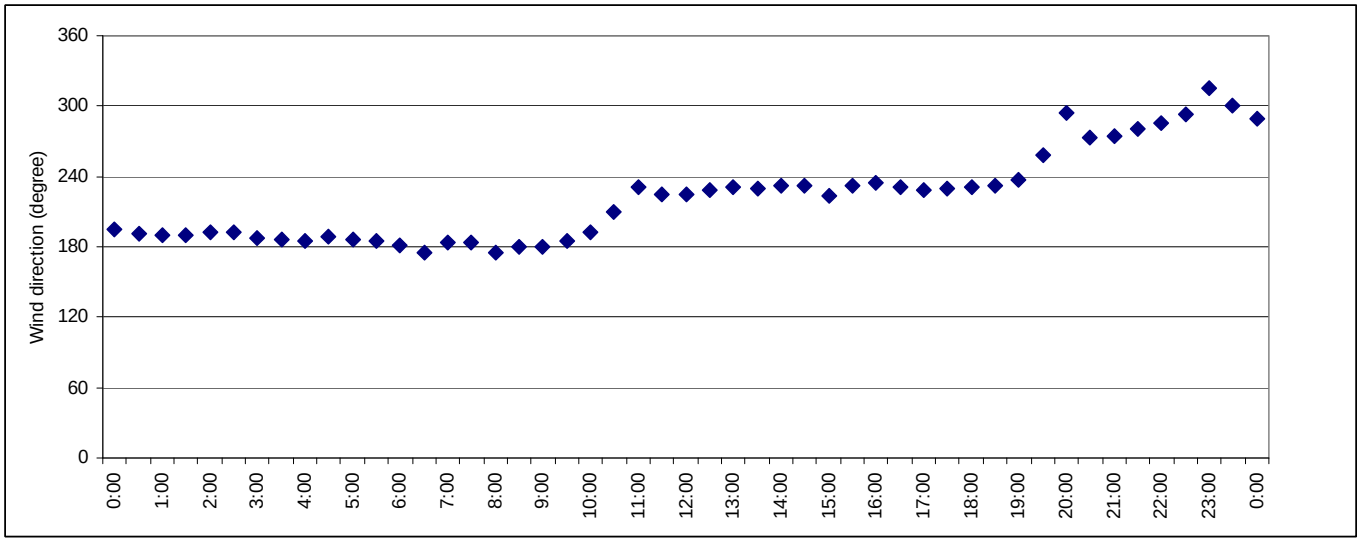
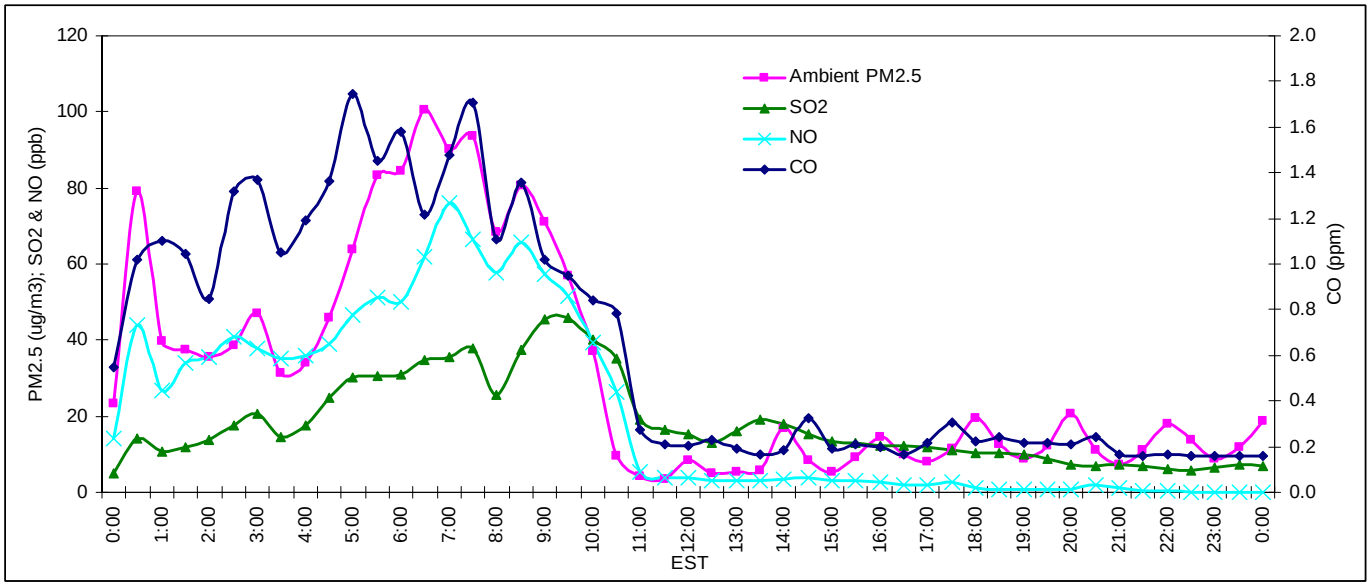


Figure 7.4 Average factor contributions versus wind direction for 190 SEAS samples collected in Steubenville, February 2007. Different scales were used to cover a large range of $PM_{2.5}$ concentrations.

The Steubenville sampling site was often impacted by short-term increases of ambient $PM_{2.5}$ and primary gaseous pollutants. For example, the highest ambient $PM_{2.5}$ concentrations were observed on February 17th as shown in Figure 7.5b. Figure 7.5b also shows simultaneous increases of primary gaseous pollutants including CO, NO and SO_2 concentrations on the morning of February 17th. Meteorological data showed that this pollution episode was associated with southerly winds (Figure 7.5a). As the wind shifted from south to southwesterly around 11:00am, concentrations of $PM_{2.5}$ and primary gaseous pollutants dropped dramatically. As shown in Figure 1b, large coal-fired power plants as well as iron/steel manufacturing facilities are located south of the study site. Taken together, the data suggest that emissions from these local combustion sources are behind the pollution episode that impacted the site.



(a)



(b)

Figure 7.5 Temporal variations of (a) wind direction and (b) 30-min average concentrations of CO, NO and SO_2 and $PM_{2.5}$ on February 17, 2007.

7.3 Bronchoalveolar Lavage Fluid, Tissue, and Serum Analysis

Inhalation exposure to CAPs for 13 consecutive days did not significantly alter the number or type of inflammatory cells collected in bronchoalveolar lavage fluid from either SH or WKY rats (Fig 7.6). Differences in BAL cells between strains were also not significant.

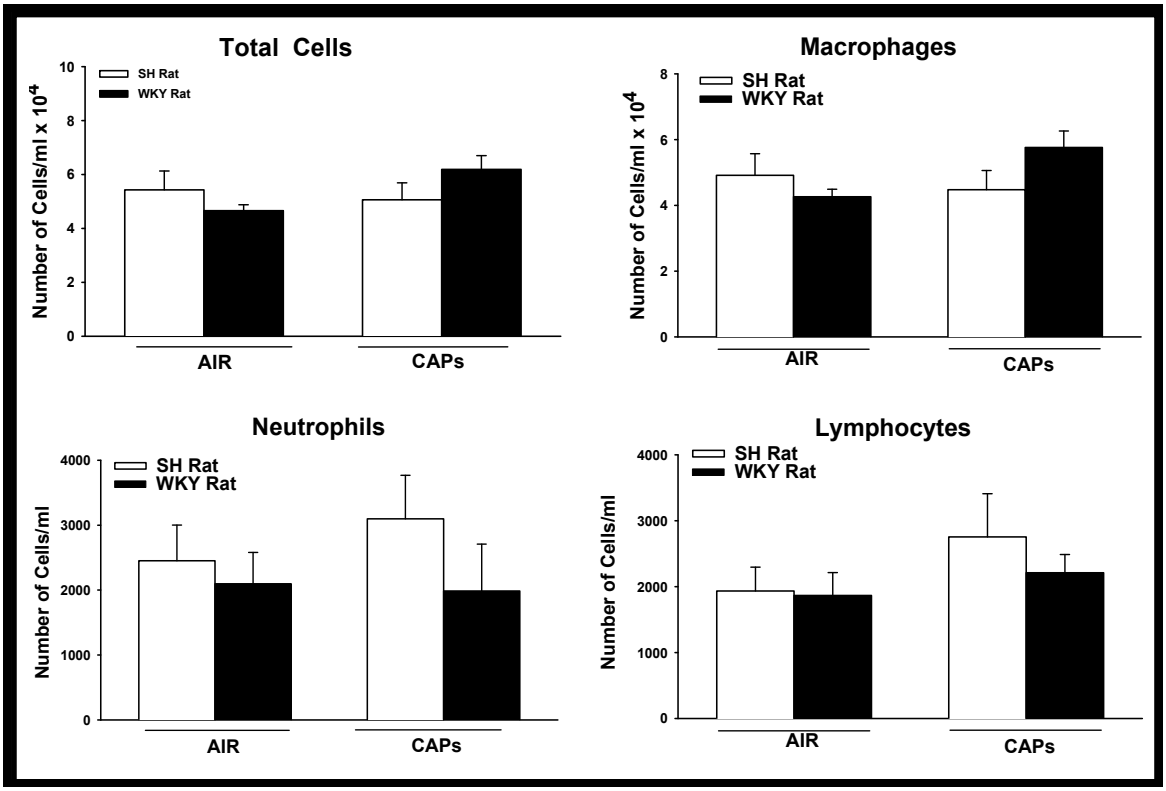


Figure 7.6 Effect of CAPs exposure on bronchoalveolar lavage cellularity, Steubenville, Winter 2007.

Modest differences in the amount of intraepithelial mucosubstances in proximal pulmonary airways were detected in WKY rats versus SH rats after exposure to filtered air (Fig 7.7). CAPs-induced decreases in IM in proximal airways were not significant in either strain. No detectable differences in mucosubstances were found in distal airways.

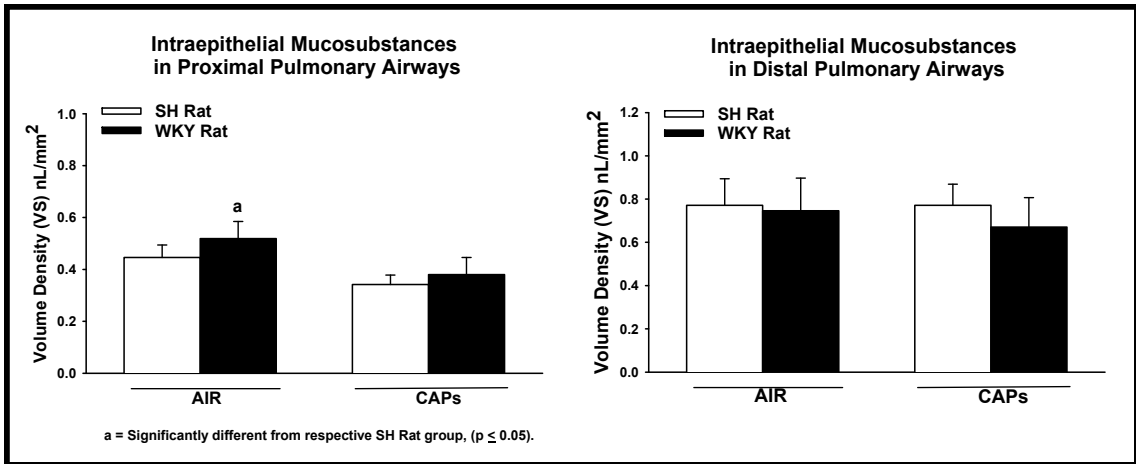


Figure 7.7 Effect of CAPs exposure on intraepithelial mucosubstances in proximal and distal pulmonary airways, Steubenville, Winter 2007.

Exposure to CAPs had no effect on serum CRP levels in either rat strain (Fig. 7.8). No strain differences in CRP were detected between SH and WKY rats. Furthermore, serum levels of TNFα, IFNγ, IL-10 and IL-6, where detectable, were not significantly different across strains or exposures (data not shown).

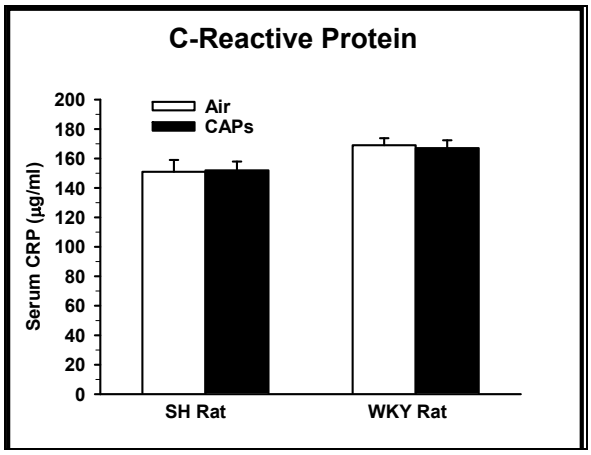


Figure 7.8 Effect of CAPs exposure on serum C-reactive protein, Steubenville, Winter 2007.

7.4 Heart Rate and Heart Rate Variability Responses

Mixed modeling results comparing Air- and CAPs-exposed SH rats during the winter were not significant for any HRV parameter using either the 8-hour or 30-minute datasets. These data are summarized in Tables 7.4 (8-hour) and 7.5 (30-minute). As in our other studies, the 8-hour integrated averages in air pollution concentrations did not have sufficient variability to detect changes in HRV. But unlike the summer study at this site, high-resolution data also failed to detect significant differences between AIR and CAPs rats. With 8-hour sampling, differences in SDNN did approach significance (p=0.0506), but with the higher temporally resolved 30-minute data this relationship was not evident (p=0.1776).

Table 7.4 Steubenville winter daily (8-hour) average statistics for unexposed (AIR) and exposed (CAPs) rats.

	AIR Rats	**CAPs Rats**	**Mixed Modeling Significance (p<0.05)**		
	N=95	N=84	Group	Date	Group*Date
Heart Rate	307.7	301.8	0.1832	<.0001	0.7354
ln(SDNN)	2.74	2.79	0.2533	<.0001	0.0506
ln(rMSSD)	1.44	1.47	0.6833	<.0001	0.7443

Table 7.5 Steubenville winter study mixed modeling results for the 30-minute HRV parameters.

	Mixed Modeling Significance (p<0.05)		
	Group	Date	Group*Date
Heart Rate	0.1801	<.0001	0.9060
ln(SDNN)	0.2822	<.0001	0.1776
ln(rMSSD)	0.7075	<.0001	0.9147

Despite the lack of significant differences between exposure groups, CAPs-exposed rats had on average lower heart rate and greater heart rate variability than control rats during the exposure period, as shown in Figure 7.9. With the exception of one day (February 17), daily SDNN was greater in CAPs-exposed rats than those exposed to air.

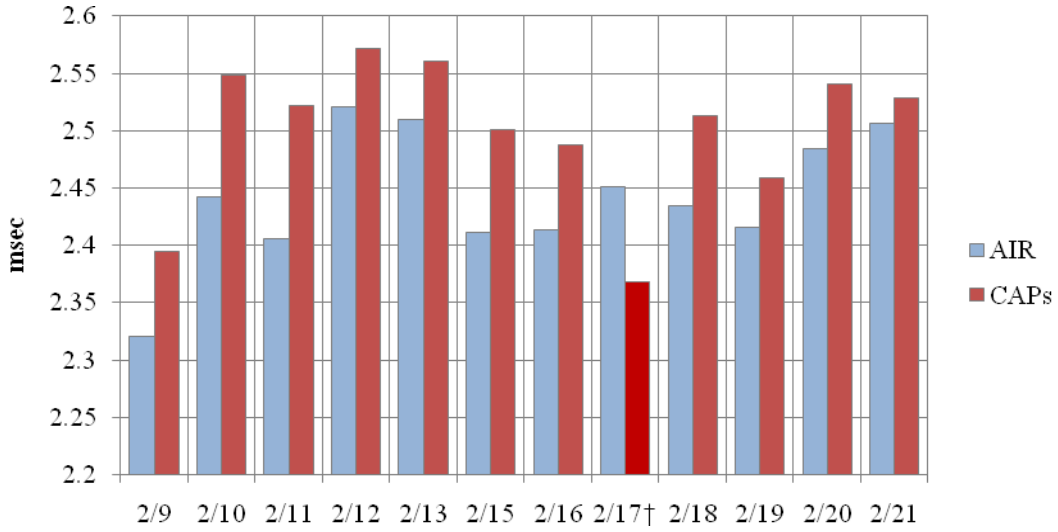


Figure 7.9 Daily ln(SDNN) in air and CAPs-exposed rats, Steubenville, Winter 2007.

Because of the lack of significant differences between exposure groups by mixed model analyses (i.e., air versus CAPs exposure), we did not evaluate associations between HRV parameters and specific CAPs components.

8.0 DISCUSSION

8.1 Detroit

We had previously conducted a similar but limited study at the same location in Detroit (Rohr et al., 2010b) using half as many animals; in that study we also only collected 8-hour integrated samples. Although we detected exposure-related differences in the lung, no significant changes in cardiac function were observed. In the work reported herein, we collected semi-continuous elemental data and were able to detect significant associations between multiple CAPs components and changes in cardiac function.

We linked factors of motor vehicles and local emission sources unique to southwest Detroit to altered HR and HRV. These findings agree with our recent reports using less robust exposure metrics, which found that local emissions sources upwind of the exposure site, including incinerators, refineries, and metal processing operations, were linked to cardiopulmonary effects, including pulmonary deposition of metals (Rohr et al., 2010b; Morishita et al., 2006). With the exception of one association we found for a coal/secondary factor in the winter (this factor would also contain primary sulfate from local sources), the present study also finds that similar local sources, including cement/lime production, sludge incineration, refinery, iron/steel, and motor vehicles influenced HRV metrics. When the predominant wind direction is from the southwest, as in summer, the site is impacted by mixtures from multiple sources. Among the numerous sources located in that general direction, it appears that those located on a heading of 210º-230º from the study site (I-75 corridor, sewage sludge incinerator, iron/steel manufacturing and cement/lime production) were most strongly associated with reduced SDNN in the summer.

There were some inconsistencies in our results that should be pointed out. First, although changes in HR were only marginally significant in the summer ($p=0.0576$), we did evaluate associations between CAPs components/source factors and cardiac functional parameters. In the summer, increased HR was significantly associated with both S and Se; however, in the winter these elements were significantly associated with reductions in HR. Similarly, EC was associated with decreased SDNN in the summer, but increased rMSSD in the winter. These kinds of inconsistencies are difficult to explain, but call for caution in interpretation of results.

As mentioned, it is interesting as well as slightly troubling that we found opposing responses to the same source factors in Detroit, albeit in different seasons. In the summer, the motor/diesel factor was linked to decreased HRV (SDNN), whereas in the winter, the combined motor vehicle/iron and steel factor was linked to increased HRV (rMSSD). Keeping in mind that the two HRV parameters do not represent exactly the same physiological process, and that the factors in the two seasons differed (the winter factor was combined with iron and steel manufacturing), this is nevertheless consistent with previously reported seasonal variations in $PM_{2.5}$-induced oxidative stress (Becker et al., 2005). Correlations between OC and EC in summer and winter resulted in R^2 values of 0.22 and 0.67, respectively. As discussed by Robinson et al. (2006), the weak correlation in summer is indicative of the larger contribution of secondary organic aerosol. In general, reductions in HRV are better predictors of poorer cardiac outcomes in clinical settings; therefore,

we can speculate that the secondary particulate products formed in the summer may be of particular importance from a health perspective.

In order to better understand source factor/HRV relationships and determine if a particular finding was robust, we looked for source factors as well as the those PM constituents most heavily loaded onto the factors to be significantly associated with the same health endpoints. In general we found these associations to be fairly consistent. For example, SDNN reductions in the summer were tied to iron/steel, sludge incineration, cement/lime, and motor vehicle factors. The iron/steel factor was loaded with Fe, Pb and Zn, all of which were significant in univariate analyses,, and the motor vehicle factor was loaded with a number of elements, including Fe, Ti, Mg, Cr, Mn, and As, some of which were significantly associated with HRV metrics. Previous studies have shown some of these metals to be associated with motor vehicle emissions: Mg, Ca, Fe, Zn, Ce, Pb (tailpipe emissions); Ba, Fe, Sb, and Mn (brake wear dust), and Zn (tire wear dust) (Schauer et al., 2006; Garg et al., 2000). Furthermore, EC is widely used as a marker for diesel emissions, and was also significantly associated with SDNN reductions. EC, though not included in the PMF modeling, had the highest correlation with the motor vehicle factor among the six factors (Morishita et al., 2010). However, there were also some discrepancies in the source and component results. For example, Al was loaded onto the motor vehicle factor but did not show a significant relationship with SDNN.

In winter, comparisons of source factor and component associations with cardiac endpoints were somewhat internally consistent, although less so for rMSSD than for HR. Only four elements, along with EC, were associated with increased rMSSD: Ba, Zn, As, and Rb. The combined motor vehicle and iron/steel factor was loaded with Rb, Al, Cr, Mn, Zn, and Ba. Therefore, 3 of 6 elements showed internal consistency. EC was also significantly associated with increased rMSSD, strengthening our confidence in the association (although the caveat mentioned earlier regarding opposing directionality of HRV effects in association with EC should be kept in mind). Of the 9 elements highly loaded on the coal/secondary factor (Rb, Cd, Sb, Pb, S, V, Zn, As, Se), which was significantly associated with rMSSD, only 3 were associated with rMSSD in univariate models. Several of these elements (e.g., S and Se) that are commonly regarded as tracers of coal combustion were not associated with rMSSD. Based on these findings, a clear and robust association between a source factor and rMSSD was not observed, although the association was stronger for the motor vehicle and iron/steel factor and its constituents than for the coal/secondary sulfate factor.

These findings, taken together, suggest the need for caution when interpreting the results of factor analyses when applied to health datasets. Both individual constituent relationships as well as source factor analyses should be considered; source factor analyses in isolation may not present a complete picture. To our knowledge, only one other toxicological study (Chen et al., 2010) and one epidemiological study (Sarnat et al., 2008) have compared the two types of analyses. Chen et al. concluded that component-specific analyses provide insights beyond those attainable considering only source factors, while Sarnat et al. observed generally similar findings. However, Sarnat et al.'s analyses were not entirely comparable to ours because of the use of tracers; we considered all elements in univariate models.

Several other toxicological studies have evaluated HRV in conjunction with PM components and/or source factors, although none with the high time resolution reported in this study. In Tuxedo, NY, Lippmann et al. (2005) reported significant associations between resuspended soil, secondary sulfate, and residual oil factors and HRV parameters. Other work at the same location (Lippmann et al., 2006) suggested a significant effect of nickel on HRV in ApoE -/- mice. We did not observe an association between HR and HRV parameters and nickel, and concentrations were relatively comparable; however, the NY exposures were much longer than our study. In our summer exposures, average Ni was 53 ng/m^3, with a peak of 211 ng/m^3 on one day, while in the NY study mean Ni concentration was 43 ng/m^3, with Ni peaks of about 175 ng/m3 on 14 days. On those 14 days, there was unusually low PM mass and vanadium, a pattern that was opposite from what we observed on the high Ni day (high V and high PM mass). In the winter season in Detroit, Ni was very low, with >60% of the values below our assay limit of detection.

Overall, we observed significant reductions in SDNN in the summer that were strongly associated with cement/lime, iron/steel, and gasoline/diesel factors, while associations with the sludge factor and components were less consistent. In winter, increases in HR were associated with a refinery factor and its components. CAPs-associated HR decreases in winter were linked to sludge incineration, cement/lime, and coal/secondary factors and the majority of their associated components. Specific relationships for increased rMSSD in winter were difficult to determine due to lack of consistency between factors and associated constituents. Our results appear to indicate that specific modulation of cardiac function in Detroit was most strongly linked to local industrial sources. However, as discussed, inconsistencies with the data and statistical results make definitive conclusions difficult to reach.

8.2 Steubenville

In Steubenville, we again linked highly time-resolved measurements of $PM_{2.5}$ elemental composition with acute cardiovascular changes in laboratory rats during inhalation exposures, and found unique wind and source factor dependences on these responses. Furthermore, we detected both increases and decreases in heart rate and HRV depending on particle composition, demonstrating that the ambient PM mixture can alter cardiac rhythm by multiple pathways. Our thirteen-day study in the middle of summer was impacted primarily by two discrete wind directions, and allowed us to identify distinct source factors and elements that were linked with acute cardiovascular changes. These significant findings were only observed in the summer; in the winter there were no significant differences in response between air- and CAPs-exposed rats.

In Steubenville, we identified a waste incineration/non-ferrous metal processing factor to be associated predominately with NE winds, in the direction of a number of waste incinerators and metal smelting and processing plants. As such we have combined non-ferrous metal processing as well as waste incineration in this factor (Morishita et al., 2010). This factor, which was loaded primarily with Zn and Cd, was linked to decreased SDNN with NE winds, and Zn and Cd were likewise associated with decreased SDNN in univariate models. Very few studies have estimated the cardiovascular health effects of PM derived from waste incineration. Chen et al. (2010 reported that an incineration source factor in Manhattan (element loadings of Zn, Pb, Cu, and Fe) was associated with increased HRV in CAPs-exposed mice, which is the opposite of what we observed.

Metal coating and processing industries located southwest of the Steubenville site were strongly linked to increased HR, while somewhat more weakly linked with decreased SDNN, with elemental contributions to this factor being defined by V, Cr, Ti, Mo, La and Ce. In a series of studies in rural New York, combustion of vanadium- and nickel-containing residual oil fly ash (ROFA), was linked to decreased HRV in ApoE- mice (Lippmann et al., 2005). These investigations were recently extended in a comparison study of rural and urban New York sites, in which nickel refinery sources had a greater impact on HRV than did oil fly ash sources (Chen et al., 2010). We did not detect significant Ni associations in Steubenville as this element was often less than detection limits; nor did we observe any associations with Ni in Detroit (Rohr et al., 2010a). Interestingly, the Ni refinery factor used in the New York study used Cr as the predominant elemental tracer, which agrees with our metal factor in Steubenville which has a high Cr contribution.

Transition and multi-valent metals such as Zn, V, Ti, Ni, Cr, Fe, and Mn have been shown to affect cardiovascular responses after airway exposure in animals (Campen et al., 2002; Kodavanti et al., 2008; LeBlanc et al., 2010; Wallenborn et al., 2007). The Steubenville area has a large concentration of metal and steel processing industries with a high outputs of Fe, Ni, Zn, and Mn among other metals (Connell et al., 2006; Koutrakis and Spengler, 1987). It is not surprising that in addition to the metal factor we also found associations with the steel and iron processing factor in Steubenville with NE winds, which were linked to bradycardic responses but not to changes in HRV. Decreased heart rate and increased HRV in response to particle inhalation, while antithetical to the majority of epidemiological reports that show the opposite effects, is not unprecedented in animal models. Chen and coworkers demonstrated opposing HRV responses to ambient $PM_{2.5}$ inhalation in mice that was dependent on whether exposures took place urban versus rural settings (Chen et al. 2010). We recently detected bradycardia and increased HRV in rats during exposure to CAPs in winter, and opposing responses during the summer at the same urban Detroit site (Rohr et al., 2010a). Furthermore, inhalation exposure to oil fly ash (Farraj et al., 2010), ozone and black carbon (Hamade and Tankersley, 2009), and on-road, highway aerosols (Elder et al., 2007) can all reduce heart rate and increase HRV. Given the range of exposure atmospheres in these studies, it is unclear what elements, groups of elements, or sources, promote the divergent and acute responses in autonomic tone in laboratory animals.

Despite the high concentration of coal fired-power plants in the Ohio River Valley near Steubenville and the documented impact of this source on our site as discussed in earlier sections, we did not find significant associations of cardiac regulation with the coal/secondary factor, although individual tracer elements (S, Se) and SO_2 were linked to decreased heart rate, and SO_2 to decreased rMSSD. No elements typically used as tracers for coal combustion were linked with reduced SDNN. These results are consistent with some other CAPs studies, although they differ from recent panel studies of elderly subjects in Steubenville, which describe decreased HRV and increase incidence of arrhythmia associated with increased sulfate concentrations with a one-day lag (Luttmann-Gibson et al., 2006; Sarnat et al., 2006). This association between HRV and sulfate appears to be enhanced with underlying cardiovascular inflammation as measured by C-reactive protein (CRP) in plasma (Luttmann-Gibson et al., 2010). As such, we might expect to see similar responses in the SH rat, a susceptible strain we

have previously shown to respond to CAPs exposure with increased CRP (Rohr et al., 2010b). In the sensitive ApoE- mouse for example, Chen and colleagues found the regional transport factor (S, Se, EC) in rural upstate New York had the largest impact on HRV (Chen et al., 2010). However despite contributing the most to PM mass in Steubenville, on average 39%, the effects of coal/secondary factor on HRV were not apparent.

Traffic sources of PM have been consistently implicated in decreased HRV, especially in urban studies (Schwartz et al., 2005; Zanobetti et al., 2010). Although mobile/ traffic sources in Steubenville had minor contributions to PM mass (12%), this factor showed associations with cardiac endpoints. Specifically, it was linked with reduced SDNN with NE winds, as were a number of elements that loaded onto this factor.

As with the Detroit study, it is very important to compare associations between source factors and between the individual elements loaded onto these factors. We have more confidence in a given source's impact if the elements comprising the factor also suggest impacts. In Steubenville, we found examples where PM constituents identified as most heavily loaded onto a source factor were associated with a health effect when the factor itself was not. For example S and Se were associated with decreased HR and are highly loaded onto the coal/secondary factor, yet this factor had no associated effects on HR. Contributing to this outcome is that none of the other four elements dominating this factor – Al, P, V, and Cu – were associated with decreased HR. The converse was also found when a factor was significant but many elements loaded to this factor were not. For example, we found associations between metal processing and decreased SDNN during SW winds, yet only one (Mo) of the seven elements loaded to the factor (P, Ti, V, Cr, Mo, La, Ce) was individually linked to this response from this wind direction. Of course, some elements, such as Mo and Sb, are loaded onto more than one source factor. As such, component-specific analyses may provide insights beyond those attainable considering only source factors.

As with the Detroit data, we observed some inconsistencies that deserve mention. For example, SO_2 was linked with increased HR with SW winds, but decreased HR with NE winds.

Overall, we observed significant changes in HR, SDNN, and rMSSD in Steubenville in the summer. We did not observe significant changes in the winter. Changes in HR (both increases and decreases were observed) were linked with metal processing, waste incineration, and iron/steel factors along with most of their associated elemental constituents. Reductions in SDNN were associated with metal processing, waste incineration, and mobile source factors and the majority of elements loading onto these factors. There were no consistent associations between changes in rMSSD and source factors/components. We did not observe any associations with the coal/secondary factor or with most of its associated components. As with Detroit, our Steubenville results indicate that changes in cardiac function were most strongly associated with local industrial sources.

8.3 Integrated Analysis

Our original study proposal included three locations (Detroit, Steubenville, and a state park in Pennsylvania). Due to NETL funding restrictions applied mid-way through the program, we were only able to complete our study in Detroit and Steubenville. This was unfortunate, as the

three locations were selected specifically for their influence from different PM emissions sources. The third, unstudied location, given its rural setting with no nearby industry or major traffic sources, was almost completely impacted by regionally transported pollution. This high-level contrast among locations would have provided us with one more basis for comparison of health responses. However, with the two locations we studied, each with a different air pollution profile, we were still able to generate highly valuable information regarding the role played by different PM components and source factors in cardiac functional modulation.

We began with the premise that PM composition would differ between our sites as well as seasonally. This was generally the case, as can be seen from comparing Figures 4.4, 5.5, 6.2, and 7.2 as well as the compositional data in Tables 4.1, 5.1, 6.1, and 7.1. In Detroit in the summer, 31% of PM was estimated to be derived from diesel and gasoline vehicles, whereas in Steubenville during the same season that number was only 12%. Both locations had high regional/secondary influence (33% and 39% for Detroit and Steubenville, respectively). In the winter, the differences in mobile source influence were less notable, with 24% of PM in Detroit estimated to be mobile source-associated, and 21% in Steubenville. The proportion of the secondary sulfate factor was higher in Detroit in the winter (33%) than in Steubenville (19%). However, in examining the component data, approximately 25% of total PM was comprised of sulfate in Steubenville in the winter versus about 15% in Detroit. The difference between the source factor and compositional data reflects S loadings onto an iron/steel manufacturing factor in Steubenville and thus a lower contribution from the secondary sulfate factor. It is also worth noting that in the winter, the sulfate concentrations in the two locations were relatively similar (47 $\mu g/m^3$ in Detroit and 63 $\mu g/m^3$ in Steubenville). Overall, our site selection, which was aimed at exploiting different air quality regimes, was successful.

We also achieved our goal of seasonal contrasts in air quality at the two study locations. In Detroit, there was a notable difference in sulfate contribution between summer (24% of PM mass) and winter (13%) study periods. In Steubenville, there was essentially no difference for sulfate (23% and 25% for summer and winter studies, respectively). However, organic carbon displayed significant seasonal variation in both locations. In Detroit, summer OM contribution to PM mass was 31%, versus 16% during the winter study period. In Steubenville, these numbers were 33% and 23%. Elemental carbon contribution was relatively constant (1-2%) for all sites and seasons. It should be noted that our 13-day exposure periods were only snapshots of the full season, and conclusive seasonal differences cannot be determined.

Associations between source factors and their associated components at the two locations were broadly consistent. In Detroit, we observed associations between cardiac changes and cement/lime, iron/steel, sludge incineration, refinery, coal/secondary, and gasoline/diesel factors. In Steubenville, factors of metal processing, waste incineration, iron/steel, and mobile sources were most strongly associated with HR and HRV changes. Of course, it should be noted that the factors are not directly comparable between study locations, nor between seasons. In addition, we observed several inconsistencies in our results, as previously discussed, that make definitive conclusions difficult. In both cities, results indicate that changes in cardiac function were most strongly associated with local industrial sources with high emissions of metals. Results for coal-fired power plant-derived PM were inconsistent and largely nonsignificant.

Interestingly, we observed associations between cardiac function metrics and mobile sources in both Detroit and Steubenville, although our Detroit site, with its multiple traffic sources, was selected for study to provide a contrast with Steubenville, a much smaller city. Similarly, the one association we observed between HR and a coal/secondary factor was in Detroit (although this finding was inconsistent due to opposing signs of effect estimates between seasons, and this factor would also contain primary sulfate from local sources), whereas Steubenville was selected because it is considered to be one of the most highly power plant-impacted areas in the country.

An important limitation of our study that warrants mention is that of multiple comparisons. With so many individual statistical tests, some effect estimates will be statistically significant simply due to chance. We do not know which results are in this way spurious.

9.0 CONCLUSIONS

The primary objective of the study as it was defined in 2004 was to "evaluate the potential for adverse cardiopulmonary effects from ambient exposure to realistic (environmentally relevant) coal-fired power plant and traffic-related PM". We successfully addressed this objective, and in doing so the study has generated critical information that is expected to inform the regulatory process for PM. Using novel, highly time resolved measurements of trace elements coupled with continuous ECG monitoring, we were able to link physiological responses in rats to specific PM components and source factors in both Detroit and Steubenville. Using this refined exposure assessment approach for the first time with either humans or animals, we found novel and divergent effects of CAPs exposure on cardiac rhythm that were dependent on location, season, and PM composition.

Our results suggest that multiple PM components play a role in acute cardiac responses in rats exposed to Detroit and Steubenville CAPs; however, overall our findings suggest that changes in cardiac function were most strongly associated with local industrial sources. Results for coal-fired power plant-derived PM were inconsistent and largely negative, whereas traffic and metal-related industries appeared to have a stronger impact. However, due to some inconsistencies in our results, these findings should not be viewed as definitive. Our findings also illustrate the need to use caution, and to consider in a holistic manner, both component- and source factor-based analyses when interpreting data. If only one approach is employed, less powerful conclusions might be reached.

Along with a large amount of existing literature, the work reported here further demonstrates that PM composition plays a strong role in resultant health effects. This work has been submitted for publication in the peer literature, with four manuscripts documenting (1) Detroit exposure characterization and receptor modeling; (2) Detroit toxicological results; (3) Steubenville exposure characterization and receptor modeling; and (4) Steubenville toxicological results. We have completed another field study (with non-NETL funding), the Michigan Integrated Cohort and Animal Particle Study (MICAPS) in Dearborn, which includes a CAPs animal study and a human panel study. This work will also add to the growing literature on the most important PM sources and components from a public health perspective.

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